DIFFICULT-TO-TREAT SCHIZOPHRENIA:
A CLINICAL, PSYCHOPHARMACOLOGICAL, AND
NEUROIMMUNOLOGICAL STUDY

Grigori Joffe

Academic dissertation

To be publicly discussed with the permission of the
Medical Faculty of the University of Helsinki
in the auditorium of the Department of Psychiatry,
12 noon, June 24, 1999

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ISBN 952-91-1081-2
Yliopistopaino
Helsinki, 1999

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This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:


III. Grigori Joffe, Björn Appelberg, Ranan Rimón. ADJUNCTIVE NEFAZODONE IN NEUROLEPTIC-TREATED SCHIZOPHRENIC PATIENTS WITH PREDOMINANTLY NEGATIVE SYMPTOMS: AN OPEN PROSPECTIVE PILOT STUDY. International Clinical Psychopharmacology, in press


V. Grigori Joffe, Peter Nyberg, Andres Gross, Björn Appelberg. CLOZAPINE-INDUCED DECREASE IN THE PRODUCTION OF REACTIVE OXYGEN METABOLITES BY MONOCYTES IN VITRO MAY PREDICT CLINICAL RESPONSE TO CLOZAPINE IN TREATMENT-RESISTANT SCHIZOPHRENIA. Human Psychopharmacology 1999;14:203-209
**ABBREVIATIONS**

<table>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>BPRS</td>
<td>Brief Psychiatric Rating Scale</td>
</tr>
<tr>
<td>CAT</td>
<td>catalase</td>
</tr>
<tr>
<td>CGI</td>
<td>Clinical Global Impression</td>
</tr>
<tr>
<td>CLO</td>
<td>clozapine</td>
</tr>
<tr>
<td>CPRS</td>
<td>Comprehensive Psychiatric Rating Scale</td>
</tr>
<tr>
<td>CYP</td>
<td>cytochrome P450</td>
</tr>
<tr>
<td>D2</td>
<td>dopamine receptors 2</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSM-III-R</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, revised</td>
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<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, 4th edition</td>
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<tr>
<td>GSH-Px</td>
<td>glutathione peroxidase</td>
</tr>
<tr>
<td>SHT</td>
<td>5-hydroxytryptamine (serotonin)</td>
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<tr>
<td>IL</td>
<td>interleukine</td>
</tr>
<tr>
<td>MADRS</td>
<td>Montgomery-Åsberg Depression Rating Scale</td>
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<tr>
<td>MGG</td>
<td>May-Grünwald-Giemsa</td>
</tr>
<tr>
<td>MO</td>
<td>monocytes</td>
</tr>
<tr>
<td>MOn</td>
<td>non-stimulated monocytes</td>
</tr>
<tr>
<td>MOs</td>
<td>monocytes, stimulated with phorbol myristate acetate</td>
</tr>
<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>PANSS</td>
<td>Positive and Negative Syndrome Scale</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
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<tr>
<td>PMA</td>
<td>phorbol myristate acetate</td>
</tr>
<tr>
<td>PMNL</td>
<td>polymorphonuclear phagocytes</td>
</tr>
<tr>
<td>PMNLn</td>
<td>non-stimulated polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>PMNLs</td>
<td>polymorphonuclear leukocytes, stimulated with phorbol myristate acetate</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acids</td>
</tr>
<tr>
<td>QLS</td>
<td>Quality of Life Scale</td>
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<tr>
<td>ROM</td>
<td>reactive oxygen metabolites</td>
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<td>SAS</td>
<td>Simpson-Angus Scale</td>
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<tr>
<td>sILR</td>
<td>soluble interleukine receptors</td>
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<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
</tr>
<tr>
<td>TNF-a</td>
<td>tumor necrosis factor alfa</td>
</tr>
<tr>
<td>UKU</td>
<td>Committee for Clinical Investigations (Udvalg for kliniske undersøgelser) Side Effect Rating Scale</td>
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1. INTRODUCTION

1.1. Schizophrenia

Schizophrenia is a chronic and disabling disintegrative psychotic illness. Symptoms of schizophrenia fall into three clusters: positive symptoms, which include delusions and hallucinations; disorganized thought, speech, and behavior; and negative, or deficit symptoms, such as reduced thought and speech, blunted affect, and decreased initiation of goal-directed behavior. Although presenting with somewhat similar clinical features, schizophrenia is likely a group of disorders with heterogeneous prognosis and causes.

The worldwide prevalence of schizophrenia is about 1%, with somewhat higher figures (1.5%) reported in Finland (Lehtinen, 1996). Being long-lasting and incapacitating, schizophrenia exacts disastrous costs from patients, their families, and society. Despite tremendous progress in understanding schizophrenia and the rapid development of biological and psychosocial therapeutic interventions during the last decades, the fight against this illness is still far from its victorious completion.

1.2. Treatment-resistant schizophrenia

Between one-fifth and one-third of patients with schizophrenia do not show an adequate response to neuroleptic medication (Prien and Cole, 1968; Essock et al., 1996). These "treatment-resistant" patients create a serious public health problem due to their extensive hospitalization needs (McGlashan, 1988) and consequent high costs of the treatment (Revicki et al., 1990).

Treatment-resistance has been defined in different ways. The most restrictive criteria have been introduced by Kane et al. (1988):

1. Persistent positive psychotic symptoms: Item score ≥ 4 (moderate) on at least two of four positive symptom items (rated on a 1 – 7 scale) on the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) – hallucinatory behavior, suspiciousness, unusual thought content, and conceptual disorganization.

2. Current presence of at least moderately severe illness: Total BPRS score ≥ 45 on the 18-item scale and a score ≥ 4 (moderate) on the Clinical Global Impression scale (CGI) (Guy, 1976).

3. Persistence of illness: No period of good social or occupational functioning within the last 5 years.

4. Drug-refractory condition: At least three periods in the preceding 5 years of treatment with conventional antipsychotics from at least two chemical classes at doses ≥ 1000 mg per day of chlorpromazine equivalents for 6 weeks, each without
significant symptom relief, and failure to improve by at least 20 percent as measured by total BPRS score or intolerance of haloperidol at 10 to 60 mg per day during a 6-week prospective trial.

Different broader definitions of treatment-resistance have been used by others (Breier et al., 1994; Juarez-Reyes et al., 1995), and today treatment-, or neuroleptic-resistance is often defined as a failure to respond to the usual drug treatment (Wahlbeck et al., 1998). For the term “treatment-resistant schizophrenia” has frequently been substituted the term “difficult-to-treat schizophrenia”, especially when used in a broader sense.

Although most definitions of treatment resistance consider positive symptoms, there has been an awareness of the problem of persistent deficit symptoms. Negative symptoms of schizophrenia (lack of normal mental activities such as thought, speech and motivation) are well known as especially resistant to treatment interventions (McPhillips and Barnes, 1997). In addition, depression and extrapyramidal side-effects (EPS) of neuroleptics, which are often indistinguishable from each other and from the negative symptoms of schizophrenia (Coffey, 1994; Sax et al., 1996) can make schizophrenic patients treatment-resistant (Kane, 1996) – either directly or via EPS-related in compliance with antipsychotic medication. To distinguish enduring (“primary”, “deficit”, or “core”) negative symptoms from less stable secondary negative features is theoretically and prognostically important, but difficult due to the lack of valid measures (McPhillips and Barnes, 1997). Negative symptoms scores correlate strongly with the duration of initially untreated psychosis (Waddington, 1996).

1.3. **Active brain process as plausible background of schizophrenia and treatment-resistance**

Gradual deterioration in schizophrenic patients was observed by Kraepelin before the neuroleptic era. However, the convincing evidence for neurodevelopmental pathology in schizophrenia has since the 1980s led to general abandonment of the classical chronic deterioration disease concept. It is interesting, however, that while many patients recover almost completely from their first psychotic episode, the majority subsequently experience additional episodes of psychosis with an increasing, often persistent morbidity. In contrast to Alzheimer’s disease, which is characterized by a continuing inexorable progression, the progression in schizophrenia occurs mostly during the initial years of the disease. McGlashan and Fenton (1993) found an instability of clinical picture with a drift toward disorganized, non-specified, and deficit subtypes of schizophrenia and significant worsening of negative symptoms during the first 5 years of active psychosis with a plateau later on. These changes were associated with poor functional outcome 15 years later. Negative symptoms, although
most variable at the initial phase of the illness increased in severity, stability, and adverse prognostic weight during the later course of the disease. Consistent with the view that the decline in schizophrenia occurs early in the illness and then plateaus, Harrison et al. (1996) found that the illness course over the 2 years after onset is strongly associated with the course over the ensuing 10 years. Progression of negative symptoms, accompanied by relatively stable positive symptoms was also found by Peralta et al., 1995. After the first years, the patients tend to reach a relatively stable, symptomatic treatment-resistant phase. Although the etiology of this process is obscure, timely effective intervention at the early stage is essential for the outcome (Wyatt, 1991; Loebel et al., 1992; McGlashan and Fenton, 1993; Lieberman et al., 1993; Waddington et al., 1996; Wyatt et al., 1997). This evidence proposes that although the disease may be developmental, a neurodegenerative component is involved in treatment resistance.

A convincing body of evidence indicates that the treatment response and the level of recovery in early stages of the disease are improved despite only low doses of neuroleptics (McEvoy et al., 1991; Lieberman et al., 1996). In addition, the intervention in this stage is beneficial also in the long term (Schooler et al., 1997; Lieberman et al., 1992). A long duration of untreated psychosis as a predictor of poor outcome has been reported in numerous studies (Loebel et al., 1992; Lieberman et al., 1992; Waddington et al., 1995; Szymanski et al., 1996). Also several modern neuroimaging studies have demonstrated a more rapid loss of brain volume in schizophrenic patients than in controls during the first years of the disease (Delisi et al., 1997; Gur et al., 1998). Nair et al. (1997) have found two different groups of patients – with or without progression of such brain volume loss, possibly due to at least two etiologically distinct processes. Loss of volume was associated with neurobehavioral decline (Gur et al., 1998). It has been concluded that periods of active psychosis involve neurodegenerative and/or neurotoxic processes (Wyatt, 1995; Nair et al., 1997; Lieberman et al., 1997), which, if not ameliorated by neuroleptics, may result in negative symptoms and treatment refractoriness.

It has been shown that along with a prolonged untreated psychosis tardive dyskinesia is a predictor of poor outcome in first-episode schizophrenia, and that subsequent non-responders are more liable to develop it (Lieberman et al., 1992). In addition, an association between tardive dyskinesia and cognitive deterioration has been observed (Waddington et al., 1997). Tardive dyskinesia therefore has been proposed to be a phenotypic marker of the illness, associated with treatment resistance (Lieberman et al., 1992). The neurobehavioral decline associated with negative symptoms seems to
be interrelated with tardive dyskinesia (Lieberman et al., 1992) and with the loss of brain volume during the course of the disease, manifesting the neurotoxicity of the schizophrenic process (Gur et al., 1998).

Consequently, it can be postulated that the most effective treatment modes available with beneficial effects on positive and (even more important) negative symptoms and tardive dyskinesia should be used aggressively at the very beginning of the disease in order to protect the brain and to avoid the development of treatment-resistance. In this respect, clozapine (CLO) and the other “atypical” neuroleptics ought to be discussed.

1.4. Clozapine

CLO, a prototypic “atypical” neuroleptic, is a tricyclic dibenzazepine compound which was developed in 1957 by Wander, a small Swiss pharmaceutic company. Initially designed as an antidepressant, CLO surprisingly showed a potent antipsychotic action without noticeable extrapyramidal side-effects. Since CLO did not have cataleptic and amphetamine antagonism properties (believed at that time to be necessary for an antipsychotic), it was initially not introduced to clinical practice, and the first clinical studies were published only in the late 60s (Berzewska et al., 1969; Gross and Kaltenbük, 1970; Angst et al., 1971). In Finland CLO was registered in 1975. However, when, during a relatively short period, 8 out of 16 Finnish patients developing leukopenia during CLO treatment died because of agranulocytosis (Amsler et al., 1977; Idänpää-Heikilä et al., 1977), the drug was withdrawn from the market in Finland and most other countries. Nevertheless, re-introduction of CLO was shortly thereafter permitted for many patients who did not respond to any other drug. Hence, considerable clinical experience was obtained until the middle 80s, when CLO was shown to be more effective than chlorpromazine in a controlled double-blind study by Honigfeld et al. (1984). However, only when a methodologically more restrictive study by Kane et al. (1988) revealed the superiority of CLO over haloperidol in a group of 286 patients with treatment-resistant schizophrenia, was CLO launched in the United States and the UK - in 1990. This manifested a new breakthrough in the pharmacotherapy of schizophrenia. Nevertheless, due to the 1 – 3% risk of agranulocytosis (Fitzon and Heel, 1990; Krupp and Barnes, 1992; Alvir et al., 1993; Owens, 1996), CLO in most countries was reserved for treatment-resistant cases only - a situation requiring a clear definition of this condition. Hereby, the whole concept of treatment-resistance gained a new meaning, relevant to clinical practice.

CLO differs from conventional neuroleptics in many ways. It is claimed to be a more potent antipsychotic with a degree of response of 30 to 60% in schizophrenia resistant
to conventional neuroleptics (Kane et al., 1988; Christison et al., 1991; Szymanski et al., 1994; Breier et al., 1994; Schooler et al., 1994; Barnes and McEvoy, 1996; Wahlbeck et al., 1993). It is effective against both positive and negative symptoms of schizophrenia (Kane et al., 1988), including the primary negative symptoms (Miller et al., 1994; Brar at al., 1997), it postpones relapses (Wahlbeck and Cheine, 1998), and has beneficial effects on neurocognition in schizophrenia (Lee et al., 1994). CLO appears to be a potent mood stabilizer (Zarate et al., 1995; Vestergaard, 1997) with beneficial effects on impulsive, aggressive (Garmenda et al., 1992; Mallya et al., 1992), and suicidal (Okayli et al., 1992; Meltzer and Okayli, 1995) behaviors. Treatment with CLO seems to improve the quality of life of schizophrenic patients (Meltzer, 1992a) and is cost-effective despite expensive mandatory hospitalization at the beginning of the treatment and frequent white blood cell count monitoring (Honigfeld and Patin, 1990; Morris et al., 1998).

The side-effect profile of CLO differs as well from that of conventional compounds. In addition to agranulocytosis, CLO more frequently causes troublesome hypersalivation (Lieberman et al., 1989), weight gain (Leadbetter et al., 1992), and seizures (Devinsky et al., 1991), but it is almost free from extrapyramidal side-effects, tardive dyskinesia, and hyperprolactinemia (Meltzer, 1992b; Tamminga et al., 1994; Owens, 1996).

To summarize, the superior efficacy of CLO, primarily with regard to negative symptoms and tardive dyskinesia could be an argument for its early use in schizophrenia in terms of overall clinical outcome and prevention of treatment-resistance (Lieberman, 1996; Edwards et al., 1998). However, the CLO-related risk of a life-threatening agranulocytosis makes it a drug of reserve and thus exerts pressure toward creating safer alternative compounds or drug strategies with at least equal efficacy, or both.

1.5. Serotonin-dopamine antagonism and the atypicality of neuroleptics

The antipsychotic potency of the conventional neuroleptics has been related to their ability to inhibit the dopamine D2 receptors (Connell, 1958; Randrup and Munkvad, 1972; Carlsson, 1977; Kapur et al., 1996). This observation has led to the development of the dopamine theory of schizophrenia. Numerous D2 inhibitors of different chemical classes have been developed since the invention of chlorpromazine in 1952. Since they are of equal antipsychotic efficacy and differ from each other mainly in terms of side-effects, the change from one D2 blocking neuroleptic to another usually offers no additional gain in treatment outcome (Kane et al., 1988;
Kinon et al., 1993). In addition, chronic treatment with conventional dopaminergic drugs may result in persistent neural dysfunction (Lieberman et al., 1990) and poor clinical outcome (Chouinard, 1991; McEvoy, 1991; Lieberman et al., 1993).

The remarkable properties of CLO have been mostly attributed to its specific receptor affinity spectrum, different from that of conventional neuroleptics. CLO shows higher affinity to D1 (Farde and Nordström, 1992; Farde et al., 1992) and D4 (Van Tol et al., 1991) receptors than to D2 receptors. It antagonizes also 5HT3 serotonin receptors, α1 and α2 adrenoreceptors, H1 histamine receptors, and muscarinic acetylcholine receptors (Beerpoort et al., 1996; Wirshing et al., 1997) (with the exception of M4 receptors, which are stimulated by CLO) (Zorn et al., 1994), and shows a partial agonism in 5HT1A receptors (Meltzer and Roth, 1998). However, the higher affinity of CLO to serotonin 5HT2a and 5HT2c than to D2 receptors (Meltzer, 1989a; Roth et al., 1992; Meltzer, 1994; Nordström et al., 1995; Kapur and Remington, 1996; Schotte et al., 1996; Meltzer and Roth, 1998) as an explanation of its unique efficacy has accumulated the most convincing empirical support. Indeed, the novel atypical neuroleptics risperidone, olanzapine, and sertrindole, designed on the basis of this CLO-like serotonin-dopamine antagonism (SDA) concept, have each shown in double-blind clinical trials an efficacy superior to conventional neuroleptics in reducing negative symptoms (Marder and Meibach, 1994; Beasley et al., 1996; Zimbroff et al., 1997). These drugs may also prove to be more effective in producing remission of psychosis and have been promoted as first-line antipsychotics. However, their role in treatment-resistant schizophrenia has yet to be established. Today CLO remains the only neuroleptic with established efficacy in rigorously defined treatment-resistant schizophrenia (Christison et al., 1991; Barnes and McEvedy, 1996; Fleischhacker, 1999). Despite the markedly well-developed knowledge of the receptor-level effects of CLO, the exact mechanism of its novel action is still far from clear (Barnes and Kane, 1996). Neither is it known whether the desirable clinical effects of CLO and its blood dyscrasia-provoking property are interrelated.

1.6. Serotonin-dopamine antagonism concept and antidepressants in schizophrenia with negative and depressive components

Both the antidepressants (Siris et al., 1987; Silver and Nassar, 1991; Delle Chiave et al., 1994; Goff et al., 1990; Goff et al., 1995; Salokangas et al., 1996) and the inhibitors of postsynaptic 5HT2 serotonin receptors (Strauss and Kleser, 1991; Silver et al, 1991; Duinkerke et al., 1993; Lee et al., 1995; Meltzer et al., 1996) seem to be useful adjuncts to conventional neuroleptics in some schizophrenic patients with negative and depressive symptoms. In theory, these two strategies could be used
simultaneously in a complementary fashion by means of combining conventional neuroleptics with novel antidepressants (e.g., mirtazapine or nefazodone), which are able to inhibit the post-synaptic 5HT2 receptors. In addition to enhanced efficacy, such a combination could also diminish side-effects, since, like neuroleptics, antidepressants may cause EPS (Leonard and Faherty, 1996) and sexual dysfunction (DeVane, 1995), whereas the 5HT2 blockade can counter both conditions. Moreover, the sufficient antipsychotic level of the D2 blockade by the SDA neuroleptics is lower than that by conventional neuroleptics (Farde et al., 1994; Goyer et al., 1996).

Thus, the 5HT2 blockade by the add-on nefazodone or mirtazapine might allow a reduction in neuroleptic doses with enhanced efficacy, a further lessening of side-effects, better compliance, and improved quality of life. Two recent preclinical experiments with mirtazapine, performed in rodents on the basis of this assumption, support this hypothesis, since co-treatment with mirtazapine enhanced the antipsychotic-like effect and reduced extrapyramidal side-effects of conventional neuroleptics (Berendsen et al., 1998; Pinder et al., 1998).

To our knowledge, clinical trials with such combinations are lacking. Nevertheless, combinations with theoretically similar pre- and postsynaptic effects, i.e., CLO and selective serotonin reuptake inhibitors, may be useful in some patients. For example, the improved outcome for schizophrenic patients in the study of Szegedi et al. (1995) was attributed to the pharmacodynamic benefits of co-administration of CLO and fluvoxamine. Pharmacokinetic interactions between study drugs might, however, contaminate these results (Koponen et al., 1995).

1.7. Cellular immune mechanisms and free radicals as a plausible background for schizophrenia and its neurotoxicity

1.7.1. MONONUCLEAR PHAGOCYTES AND SCHIZOPHRENIA

Immune mechanisms in schizophrenia have been extensively investigated throughout the decades. An increasing number of studies indicate that immune mechanisms, e.g., an autoimmune process (Heath et al., 1967; Kirch, 1993; Ganguli et al., 1993) or a viral infection (DeLisi and Crow, 1986; Kirch, 1993) underlie the pathophysiology of schizophrenia, at least as a contributing factor. One of the most consistent findings in this field has been a defect in interleukin (IL)-2. Increased (O’Donnell et al., 1996) or decreased (Ganguli et al., 1995) levels of IL-2, increased soluble IL-2 receptors (sIL-2R) in peripheral blood (Rapaport et al., 1989; Ganguli and Rabin, 1989), and increased IL-2 in CSF (Licinio et al., 1993; McAllister et al., 1995) have been reported in schizophrenia. IL-2, a natural stimulant of mononuclear phagocytes (e.g., microglia in the brain and their precursor, peripheral blood MO) is
produced by T-lymphocytes. Microglia and its activated form, brain macrophages, are also capable of production of cytokines, some of which (e.g., IL-1 and transforming growth factor-beta) can (respectively) stimulate or suppress T-lymphocytes. Thus, macrophages and T-lymphocytes are functionally entangled (Roitt et al., 1993). IL-2 from leukocytes has been shown to induce positive schizophreniform symptoms, such as hallucinations, delusions, paranoia, agitation, and irritability in healthy subjects (Denicoff et al., 1987). In addition, negative symptoms may relate to an immune defect in schizophrenia, since low IL-2 production is associated with an earlier age of onset and more severe negative symptoms (Ganguli et al., 1995). Furthermore, macrophage-produced cytokines (e.g., alpha-interferon) may have behavioral effects resembling prodromal and/or negative symptoms of schizophrenia, i.e., fatigue, depression, signs of frontal lobe pathology, slowing of behavior, and motor perseveration (Adams et al., 1984; Renault et al., 1987; Niiranen et al., 1988); this evidence, however, is inconsistent (Katila et al., 1993). These observations have generated the macrophage-T-lymphocyte theory of schizophrenia (Smith, 1992). According to this theory, an activation of macrophages (via consequent hyperactivation of T-lymphocytes, who thereafter wrench themselves free of the control of macrophages) initiates the schizophrenic process. An acute phase response, as well, has been shown in schizophrenia (Smidt et al., 1988; Wong et al., 1996). Based on the model for acute phase response in liver (Heinrich et al., 1990), this observation also in schizophrenia would prescribe a role for monocyte (MO)/macrophage-produced cytokines, such as IL-1, IL-6, tumor necrosis factor, and perhaps nerve growth factor, and hence the involvement of MO/macrophages or their products also in the pathogenesis of schizophrenia. Indeed, enhanced levels of IL-6 and sIL-6R receptors have been measured in the plasma of young schizophrenic patients (Maes et al., 1994), giving support to this monocyte/macrophage theory. Moreover, elevated proportions of macrophages in the CSF (Nikkilä, 1997) and an increase in microglia in the frontal and temporal cortex of schizophrenic patients (Radevitz et al., 1998) indicate an involvement of microglia/macrobes in the schizophrenic process.

1.7.2 FREE RADICALS AND SCHIZOPHRENIA

1.7.2.1 Free radicals and the brain

Free radicals are species that contain one or more unpaired electrons and are capable of independent existence (Halliwell and Gutteridge, 1989). Such species – in the human mainly reactive oxygen metabolites (ROM) (see APPENDIX 1) – are unstable and highly reactive, and they achieve stability by the annexing of electrons from, meaning oxidation of surrounding molecules. These molecules in turn become free
radicals and may thus initiate a chain ("redox") reaction (Maxwell, 1995). ROM function as intra- and extracellular signaling molecules and can directly affect the cellular signaling apparatus and control of gene expression (Palmer and Paulson, 1997). ROM in turn are controlled by the antioxidant defense systems. Although ROM are generated under physiological conditions, their excessive amount, due to either ROM hyperproduction or to the insufficiency of antioxidant defense systems, may lead to oxidative stress (Mahadik and Scheffer, 1996). Oxidative stress due to such an excess can initiate apoptosis in some cell types (Sugaya et al., 1997). Polyunsaturated fatty acids (PUFA) are especially sensitive to oxidative stress, and their long chain reactions can rapidly lead to cell membrane dysfunctions: in addition to their role in the transport of ions and nutrients, PUFAs serve as second messengers in neuronal transduction (Mahadik and Scheffer, 1996). Proteins, as well, when attacked by ROM, may lose their normal structure with consequent functional disturbances in, for example, ion channels or receptors. ROM-induced damage in PUFAs, proteins, and deoxyribonucleic acid (DNA) can cause cell dysfunctions and even death. ROM have a pathogenic impact in a variety of human diseases. The nervous system is particularly vulnerable to the ROM attack due to its special biochemistry, anatomy, and physiology; it has:

1) a high rate of oxidative metabolism
2) high concentrations of membrane lipid PUFAs, which are highly oxidizable
3) low levels of antioxidant enzymes catalase (CAT) and glutathione peroxidase (GSH-Px)
4) high endogenic ROM generation in, for example, monoamine oxidase-catalyzed oxidation of catecholamines, metabolism of prostaglandines, or active production by macrophage-type microglia cells
5) highly specialized neuronal signal transduction dependent on faultless membrane function
6) a high surface area/cytoplasmic volume ratio
7) long axons endangered by peripheral injury
8) a disruption-sensitive neuronal network
9) a lack of cell turn-over (Evans, 1993)
10) a high content of iron and the inability of CSF to bind released iron ions (Haliwell, 1992)

Evidence is mounting that ROM are involved in the central nervous system (CNS) membrane pathology, e.g., in Parkinson’s and Alzheimer’s diseases, Down’s syndrome, multiple sclerosis, trauma, and ischemia (Evans 1993).
1.7.2.2. Free radicals in schizophrenia

The theory of a pathological role for free radicals in schizophrenia, proposed originally in the mid 1950s by Hoffer et al. (1954), has recently been supported by a rapidly growing body of findings (Reddy and Yao, 1996):

1. There is evidence (although not unequivocal, Katila et al., 1997) for peroxidative damage of membranes, e.g., increased levels of malondialdehyde, pentane, and phospholipase A2 in medicated (Prilipko, 1984; Phillips et al., 1993; Gattaz et al., 1987; McCreadie et al., 1995) and drug-naive (Scheffer et al., 1995) patients, especially those with tardive dyskinesia (Lohr et al., 1990). Interestingly, negative symptoms are associated with high levels of saturated and low levels of long-chain unsaturated fatty acids, whereas the picture with regard to positive symptoms is the opposite (Glen et al., 1994). Altered antioxidant defence has been demonstrated: decreased or increased superoxide dismutase (SOD) and GSH-Px activity in neuroleptic-treated and drug-free patients, as well as decreased CAT activity (Abdalla et al., 1986; Reddy et al., 1991; Mukherjee et al., 1994; Zhang et al., 1998) and decreased E-vitamin/cholesterol rate in schizophrenia (McCreadie et al., 1995) and tardive dyskinesia (Cadet and Kahler, 1994).

2. Promising results of clinical efficacy trials with supplementation of:
   a) E-vitamin (a dietary lipid-soluble chain-breaking antioxidant) in schizophrenia (Sram and Blinkova, 1992) and (especially when used early, according to Reddy and Yao, 1996) in tardive dyskinesia (Adler et al., 1993; Peet et al., 1998) or
   b) essential fatty acids (EFAs) (eicosanoids) in schizophrenia (Vaddadi, 1992; Puri and Richardson, 1998) and tardive dyskinesia (Vaddadi et al., 1989) have been reported.

3. Prognosis of schizophrenia in developing countries is better despite the lack of maintenance treatment, possibly due to a low consumption of animal fats (which is associated with increased oxidative tone) (Christensen and Christensen, 1988).

1.7.2.3. Free radicals and conventional neuroleptics

Some conventional antipsychotics are able to exert pro- (e.g., haloperidol) or antioxidant (e.g., chlorpromazine and prochlorperazine) activity in vitro and possibly in vivo (Jeding et al., 1995). In a recent study by Dalla Libera et al. (1998), the conventional neuroleptics chlorpromazine and trifluoperazine acted as good antioxidants, as did serotonin and CLO. Interestingly, CLO was the most potent of all four substances in a hydrophobic environment of the type present in biological membranes. Most data, however, suggest that the conventional neuroleptics themselves are a direct source of free radicals (Chignell et al., 1985) and correspondingly are a cause of enhanced lipid peroxidation (Pall et al., 1987).
1.7.3. MONOCYTES AND MICROGLIA/MACROPHAGES AS A POSSIBLE LINK BETWEEN IMMUNE AND FREE RADICAL MECHANISMS IN SCHIZOPHRENIA

One of the major sources of ROM in human are phagocytes: neutrophils (polymorphonuclear leukocytes, PMNL) and MO in the peripheral blood, and macrophages in organs. For instance, in the brain ROM are modified (along with the processes of incomplete reduction of oxygen in mitochondria, monoamine oxidase catalyzed oxidation of dopamine and noradrenaline, and metabolism of prostaglandins) (Evans, 1993) by microglia or their activated state, called brain macrophages. The ability to produce ROM is an important function of phagocytes, necessary for destroying microbes, parasites, and altered cells (Babior, 1978; Karnovsky and Badwey, 1983). The ability of microglia/brain macrophages to produce ROM might provide a link between the free radical and immune theories of schizophrenia: the phagocyte-produced ROM may be neurotoxic and seem to participate in diverse brain pathology, accompanied by behavior disturbances (Fisher, 1988; Thery et al., 1993; Banati et al., 1993; Oken, 1995; Benveniste, 1997). It has been hypothesized that brain damage by activated microglia-produced ROM is a common pathophysiological mechanism for various brain diseases characterized by behavioral disturbances, including schizophrenia (Oken, 1995). While microglia from a living person are difficult to obtain for experiments, the peripheral blood phagocytes are easily available, MO are of particular interest in this respect. First, they constitute a precursor of tissue macrophages, including microglia, and share with the latter numerous functions, e.g., ROM production (Lydyard and Grossi, 1993; Langemans et al., 1994). Second, they are capable of penetrating the blood-brain barrier (BBB) under normal conditions (Lassmann et al., 1993), and especially so when the BBB is altered, as it is in about one-third of patients with schizophrenia (Müller and Ackenheil, 1995). “Altered” MO have been proposed even as an etiologic factor in schizophrenia (Smith, 1991).

Despite these considerations, the ROM production by phagocytes in schizophrenia has been insufficiently explored. Two depression studies from Ireland, comprising small control groups of schizophrenic patients, are the only available reports on this issue (O’Neill and Leonard, 1990; McAdmas and Leonard, 1992). In the first study, the ROM production by PMNL from six drug-free schizophrenic patients was measured cross-sectionally by luminol-dependent chemiluminescence with no differences evident between patients and controls. The ROM production by MO was not assessed. In the second study, the ROM production by MO and PMNL of 10 drug-free schizophrenic patients (3 females and 7 males) was assessed longitudinally before and after successful treatment with conventional neuroleptics. The ROM production by both cell populations increased during follow-up. However,
during the time that the initially low values for PMNL normalized, those for MO, normal before the neuroleptic treatment, increased significantly to levels twice as high as in the controls. The investigators, however, found no changes in the ROM production by blood phagocytes from their patients after incubation of the cells in vitro with the patients’ current neuroleptics. In both studies the cells were challenged by opsonized zymosan. No data for non-stimulated cells were reported.

1.7.4. NEUROLEPTICS, FREE RADICALS, AND CELL IMMUNITY

1.7.4.1 Conventional neuroleptics

The antipsychotic activity of neuroleptics, although associated with receptor-level phenomena, can not be attributed exclusively to them. For instance, the delay between receptor occupancy and clinical response calls for clarification. One possible explanation may be an immunosuppressive effect of neuroleptics that may differ between conventional compounds and CLO (Leykin et al., 1997).

Some authors have reported suppressive (Ruutu, 1972a; Ruutu, 1972b; Salovera et al., 1987; Boukhris, 1988; Bessler et al., 1995) or stimulatory (Ferguson et al., 1978; Goldstein et al., 1980) effects of conventional neuroleptics on cell immunity, while others have failed to demonstrate any significant effects (Pollmacher et al., 1997). Conventional neuroleptics have been shown to inhibit in vitro various types of phagocytes from the human and other species (Ruutu, 1972a; Ruutu 1972b; Horwitz et al., 1981; Pfister et al., 1984; Baciu et al., 1988; Brewton and MacCabe, 1988; Watanabe et al., 1988; Krumholz et al., 1995). Little is, however, known about their effects on the ROM production by phagocytes, although the sparse data published give some evidence for an inhibitory effect of phenothiazines on the ROM production by animal alveolar and peritoneal macrophages in vitro (Chang et al., 1983; Traykov et al., 1997).

Few patient data exist on effects of neuroleptics on macrophages in schizophrenia. Nikkilä (1997) found that elevated proportions of macrophages in CSF from schizophrenic patients tend to normalize following neuroleptic treatment. The direct in vivo effect of neuroleptics on the ROM production by phagocytes in schizophrenia remains to be explored, although treatment with conventional antipsychotics seems to increase ROM production (O’Neill and Leonard, 1992).

1.7.4.2. Clozapine, cell immunity, and free radicals

Side-effects of CLO such as hyperthermia, transient leukocytosis, eosinophilia, and agranulocytosis, along with its unique efficacy, have raised particular interest in its possible immunological effects. In the study of Pollmacher et al. (1996), CLO was
found to enhance tumor necrosis factor-alpha (TNF-alpha), soluble TNF receptors p55 and p75, and sIL-2R in schizophrenic patients, while haloperidol showed no such effects (Pollmacher et al., 1997). Another group reported CLO in vivo to elevate sIL-2R (which may facilitate immunosuppressive effects) while not affecting IL-6 levels (Maes et al., 1994). The same authors found an increase in plasma sCD8 antigen, IL-1R antagonist, IL-6, and clara cell protein at different phases of CLO treatment (Maes et al., 1997). Sperner-Unterweger et al. (1993) demonstrated a suppression of granulocyte-macrophage colony-stimulating factor by CLO in vitro and interpreted this finding as an indicator of a mediator role for cytokines in CLO-induced agranulocytosis. Pollmacher et al. (1997) observed a transient increase in granulocyte colony-stimulating factor at the second week of a CLO trial in 55% of their patients, accompanied by increases in MO and PMNL counts, in rectal temperature, and in plasma levels of cytokines. CLO showed a significant immunosuppressive effect, equal to that of haloperidol - an effect assessed by the production in vitro of IL-2, IL-4, and interferon-gamma by healthy subjects’ lymphocytes stimulated with phytohemagglutinin (Leykin et al. 1997). Thus, although no exact knowledge yet exists as to immune effects of CLO, the conclusion is warranted that in schizophrenia CLO may have complex immunomodulatory effects.

The direct effects of CLO on human phagocytes have been studied mainly from the point of view of agranulocytosis and therefore apply mostly to PMNL (Pisciotta et al., 1992; Liu and Uetrecht, 1995), without simultaneous analysis of clinical modalities. In these studies it has been found (and consistently replicated) that CLO is oxidized by the myeloperoxidase and the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system of PMNL to a free radical (Fisher et al., 1991; Mason and Fisher, 1992; Liu and Uetrecht, 1995; Uetrecht, 1995), presumably a relatively stable nitrenium ion. These findings, supported by preliminary data on changes in the antioxidant defense (low plasma and red blood cells’ GSH-Px and selenium as factors predisposing to agranulocytosis) in CLO-induced agranulocytosis survivors (Linday et al., 1995) suppose an involvement of free radical mechanisms in this complication. The authors did not study or discuss the metabolism of CLO to a free radical in regard to the clinical effects of CLO. It is known, however, that reactive metabolites of such substances as arylamines formed, via the same mechanism by PMNL, can inhibit phagocyte function and mediate some of the therapeutic effects of these drugs (Uetrecht, 1995). Furthermore, the chain-breaking, or “sacrificial” antioxidants, in contact with toxic free radicals, become themselves relatively inactive free radicals; through this, the toxic ones are detoxified (Maxwell, 1995). The exact role of the CLO-derived free radicals in the interplay with the phagocyte-produced ROM remains to be investigated.
1.7.5. CLOZAPINE AND THE PRODUCTION OF REACTIVE OXYGEN METABOLITES BY PHAGOCYTES

If a link between the phagocyte and free radicals mechanisms does exist and underlie the pathophysiology of schizophrenia and/or tardive dyskinesia, this could be revealed by longitudinal intraindividual studies with parallel monitoring of changes in clinical picture and in the phagocyte-produced ROM status during the course of a neuroleptic-treated schizophrenic psychosis. To the best of our knowledge, virtually no such studies have been published thus far. CLO seems to be a convenient drug for a study of this kind. It is effective in treatment-resistant schizophrenia and against negative symptoms, while not causing tardive dyskinesia - all of these being conditions in which free radicals may play a role (Cadet and Kahler, 1994; Horrobin et al., 1994). Some authors have observed a complex free-radical interplay between CLO and PMNL in their agranulocytosis-focused studies (Fisher, 1991; Lindsay, 1995; Liu and Utrecht, 1995).
2. AIMS OF THE STUDY

The aims of the present research, study by study, were:

I. To investigate retrospectively the effects of long-term CLO treatment in out-patients with treatment-resistant schizophrenia

II. To examine prospectively the clinical effects of CLO in schizophrenic patients with early signs of developing treatment resistance

III. To study prospectively whether a combination of conventional neuroleptics with nefazodone leads to CLO-like beneficial effects in difficult-to-treat schizophrenic patients with prominent negative or depressive symptoms or both

IV and V. To explore the in vivo (IV) and in vitro (V) effects of CLO on the ROM production by blood phagocytes in treatment-resistant schizophrenia
3. PATIENTS AND METHODS

3.1. Patients

The series of studies included 71 patients with difficult-to-treat schizophrenia, whose general characteristics are presented in Table 1.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Patients</th>
<th>Age, years</th>
<th>Sex, males/females</th>
<th>Duration of illness prior to study medication, years</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>44</td>
<td>42/42 (24 – 62)</td>
<td>27/17</td>
<td>11.8/11.8 (1 – 33)</td>
</tr>
<tr>
<td>II</td>
<td>11</td>
<td>25/25 (18 – 44)</td>
<td>7/4</td>
<td>2.3/2.6 (0.9 – 3.8)</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>36/36 (18 – 51)</td>
<td>4/4</td>
<td>7.9/8 (1 – 16)</td>
</tr>
<tr>
<td>IV and V</td>
<td>8</td>
<td>31/32 (24 – 41)</td>
<td>1/7</td>
<td>9/7.5 (2 – 22)</td>
</tr>
<tr>
<td>Entire population</td>
<td>71</td>
<td>38/38 (18 – 62)</td>
<td>39/32</td>
<td>10.9/9 (0.9 – 45)</td>
</tr>
</tbody>
</table>

Study I

Patients with DSM-III-R (APA, 1987) schizophrenia (n = 43) or schizo-affective disorder (n = 1) fulfilled the inclusion criteria (duration of illness at least 2 years and duration of CLO treatment at least 1 year) and were enrolled into the study. Five of these 44 were in-patients with disorganized schizophrenia (further referred to as hebephrenic, according to the Finnish version of DSM-III-R). They had a history of continuous or almost continuous long-term hospital treatment before and after the initiation of CLO, and their CLO medication was continued due to a slight improvement despite a lack of optimal treatment response. The other 39 were outpatients.

The main focus of the study was on these 39 out-patients: 24 men and 15 women, mean/median age 41.3/42 (range 24 – 61) years, of whom 27 had hebephrenic, 10 paranoid, one undifferentiated schizophrenia, and one schizo-affective disorder. These patients were treated with CLO for 8.0/7.8 (3 – 14.8) years, CLO daily doses at the endpoint were 436/400 (200 – 700) mg. In these 39 patients duration of illness prior to CLO was 13.1/11.4 (3 – 33) years and duration of hospitalization 4.1/3.2 (0.08 – 11) years before and 2.5/2.2 (0.04 – 9.2) years after introduction of CLO.
Study II
This study comprised 11 schizophrenic (DSM-III-R) (APA, 1987) patients suffering from overt psychotic illness with a total score of at least 18 on the 18-item Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) (each item rated from 0 to 6), despite routine treatment with one or two conventional neuroleptics (haloperidol, sulphiride, trifluoperazine, or chlorpromazine) for 2 - 12 months. These 11 patients were shifted to a CLO trial due to their lack of response to adequate conventional neuroleptic medication (7 patients had, in addition, disturbing side-effects). The current episode of illness had to be the first or second one, with a duration of less than one year. Established therapy-resistant patients were excluded, specifically those who had failed to show any significant clinical response to two neuroleptics of two different classes at a daily dose of 800 mg chlorpromazine equivalents (Kaplan and Sadock, 1993) or more for at least three weeks each.

One patient soon withdrew his informed consent. The remaining 10 patients (six men and four women, four in- and six out-patients) had a mean/median age of 25/24 (18 – 44) years. Their duration of illness was 2.2/2.3 (0.9 – 3.8) years, duration of the current episode was 0.61/0.7 (0.25 – 0.9) years, and duration of the last conventional neuroleptic trial was 0.42/0.4 (0.25 – 0.75) years. Seven patients had paranoid, one catatonic, and two undifferentiated schizophrenia. Four patients had previously received one and six patients consecutively two conventional neuroleptics. At the screening phase all patients were on either haloperidol (15 to 35 mg per day) or trifluoperazine (12.5 to 25 mg per day).

Study III
Eight schizophrenic (DSM-IV) (APA, 1994) patients (four males and four females, two in- and six out-patients) were enrolled in the study. They exhibited a remission into either a non-psychotic or a residually psychotic state after their last episode of schizophrenia and, despite an adequate conventional neuroleptic medication, suffered from long-standing disabling negative and/or depressive symptoms. Treatment with any antidepressant within the last weeks prior to the trial was forbidden. Five patients had residual, two patients undifferentiated, and one patient paranoid schizophrenia. These patients had previously suffered 3.5/2 (0 – 13) episodes of the disease, and at the screening phase were on conventional neuroleptics at doses of 415/175 (30 – 1700) mg chlorpromazine equivalents.

Studies IV and V
Eight in-patients (one male and seven females) with chronic schizophrenia (DSM-III-R) (APA, 1987), who suffered from an active psychosis despite standard conventional
medication, were enrolled in a CLO trial. Seven patients had undifferentiated, and one patient disorganized schizophrenia. The patients had previously received 5.5/5 (2 - 9) conventional neuroleptics for 8.8/7.5 (1 - 22) years. Each patient had received at least one neuroleptic at doses of 500 mg chlorpromazine equivalents (six patients over 700 mg and one patient over 600) or more for at least two months. Allergic states, acute infection, hyperthermia, or steroid and/or antimicrobial medication formed the exclusion criteria.

3.2. Methods

3.2.1. CLINICAL ASSESSMENTS

Psychopathology was assessed by the 12-item version of the Comprehensive Psychiatric Rating Scale (CPRS) modified for patients with schizophrenia (Montgomery et al., 1978) (Study I), the 18-item Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) (II), and the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) (II – V). In addition, the deficit syndrome (impaired intrapsychic, interpersonal, and instrumental role functioning) was assessed with the Quality of Life Scale (QLS) (Heinrichs et al., 1984) (II, III).

The Montgomery-Åsberg Depression Rating Scale (MADRS) (Montgomery and Åsberg, 1979) was used for a more thorough evaluation of the depressive symptoms (II – V).

A retrospective analysis of improvement in terms of clinical symptoms (clinical improvement) and social functioning (social improvement) during clozapine treatment was performed with a 6-point global assessment rating scale, derived from the item for global improvement of the CGI (Guy, 1976) with two additional intermediate stages. The eventual scale was as follows: 0. no change; 1. minimally improved; 2. much/minimally improved; 3. much improved; 4. very much/much improved; 5. very much improved (I). The retrospective assessment was based on available data on the need for psychiatric hospitalization and other types of health care, and on occupational, educational, and marital status, as well as social and intimate relations.

The Simpson-Angus Scale (the Neurological Rating Scale for Extrapyramidal Side Effects)\(^1\) (Simpson and Angus, 1970) was used for assessment of extrapyramidal side-effects of medication (III). Furthermore, the overall clinical severity of illness was rated by the Clinical Global Impression (CGI) severity item (Guy, 1976) (III). Five items for sexual functioning (Increased Sexual Desire, Diminished Sexual Desire, Erectile Dysfunction, Orgastic Dysfunction, and for the male participants also

\(^1\) Item 10 (salivation), although rated, was not included in the statistical calculations and is reported separately for the only patient with a disturbance related to this item (see below).
Ejaculatory Dysfunction) derived from the Committee on Clinical Investigations (Udvalg for kliniske undersøgelser, UKU) Side Effect Rating Scale (Lingjaerde et al., 1987) served for assessment of sexual dysfunctions (III).

The patients’s subjective impression of the medication was evaluated by the Patient Global Impression (PGI) (Guy, 1976) (II)

The scores for the last observations were carried forward for statistical analysis (III).

3.2.2. EXPERIMENTAL ASSESSMENTS (STUDIES IV AND V)
3.2.2.1. Blood sampling
Overnight fasting blood samples were taken at every visit at 07:45 - 08:00 h. From smokers the samples were drawn immediately after their first cigarette of the day.

3.2.2.2. Quantitation of serum clozapine
Serum clozapine concentrations were determined with a high performance liquid chromatographic assay (Lovdahl et al., 1991). The intra- and interassay variation (1000 nM/L) was 3.7 and 6.2, respectively.

3.2.2.3. Cell counts
Determination of the total leukocyte count from blood anticoagulated with EDTA was performed with a Counter Coulter (Counter Electronics Ltd, UK) within one hour after blood sampling. At the same time blood smears were made, dried in room air, and later stained with May-Grünwald-Giemsa (MGG) for differential counts.

3.2.2.4. Isolation of polymorphonuclear leukocytes (PMNL) and mononuclear leukocytes
Ten ml of heparinized venous blood was layered on 7 ml Ficoll-Paque (Pharmacia, Sweden) in one hour after blood sampling and centrifuged at 450 g for 20 min at 20℃. The band in the interface between the plasma and the Ficoll layer, containing mononuclear cells (i.e., lymphocytes and MO), was aspirated and washed once in phosphate buffered saline (PBS). The leukocyte count was determined with the Counter Coulter, and the cell suspension was diluted to 5 x 10^6 mononuclear cells/ml. Cytocentrifuge preparations of the cell suspension were prepared immediately, dried in room air, and stained with MGG for determination of the MO proportion.

The pellet from the density centrifugation, containing PMNL and erythrocytes, was collected and hemolyzed during continuous mixing in a hypotonic solution for 10 min at +4℃. The cells were washed at 450 g for 5 min in PBS, the hemolysis was repeated once, and the cells were washed twice at 450 g for 5 min in PBS. The cell
count was determined with the Counter Coulter, and the suspension was diluted to 5 x $10^6$ PMNL/ml.

3.2.2.5. Chemiluminescence assay

Fifty µl of the mononuclear cell or PMNL suspension was added to a solution containing 5.6 mM luminol (Bio-Orbit, Finland). Half of the samples were stimulated with 0.1 µg/ml phorbol myristate acetate (PMA) (Sigma, USA). The light emission caused by the production of ROM was recorded for 30 min at 2-min intervals with a Bio-Orbit 1251 luminometer (Bio-Orbit, Finland) operated by the Bio-Orbit Phagocytosis Program 1251-124. The results are expressed as the areas under the light emission curves per 100,000 cells. The MO results were corrected for the actual MO count on the basis of the differential count obtained from the cytocentrifuge preparations and are further referred to as the ROM production by MO.

Prior to the chemiluminescence assay, the patients’ isolated (i.e., free from serum clozapine) MO and PMNL, both non-stimulated (MOs and PMNLn) and stimulated with PMA (MOs and PMNLs) were incubated for 1 hour in 37°C in suspensions

- with PBS only (IV, V)
- with CLO (5 mg/L) dissolved in water with 1 equivalent HCl for one hour at 37°C (V)
- with the solvent (HCl) only (V)

3.3. Design, arrangements, and regulations

Study 1

The study was performed as a chart review with a supplementary cross-sectional clinical evaluation with CPRS. The observation period comprised the time from the onset of the disease (first admission for psychosis) until discontinuation of CLO medication, death of the patient, or the end-point of the study (end of 1994). The following information was obtained from hospital records and clinical interviews: sex, age, diagnosis, duration of the disease before CLO administration, duration of hospitalization per year before and during CLO treatment. In addition, data were registered on the best level of working capacity during any period of life and at the end-point of the study (the end of 1994), number of conventional antipsychotics used prior to introduction of CLO, CLO doses at the end-point of the study, and the need for concomitant psychotropic medication at the end-point of the study. Global assessment of improvement in terms of clinical symptoms (clinical improvement) and
social functioning (social improvement) during CLO treatment, need for psychiatric hospitalization and other types of health care, occupational, educational, and marital status, as well as social and intimate relations were recorded as well.

A mirror design was used when comparing the duration of hospitalization per year before and during CLO treatment. This meant that equal numbers of complete years before and during CLO treatment, adjusted according to the shorter of these periods, were taken into consideration. For example, if the patient had been ill for 10 years before clozapine treatment and had received clozapine for more than 3 years, all the months of hospital treatment year by year within the last 3 complete years before, and correspondingly within the first 3 complete years during clozapine treatment, were summarized and compared to each other.

Study II
This study was an open prospective 26-week clinical CLO trial. The following information was obtained from hospital records and clinical interviews: sex, age, age at onset of the first psychotic symptoms, duration of illness before CLO, and number of conventional antipsychotics prior to CLO. At the screening visit, pertinent physical and psychiatric examinations were performed. A 1- to 9-day wash-out from conventional orally used neuroleptic was conducted before the base-line (week 0) evaluation (none of the patients had received depot-neuroleptics).

The recommended CLO daily dose regimen was as follows: 12.5 - 25 mg once or twice on the first day with stepwise increases of 25 to 50 mg during 7 to 14 days until 300 to 450 mg (up to 600 mg) was reached. Individual adjustment of the doses was allowed, and the use of the lowest effective dose during the maintenance period was recommended. Concomitant use of medication with primarily central nervous system activity besides short/middle-acting benzodiazepines was forbidden.

Assessments of treatment efficacy were performed weekly for the first 8 weeks and thereafter bimonthly until the end-point at week 26 (for the CGI also prior to wash-out, and for QLS at base-line and end-point). The PANSS and BPRS scores were adjusted by distracting 1 point from each item (ratings from 0 to 6). The BPRS scores and their changes were additionally counted before the adjustment for the purpose of comparability with some other studies.

Study III
This study was an open prospective add-on nefazodone trial with no wash-out period. Nefazodone was used orally at an initial daily dose of 100 mg with subsequent upward titration through 200 mg on day 3 to 300 mg on day 7 of medication. A further weekly increase of the daily dose by 100 mg up to the maximum of 600 mg was applied if possible. No concomitant use of psychotropic drugs was allowed besides the pre-
existing neuroleptic, or lithium, or long-term benzodiazepine medication. Any changes in medication were prohibited during at least the last 4 weeks prior to and the first 6 weeks of the trial. Thereafter, a reduction in the doses of concomitant drugs was allowed if clinically justified.

Studies IV and V

CLINICAL CONSIDERATIONS
The studies were conducted as two (in vivo and in vitro) aspects of an open naturalistic prospective 10-week CLO trial with no prescribed study drug dose regimen. A washout period of 36 to 48 hours preceded the study medication. Use of concomitant neuroleptics was prohibited. Clinical and experimental assessments were performed simultaneously at weeks 0, 3, and 10 of the trial.

EXPERIMENTAL CONSIDERATIONS
STUDY IV was designed to examine longitudinal in vivo changes in the ROM production during CLO treatment

IV.A. The ROM production by MON, MOs, PMNLn, and PMNLs at weeks 3 and 10 was compared to that at base-line.

IV.B. Correlations were calculated between the changes in the ROM production by the cells during the trial (the values at base-line minus those at week 3 or week 10) (Δ ROM) and serum concentrations of CLO at weeks 3 and 10.

IV.C. Correlations were calculated between Δ ROM at weeks 3 and 10 and clinical changes (the PANSS or the MADRS scores at base-line minus those at week 3 or week 10) (Δ PANSS and Δ MADRS).

STUDY V was a separate experiment designed to examine the in vitro effects of CLO on the ROM production, including the possible predictive value of these effects at baseline on the clinical outcome of CLO medication.

V.A. The ROM production by the CLO-incubated MON, MOs, PMNLn, and PMNLs was compared to that of their solvent-incubated counterparts at weeks 0, 3, and 10 of the trial. PBS-only incubated cells served as a second control.

V.B. Correlations were calculated between the in vitro CLO-induced changes in ROM production at baseline (values for the solvent-incubated minus those for the CLO-incubated cells at week 0) and Δ PANSS and Δ MADRS at weeks 3 and 10.

3.4. Safety
A thorough clinical examination preceding the start of the study drugs was performed at baseline in all clinical trials. The mandatory hematological follow-up was
conducted in the trials with CLO. In Study III also out-patients were hospitalized for at least the 2 initial weeks of nefazodone medication for safety reasons. Rating scales for assessment of safety have been described (see 3.2.1.)

3.5. Ethics
All the studies were performed in accordance with the principles of the Helsinki and Madrid Declarations and Good Clinical Practice. The patients had a level of understanding sufficient to communicate intelligently with the investigators and gave their written (Studies III - V) or oral (Studies I and II) consent. It was underlined that the patients could withdraw at any time from the trial with no negative consequences for their treatments. The study protocols of the clinical trials were approved by the ethics committees of the pertinent institutions.

3.6. Statistics
Descriptive statistical data are expressed as means/medians with the ranges in parenthesis. For non-completers the scores for the last observations were carried forward (LOCF) (III). Due to the small or disproportional sizes of the groups studied, non-parametric tests were chosen for statistical calculations - Kruskal-Wallis one-way ANOVA (I and II), Wilcoxon’s non-parametric test for matched pairs (III - V), and Spearman’s correlation coefficient ($r_s$) (I, II, IV,V). General Linear Modeling (Dillon and Goldstein, 1984) was used as the means for studying interactions between the cross-sectional in vitro effects of CLO and longitudinal clinical aspects and localization of significant differences (V).
4. RESULTS

The main results of the series of studies are presented in APPENDIX 2.

Study I

The duration of illness and duration of hospitalization (but not the duration of illness prior to CLO) before and after start of CLO were significantly longer in the in-patients (U = 32.0, p = 0.015*; U = 21.5, p = 0.005**, respectively) than in the out-patients. The in-patients were, as expected, more severely ill, as assessed by their total CPRS scores (U = 7.00, p = 0.001**).

While for the in-patient group clinical improvement during CLO was only minimal and no social improvement was observed, in all out-patients social improvement was at least minimal and the clinical improvement more than minimal.

OUT-PATIENTS

Of the 39 out-patients the clinical improvement in 35, and the social improvement in 26 were rated as much, very much/much, or very much. The age at the onset of the disease showed a negative correlation with the level of clinical (r = - 0.434, p = 0.006) and social (r = - 0.399, p = 0.012) improvement. The social improvement correlated positively with the duration of CLO treatment (r = 0.384, p = 0.016) and with the duration of hospitalization after the initiation of CLO (r = 0.372, p = 0.020).

The duration of hospitalization per year showed a peak within the period from 1 year before to 1 year after the start of CLO with a subsequent continuous and significant decline during the following 10 years. Although 33 patients had been capable of work during some period of their lives, at the time of the study only one patient was capable of work comparable to work at pre-illness level and seven patients were in sheltered work, while the remaining 31 patients were disabled.

OUT-PATIENTS WITH HEBEPHRENIC SCHIZOPHRENIA

Despite an earlier age at the onset of schizophrenia and a longer duration of illness prior to, and duration of hospitalization prior to and after the initiation of CLO, the patients with hebephrenic schizophrenia improved significantly more than their non-hebephrenic counterparts in both clinical (U = 226.0, p = 0.032*) and social (U = 233.0, p = 0.024*) terms.

Study II

During the wash-out period no patient improved or deteriorated. Due to disturbing sedation and hypersalivation and, on the other hand, rapid improvement of the
symptoms, the mean/median daily doses of CLO of 192.5/200 (range 100.0 - 350.0) mg at week 8 and 225.0/250 (50.0 - 450.0) mg at the end-point were lower and their ascent slower than initially recommended. All 10 patients exhibited hypersalivation, and nine patients additionally complained of sleepiness, sedation and/or fatigue, while minor extrapyramidal symptoms, observed in all patients at baseline, abated. During the trial none of the out-patients needed hospitalization, and five of the six in-patients could be discharged from the hospital. In all the patients, clinical improvement was seen as assessed by all rating scales, with the exception of slight deterioration on the negative PANSS score in two patients. The BPRS scores (before adjustment) showed improvement of 21/21% (13 – 36) at week 8 and 24/23% (19 – 31) at the end-point. Only one patient improved less (19%) than 20% - the degree of response proposed as clinically significant (Baldessarini and Frankenburg, 1991). In six patients (defined below as good responders) the decrease in total PANSS scores (30 - 50%) exceeded 30%. The remaining four patients (fair responders) showed 21 - 26% improvement. The clinical improvement was observed mostly within the first 8 weeks with only minimal changes thereafter. The QLS assessments also showed a marked improvement of 81.7/73% (6.3 – 181.0).

The duration of illness correlated negatively ($r = -0.639$, $p = 0.047^*$) with the reduction in the positive PANSS scores. Duration of illness also showed a tendency towards a negative correlation with improvement in total PANSS ($r = 0.555$, $p = 0.096$) and a positive correlation with improvement in total QLS ($r = 0.612$, $p = 0.060$) scores. Since improvement corresponds with decrease in PANSS and increase in QLS scores, a shorter duration of illness tended to correlate with better results on both scales.

The mean duration of illness in the fair responders was 3.25/3 (3.0 – 3.8) years, which was twice as long as the 1.5/1.3 (0.9 – 1.6) years in the good responders.

**LONG-TERM FOLLOW-UP**

All those six patients who continued the treatment with the Swiss-made CLO were asymptomatic at follow-up (6 – 15 months after the end-point), and five of them were capable of work. Substitution of the original medication by Ukrainian-produced CLO, tried in two patients, failed in both.

**Study III**

Nefazodone in daily doses of 537.5/600 (300 – 600) mg at week 6 and 575/600 (300 – 600) mg at endpoint was well tolerated by all five patients who completed the study.

\(^2\) The sixth in-patient had been sentenced to hospital treatment by a court and therefore regardless of his psychiatric condition could not be discharged.
protocol. Of the remaining three patients (drop-outs), only one had an adverse event likely to be related to nefazodone. These eight patients (LOCF) showed statistically significant (p < 0.05) clinical improvement as assessed by their total PANSS and all PANSS subscales, MADRS (mainly items 6, 7, and 8 - concentration difficulties, lassitude, and inability to feel), and CGI, mostly within the first 6 weeks with only modest changes thereafter. While significant favorable changes in the Quality of Life Scale scores continued throughout the study, the decrease in the SAS scores, significant at week 6, lost its significance later on due to the deterioration shown by patient 5.

Delusions, observed initially in three patients and panic attacks in two patients, vanished entirely in all cases. The sadness and pessimism expressed by two patients (in one of them also suicidality) abated rapidly. After the first 6 weeks (the phase of stable neuroleptic doses required by the study protocol) doses of neuroleptics could be significantly reduced. No patient needed benzodiazepines at the end-point. Sexual dysfunctions initially seen in five patients improved or remained unchanged, although this finding was not statistically significant.

Studies IV and V

CLINICAL RESULTS

All eight patients completed the study protocol without hematologic complications. CLO was prescribed at daily doses of 185/163 (15 – 250) mg and 400/400 (200 – 600) mg, and the serum concentration increased from 509/275 (150 – 1225) ng/ml to 886/900 (435 – 1200) ng/ml at weeks 3 and 10, respectively. The clinical improvement (mean 29% on the total PANSS scores) was observed mainly within the first 3 weeks. Five patients showed a more than 30% and one additional patient a more than 20% improvement on the total PANSS scores. The decrease in the MADRS and all the PANSS subscale (including total) scores at the end-point was statistically significant (data not shown).

EXPERIMENTAL RESULTS

IN VIVO

IV.A. ROM production by the phagocytes

Considerable inter- and intraindividual variations were observed, but no statistically significant longitudinal trends in the ROM production by MOn, MOs, PMNLn, or PMNLs.
IV.B. Serum concentrations of CLO and longitudinal changes in the ROM production

The serum concentrations of CLO at week 3 correlated positively ($r_s = 0.761, p = 0.047^*$) with changes (post- minus pre-treatment values) in the ROM production ($\Delta$ROM) for MOs (with a similar trend, $r_s = 0.692, p = 0.085$ for MO$n$) at week 3. The CLO concentrations at week 3 also correlated positively with $\Delta$ROM for MOs ($r_s = 0.985, p < 0.001^{***}$) and MO$n$ ($r_s = 0.903, p = 0.005^{**}$) at week 10.

IV.C. Longitudinal changes in ROM production and in clinical rating scale scores

$\Delta$ROM for MO$n$ and MOs correlated positively with $\Delta$PANSS total and negative scores at week 3, and $\Delta$ROM for MOs with $\Delta$PANSS positive scores at week 10 (see Table 2). Although other correlations for MO were not statistically significant, all of them were positive, whereas those for PMNL showed no any consistent trend, and at no point did they reach statistical significance.

<table>
<thead>
<tr>
<th>Table 2. Correlations between changes in ROM ($\Delta$ ROM) production by MO$n$ and MOs, and changes in PANSS ($\Delta$ PANSS) scores during the CLO trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>week 0 vs. week 3</td>
</tr>
<tr>
<td>$\Delta$ ROM, MO$n$</td>
</tr>
<tr>
<td>$r_s = 0.590$</td>
</tr>
<tr>
<td>$p = 0.123$</td>
</tr>
<tr>
<td>$r_s = 0.479$</td>
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<tr>
<td>$p = 0.230$</td>
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<tr>
<td>$r_s = 0.719$</td>
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<tr>
<td>$p = 0.045^*$</td>
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<tr>
<td>$r_s = 0.371$</td>
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<tr>
<td>$p = 0.365$</td>
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<tr>
<td>$r_s = 0.476$</td>
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<tr>
<td>$p = 0.233$</td>
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<tr>
<td>$r_s = 0.515$</td>
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<tr>
<td>$p = 0.192$</td>
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<tr>
<td>$r_s = 0.473$</td>
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<tr>
<td>$p = 0.035^*$</td>
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<tr>
<td>$r_s = 0.429$</td>
</tr>
<tr>
<td>$p = 0.289$</td>
</tr>
</tbody>
</table>

IN VITRO

V.A. Effect of clozapine on ROM production

The overall effect of CLO on the production of ROM by the cells was significant ($p = 0.012$) only for MO$n$, with a similar trend ($p = 0.093$) for the PMNL$n$. The ROM production by the CLO-incubated MO$n$ was systematically lower than that for the solvent-incubated MO$n$ at all three time-points. The difference was statistically significant at weeks 0 and 3 with the same trend at week 10. The ROM production by the solvent- and by the PBS-incubated cells did not significantly differ from each other. No statistically significant differences appeared between the three time-points for either CLO- or solvent-incubated cells.
V.B. In vitro effect of clozapine on ROM production by monocytes and changes in clinical rating scale scores

Spearman’s correlation coefficient showed significant positive correlations for in vitro CLO-induced changes in the ROM production by MOH at baseline with ΔPANSS total and with almost all subscale scores at weeks 3 and 10. ΔPANSS positive scores at week 3 and ΔPANSS negative scores at week 10 (with a similar trend for the latter) were the only exceptions. All the correlations were positive, indicating that the degree of the CLO-induced decrease in the ROM production by MOH in vitro at baseline was associated with the degree of the subsequent longitudinal decrease in the PANSS scores (i.e., clinical improvement) during CLO treatment. General Linear Modeling found Δ PANSS negative scores at week 3 (multiple $R^2 = 0.789$, $p = 0.003$) to be the most significant single explanatory variable for the changes in the ROM production by MOH. No other variables were needed.
5. DISCUSSION

5.1. Methodological limitations of own study

The results of all five studies should be viewed with some caution due to methodological limitations. While the first part of the work was a retrospective study, the other four parts were clinical trials, performed on small patient samples without control groups. More extensive studies are needed before conclusions can be made with more confidence.

5.2. Long-term effects of CLO in out-patients with treatment-resistant schizophrenia

Our naturalistic study on CLO-treated out-patients with chronic schizophrenia represents possibly one of the longest (up to 15 years) published observations on this kind of patient population. Of the patients treated with CLO for more than one year, nearly 80% were able to be discharged from the hospital in 2.3/2.2 (0.1 – 9.0) years, which is in agreement with a previous Finnish report (Kuoppasalmi et al., 1993). The shift to CLO led in the present study to a desirable turning point in the previously unfavorable course of the disease, as illustrated by increased yearly hospitalization time prior to the shift and its gradual decrease after one year of CLO treatment. For those eight patients who continued on CLO for 10 years or more, no re-hospitalizations were any longer needed. These findings are in line with those from a retrospective study by Connelly and Fullick (1998), whose CLO-treated out-patients’ hospital days dropped from 7.9 to 1.8 for comparable time periods. It is unlikely that the higher proportion of at least moderately improved patients in our study (almost 90%, versus the 63% of Connelly and Fullick, 1998) was due to our longer follow-up, since the duration of CLO treatment in our patients did not correlate with their clinical outcome. Possible explanations may involve different patient samples and schizophrenia subtypes. Indeed, Leppig et al. (1989) reported marked or almost complete improvement in 78% of their retrospectively studied 69 out-patients with schizophrenia, which corresponds relatively closely with our results.

While all our patients with paranoid schizophrenia after the transfer to CLO improved enough to be discharged from the hospital, the hebeplrenic patients fell in two groups with clearly different courses. One group of 27 patients evidenced – unexpectedly, due to the notoriously poor prognosis of this type of schizophrenia - even a more pronounced clinical and social improvement than did the non-hebeplrenic patients, while another five patients remained chronically psychotic in-patients. Thus, CLO seems to make it possible to distinguish two subtypes of chronic schizophrenia – one to whom CLO was especially beneficial, and another, possibly
the so-called devastating subtype (Kaplan and Sadock, 1995), showing no response to any medication. The clinical or theoretical relevance of this finding is thus far unclear.

Prolonged hospitalization for out-patients after the shift to CLO was, in the present study, associated with better social outcome - perhaps because a more intensive rehabilitation effort became possible in this patient subgroup. This explanation is supported by a recent report by Rosenheck et al. (1998); their CLO-treated patients were more likely to participate in psychosocial treatment, which augmented independently the pharmacotherapeutic benefit of CLO at 12 months both in reduced symptoms and in improved quality of life.

Our finding of a more pronounced clinical and social improvement in patients of a younger age at the onset of the disease shows a discrepancy with the generally known age-outcome predictor (Frangou and Murray, 1996). In this sample this discrepancy was statistically explained by the hebephrenic patients, who experienced a more noticeable improvement. However, Breier et al. (1994) also found an association of younger age at first psychotic symptoms with good response to CLO in out-patients. Hence, those authors might have dealt with a similar patient subgroup.

Unlike clinical outcome, social outcome was directly associated with the duration of CLO treatment. This supports the suggestion of Meltzer (1989b) as to the necessity of a prolonged (up to one year) CLO trial for each individual patient, and that of the Swedish group (Lindström, 1988; Lindström & Lundberg, 1995) as to the need for a separate follow-up of social outcome, which chronologically may be delayed after clinical outcome.

Duration of illness before CLO in our study was not associated with clinical outcome. This was not surprising in this set of patients with a mean duration of illness of 11.8 years (in the out-patient group, 13.1 years), i.e., far longer than the proposed period of an active brain process (McGlashan and Fenton, 1993). However, the patients in the Swedish studies (Lindström, 1988; Lindström and Lundberg, 1995), for whom CLO was started earlier in the course of the disease (8.7 and 9.6 years), had a much higher rate of employment (up to 40%) than our patients. This indirectly speaks in favor of an earlier introduction of CLO, proposed by some authors (Lieberman, 1996; Edwards, 1998).

5.3. CLO in early treatment-resistant schizophrenia

In contrast to earlier CLO studies on treatment-resistant schizophrenia, including our own (I), duration of illness in our 10 patients with early signs of treatment-resistance showed inverse correlations with clinical improvement ($r = -0.639$, $p = 0.047^*$ for the positive PANSS scores, with a similar trend for the total PANSS scores). It is plausible that such a correlation can be observed only within the first years of
psychosis, which is in line with the theory of the neurotoxicity of the initial period of 
the schizophrenic process. While the proportion of CLO-responders in treatment-
resistant schizophrenia remains between 30 and 60% (see 1.A.), nine of ten patients in 
this study responded to CLO medication. The effects of CLO in our patients 
resembled those of conventional neuroleptics in drug-naive patients in terms both of 
lower sufficient antipsychotic doses and of enhanced incidence of side-effects.

The minimum standard for an unsatisfactory response to neuroleptic drugs has not 
been established. It has been proposed that if even moderate social impairment, 
persistent negative symptoms, and mild-to-moderate positive symptoms are present 
despite treatment with neuroleptics, CLO should be considered, because CLO should 
produce clinically significant benefits in the majority of such patients (Meltzer, 1998). 
This study was focused on the duration of active psychosis and number of previous 
nuroleptic trials rather than on the number of previous episodes. This is in contrast to 
a growing body of research on first-episode schizophrenia, in which duration of illness 
has not been delimited. Indeed, CLO-treated first-episode patients studied until now 
have been ill for many years (e.g., 4.4 years in the study of Szymanski et al., 1994) 
and have undergone numerous conventional neuroleptic trials, missing possibly the 
opimal period of the disease, when CLO might interrupt the development of 
treatment-resistance. There are few CLO studies with a duration of psychosis of less 
than 3.0 years. The patients of Claghorn et al. (1987) were ill for 2.0 years but were 
nuroleptic-intolerant rather than neuroleptic-resistant. The patients of Singer and Law 
(1974) with a duration of illness of 2.5 years were acute patients, not selected as non-
responders to other medications. Thus, our patients may represent a population not 
investigated earlier. The new term "early treatment-resistance" is here introduced, 
although it needs further clarification and validation before it can be used in clinical 
practice for the initiation of treatment with CLO.

5.4. Adjunctive nefazodone in schizophrenia with predominantly 
negative and depressive symptoms

In this clinical trial of add-on nefazodone in eight patients with difficult-to-treat post-
psychotic schizophrenia with predominantly negative and/or depressive symptoms, 
clear-cut clinical improvement in terms of positive, negative, depressive, and 
extrapyramidal symptoms and quality of life was observed. Sexual dysfunctions also 
showed a tendency to improve. Whereas doses of concomitant conventional 
nuroleptics could be successfully decreased in most, and benzodiazepines 
discontinued in all cases, anxiety observed in some patients (including panic attacks in 
two) abated completely. Clinical improvement occurred mostly within the initial 6
weeks, the phase of a stable neuroleptic dose regimen. The complete attenuation of residual positive symptoms despite the reduced doses of neuroleptics during the trial probably indicates an enhancement of the antipsychotic effect and/or improved tolerability of conventional neuroleptics caused by adjunctive nefazodone. This is in line with the hypothetical pharmacodynamic parallel between the drug combinations studied and the CLO-like atypicality of SDA-neuroleptics, i.e., a high 5HT2/D2 inhibition ratio, with an additional increase in serotonin and noradrenaline turnover. A modest increase in the serum levels of neuroleptics as the pharmacokinetic background of the improved outcome cannot be ruled out (Barbhaiya et al., 1996). However, this seems unlikely, since nefazodone, a potent inhibitor of the cytochrome P-450 (CYP) isoenzyme 3A4, is only a weak inhibitor of the CYP2D6 isoenzyme, which is central in the metabolism of conventional neuroleptics (Owen and Nemeroff, 1998). Moreover, the attenuation of the side-effects of neuroleptics observed during co-administration of nefazodone in the present study provides support for the pharmacodynamic rather than the pharmacokinetic interpretation.

It seems that in clinical practice, add-on nefazodone can become a treatment option, e.g., when the psychiatrist and the patient are reluctant to undertake the risk of a shift to a different neuroleptic in cases with well-controlled psychosis but negative or depressive symptoms remaining. Adjunctive nefazodone can possibly be considered also in more active psychotic states. Liver function monitoring is, however, justified during nefazodone treatment, since some patients may experience idiosyncratic, potentially fatal liver damage (Aranda-Michel et al., 1999).

5.5. CLO and production of ROM by monocytes in treatment-resistant schizophrenia

CLO treatment led to in vivo concentration-dependent changes in the ROM production by MO (but not PMNL) during the first 3 weeks of medication in our eight treatment-resistant schizophrenic patients. Serum concentrations of CLO at week 3 correlated even more powerfully with subsequent (at week 10) changes in the ROM production by MO. The changes in the ROM production by MOn and MOs also demonstrated several positive correlations with the decrease in clinical rating scale scores measuring psychotic (PANSS), but not depressive (MADRS) symptomatology. These correlations imply that a decrease or relatively small increase in the ROM production by MOn and MOs rather than a clear-cut increase was associated both with more favorable clinical outcome, and at week 3 with higher concentrations of CLO. Interestingly, CLO concentrations exceeding at week 3 the therapeutic level of 350 ng/ml (Jann et al., 1993; Potkin et al., 1994) were associated with a tendency to decrease, while concentrations below this level corresponded instead with an increase
in the ROM production by MOs. Thus, CLO may have a biphasic concentration-dependent effect, in which relatively low serum concentrations of the drug are associated with an increase, but the high therapeutic concentrations with a decrease in ROM production. It is possible that CLO can modulate the ROM production by MO in the same fashion as melatonin modulates that of PMNL, i.e., dampens it in high but stimulates it in low concentrations (Pieri et al., 1998).

Our separate experiment revealed a clear-cut in vitro CLO-induced decrease in the ROM production by MOs in these eight patients with treatment-resistant schizophrenia throughout the CLO trial. The grade of sensitivity of MOs to this effect at the baseline of the CLO trial predicted subsequent clinical response to CLO medication. This connection was mostly due to negative symptoms.

Our findings are not directly comparable with those of McAdams and Leonard (1993) (see 1.7.3), since the patients of that Irish group were not treatment-resistant, and all their neuroleptics were of the conventional type. In addition, their experiments (also the in vitro one) considered only zymosan-stimulated cells. We failed to find in the literature any other studies on this topic.

The results of the present study support the hypothesis of an association between the ROM production by mononuclear phagocytes, neurotoxicity in schizophrenia, and the distinct efficacy of CLO against negative symptoms and treatment-resistance. It appears that the unique clinical properties of CLO may be exerted partly via modulation of the ROM production by MO.
6. CONCLUSIONS

6.1. CLO may be especially beneficial for a substantial subgroup of treatment-resistant patients with disorganized schizophrenia, especially in combination with a sufficiently long hospitalization, allowing intensive rehabilitation. Despite the high CLO cost and blood monitoring expense, CLO medication is presumably cost-saving because of the decreased need for re-hospitalization.

6.2. CLO in early treatment-resistant schizophrenia is safe and effective, and lower doses may suffice. A change in the current practice towards introduction of CLO earlier in the course of the disease may be desirable to gain all possible benefits.

6.3. Add-on nefazodone appears to enhance the antipsychotic efficacy of conventional neuroleptics and counter their side-effects – presumably due to the CLO-like postsynaptic pharmacodynamic profile of such a combination. Hence, adjunctive nefazodone may become a valuable treatment option, e.g., when the psychiatrist or patient is reluctant to undertake the risk of a shift to a different neuroleptic in a well-controlled psychosis with difficult-to-treat negative or depressive symptoms remaining.

6.4. IV. In vivo, after 3 weeks of treatment, therapeutic serum concentrations of CLO seem to dampen the production of ROM by MO, and this effect correlates positively with clinical outcome in treatment-resistant schizophrenia. Modulation of the ROM production by MO appears to have an impact in the mechanism of action of CLO.

6.5. V. In vitro, CLO reduces the ROM production by the non-stimulated MO, and the degree of this effect may be predictable for clinical outcome, a finding which may have important clinical implications.
7. SUMMARY

Treatment-resistant schizophrenia is still an unresolved problem. Today, CLO is the only drug with established efficacy in treatment-resistant schizophrenia. Unfortunately, it may cause life-threatening agranulocytosis. The mechanisms of the clinical action of CLO and the pathophysiology of CLO-induced agranulocytosis are still poorly understood; neither is it clear whether these are interrelated. Studying CLO may bring us nearer to the understanding of schizophrenia and treatment-resistance and discovery of new, better compounds or drug treatment strategies.

A series of studies was undertaken with CLO or a CLO-like drug combination in schizophrenic patients who did not respond optimally to conventional neuroleptic medication.

In a long-term (up to almost 15 years) naturalistic retrospective follow-up of 39 out-patients, with a small control group of in-patients, an earlier finding was replicated that CLO substantially decreases the need for hospitalization in treatment-resistant schizophrenia. CLO treatment was thus presumably cost-saving. Prolonged duration of hospitalization after the start of CLO was associated with better social outcome, plausibly due to the more intensive rehabilitation permitted by CLO. Furthermore, duration of CLO treatment correlated positively also with social improvement. Surprisingly, the out-patients with disorganized schizophrenia (hebephrenic schizophrenia according to the Finnish version of DSM-III-R) displayed more noticeable improvement than did those with other types of schizophrenia. All the severely ill chronic in-patients suffered also from hebephrenic schizophrenia, probably pointing to the heterogeneity of this diagnostic category.

Since schizophrenia is evidently not only a neurodevelopmental but also a neurotoxic disease, the most effective intervention, used as early as possible, is essential for a good outcome. A prospective open CLO study was performed in “early treatment-resistant schizophrenia”. Nine out of 10 patients with the first emerging signs of resistance to their conventional neuroleptics responded to CLO, in contrast to the 30 to 60% reported in earlier CLO studies of rigorously established treatment-resistant schizophrenia. Duration of psychosis prior to CLO correlated inversely with clinical improvement. This study advocates transfer to CLO earlier in the course of schizophrenia than is the general practice today.
Nefazodone is a novel antidepressant which, in addition to increasing serotonin turnover, also inhibits postsynaptic 5HT2 receptors in a CLO-like fashion. Addition of nefazodone to conventional neuroleptics in our prospective open study appeared to influence favorably the course of the disease and diminish side-effects of the neuroleptics in eight patients with mainly postpsychotic schizophrenia and prominent negative and depressive symptoms. After the acute phase of the study, during which the study protocol required the doses of neuroleptics to remain stable, adjunctive nefazodone allowed us to substantially diminish the neuroleptic dosage. Adjunctive nefazodone may become a valuable treatment option, for instance, when a shift to a different neuroleptic is risky in a patient with a well-controlled psychosis but with remaining difficult-to-treat negative or depressive symptoms.

Both the immune system, including mononuclear leukocytes, and free radicals (mainly ROM) have been proposed as underlying the pathophysiology and possibly the neurotoxicity of schizophrenia and treatment-resistance. Since human phagocytes are able to produce ROM, they may be a link between the immune and free radical theories. CLO appears to be not only the most potent neuroleptic, but also the most dangerous in terms of hematological side-effects. In contact with phagocytes, CLO is involved in a complex free radical interplay. We hypothesized that the desirable and hazardous features of CLO are interrelated and are exerted partly via modulation of the production of ROM by phagocytes. Unlike microglia/brain macrophages, mature descendants of MO, peripheral blood MO from schizophrenic patients are easily available for exploration. Eight patients with treatment-resistant schizophrenia were studied prospectively before and after their shift to CLO. The ROM production by their blood phagocytes was measured and clinical assessments were performed simultaneously before and during the CLO trial. The serum concentrations of CLO at week 3 correlated positively with the changes in the ROM production by MO, i.e., high concentrations were associated with a clear-cut decrease in the ROM production. Furthermore, this decrease in ROM production was associated with a decrease in psychotic symptoms. In addition, CLO dampened the ROM production by non-stimulated MO in vitro, and the degree of this effect at baseline predicted subsequent clinical outcome. Thus, these data support our hypothesis and may have clinical implications.

All the studies in this series have some methodological limitations, which makes additional investigations necessary. However, our work has outlined some novel theoretical and practical approaches which may be of interest to the research community.
8. ACKNOWLEDGEMENTS

First and foremost, I am thankful to all the patients who participated in our work for remaining with us throughout all the troublesome research procedures.

I wish to express my sincere gratitude to Professor Ranan Rimón, chief of our department, who introduced me not only to research in the sphere of psychopharmacology but also to the realm of modern psychiatry in its entirety. He has been generous in generating the directions of the work and has taught me much about scientific thinking and writing.

I am deeply indebted to my supervisor and co-author, Acting Professor Björn Appelberg for the most constructive and amicable guidance throughout all the phases of this work. Without him I would undoubtedly have given up many times, and this thesis would never have been completed.

I am grateful to my supervisor Docent Esa Leinonen for his comments and his attitude, both critical and warm.

I wish to express my warmest thanks to Professor Ulf-Göran Ahlfors and Docent Kimmo Kuoppasalmi, the referees of this dissertation, for their most welcome constructive criticism.

Professor Brian Leonard, Dr. Cai Song and Dr. Peter Nyberg have introduced me to the exciting field of psychoneuroimmunology and taught me the laboratory techniques used in this work, for which I am deeply indebted.

Doctor Peter Nyberg has also been one of my co-authors, and I appreciate very much his invaluable contributions. I also express my deepest gratitude to all my other co-workers in Finland and Russia.

I thank Docent Heikki Kallia for his very instrumental impact in ideation of the immunological branch of the study in the preliminary phase of the work.

I want also to sincerely thank Novartis OY (former Sandoz OY), which for me has been personified in Rainer Gädeke (also one of my co-authors) and Outi Wilén. Without the gift of the clozapine preparation, a substantial part of the work would have been impossible, and without financial support the realization of part of the study abroad as well as several presentations of our preliminary results in international research circles would have been difficult.
My special thanks to Bristol-Myers Squibb OY and personally to Docent Timo Muhonen for the open-handed gift of nefazodone and the financial support which made it possible to present our results to the international research community.

I express my gratitude to the foundation Liv och Hälsa and the Mjölbolsta Foundation for Medical Research for funding a substantial part of the laboratory expenses.

I want to thank sincerely pharmaceutical companies Janssen-Cilag OY, Eli Lilly Finland OY, Wyeth Lederle Finland, and Organon OY for financial support.

I wish to cordially acknowledge the hospital staff of Ekäsen hospital (Ekenäs, Finland) and Departments of Psychiatry of the Universities of Helsinki (Finland) and Petrozavodsk (Russia) for their selfless cooperation. Dr. Kaj Palmgren and Professor Mark Burkin, Chiefs of Ekäsen Hospital and the Department of Psychiatry of the University of Petrozavodsk are warmly acknowledged for their help and kind companionship.

I warmly thank librarian Anja Roilas, whose help was inestimable.

For editing of these theses in a very constructive and fascinating atmosphere I am greatly indebted to Dr. Carol Norris.

I also desire to acknowledge you, Nadezhda Bronzova, for the wonderful illustrations to this book.

Last but not least, I am very happy to have been encouraged and lovingly supported during all these years by my patient family - my wife Marina, daughter Polina, and son Leo, as well as by my mother Eva and sister Eleonora.
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APPENDIX 1. REACTIVE OXYGEN METABOLITES, ANTIOXIDANT DEFENCE SYSTEMS, AND CHEMILUMINESCENCE

I. REACTIVE OXYGEN METABOLITES AND ANTIOXIDANT DEFENCE SYSTEMS

1. BASIC CHEMISTRY

In the cell aerobic metabolism oxygen is oxidized by mitochondrial cytochrome to H₂O (Reaction 1):

\[ \text{O}_2 + 4\text{H}^+ + 4\text{e}^- \rightarrow 2\text{H}_2\text{O} \]

(Weiss, 1986)

Electrons may often escape (due to, for instance, an error in mitochondria) from the electron transport chain and react with molecular oxygen with formation of a superoxide radical (Reaction 2):

\[ \text{O}_2^- + \text{e}^- \rightarrow \text{O}_2 \cdot^- \]

(Mahadik and Scheffer, 1996)

The superoxide radical is rapidly converted to hydrogen peroxide by widely distributed superoxide dismutases (Reaction 3):

\[ 2\text{O}_2^- + 2\text{H} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

(Fridovich, 1983)

\[ \text{H}_2\text{O}_2 \text{ is further reduced to a hydroxyl radical in either} \]

1) the (very) slow Haber-Weiss reaction (Reaction 4):

\[ 2\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{HO}^- + \text{OH}^- + \text{O}_2 \]

(Beauchamp and Fridovich, 1970)

or

2) its rapid modification, the Fenton reaction, that occurs in the presence of transitional metals ("Fenton reagents"), such as iron or copper (Reaction 5):

\[ \text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{HO}^- + \text{OH}^- \]

(Weiss, 1986)

The superoxide radical can also be modified in the xantine oxidase-dependent degradation of hypoxantine to xantine and further to uric acid (Reactions 6 and 7):

hypoxantine + H₂O₂ → xantine + 2O₂⁻ + 2H⁺

xantine + H₂O₂ → uric acid + 2O₂⁻ + 2H⁺

(Weiss, 1986)

A membrane-bound NADPH oxidase is a phagocyte-specific enzyme complex. It is dormant in resting phagocytes, but following stimulation (with, for instance, phagocytosis or phorbol myristate acetate, PMA, a direct NADPH oxidase stimulant) it triggers a series of metabolic events resulting in a massive production of hydrogen peroxide, superoxide anion, hydroxyl radical, and singlet oxygen (Bellavite, 1988).
This phenomenon, for historical reasons referred to as the “respiratory burst” is crucial for the microbe- and tumor-cell killing by phagocytes (Babior, 1978). In this reaction NADPH serves as a donor of electrons (Reaction 8):

\[
\text{NADPH} + 2\text{O}_2 + \text{H}^+ \rightarrow \text{NADP}^+ + 2\text{O}_2^- + 2\text{H}^+
\]

(Bellavite, 1988)

In addition, phagocytes can also produce hypochlorous acid through the action of the phagocyte-derived enzyme myeloperoxidase (Reaction 9):

\[
\text{H}_2\text{O}_2 + \text{Cl}^- + \text{H}^+ \rightarrow \text{HOCl} + \text{H}_2\text{O}
\]

(Weiss, 1989)

Hydrogen peroxide, myeloperoxidase, and chloride are known to constitute a most potent “cytad” system toward microbes, parasites, or tumor cells (Klebanoff and Clark, 1978). In phagocytes this reaction occurs in the extracellular space or in phagosomes (Karnovsky and Badwey, 1983), with no antioxidant enzymes available for its control. The magnitude of the ROM production by phagocytes depends on the species of animal, the cell type (e.g., neutrophil, macrophage) and state (“normal”, “elicted” i.e., inflammatory, “activated”, resting or stimulated), the nature of stimulant, the interplay of enzymes and substrates and so forth (Karnovsky and Badway, 1983).

ROM, if not ameliorated by antioxidant defence systems (vide infra) induce chain reactions of lipid peroxidation (Reactions 10 – 12):

- lipid-H + radical → lipid’ + radical-H
- lipid’ + O_2 → lipid-O_2
- lipid-O_2’ + lipid-H → lipid-O_2H + lipid

(Mahadik and Schefter, 1996)

2. ANTIOXIDANT DEFENCE SYSTEMS

The main antioxidant pathways are shown in Figure 1.

PREVENTIVE ANTIOXIDANTS

Transferrin (lactoferrin in milk), caeruloplasmin, and albumin bind transition metals (“sequestration”) (Maxwell, 1995)

ANTIOXIDANT ENZYMES

Superoxide dismutase is a metalloprotein that facilitates the dismutation of superoxide anion to hydrogen peroxide (vide supra, Reaction 3)

Catalase is a hem-enzyme that catalyzes the formation of water and oxygen from hydrogen peroxide:

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

(Weiss, 1986)
Glutathione peroxidase is a seleno-enzyme that removes H₂O₂ at the expense of reduced glutathione (GSH) with the formation of oxidized glutathione disulfide (GSSG):

\[ \text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow 2 \text{H}_2\text{O} + \text{GSSG} \]

(Maxwell, 1995)

**Main antioxidant defense systems**

O₂⁻ = superoxide radical; H₂O₂ = hydrogen peroxide; HO = hydroxyl radical; CAT = catalase; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase

*Preventive antioxidants bind transitional metal ions (“sequestration”), preventing their reaction with O₂⁻ and H₂O₂ to produce toxic hydroxyl radicals. Enzymes SOD, CAT, and GSH-Px facilitate breakdown of radicals in the intracellular space. “Sacrificial” (“chain-breaking”) antioxidants become oxidized in reaction with toxic radicals to become relatively inactive ones.*

There are no enzymes known to control the levels of hydroxyl radicals, and the role of the three enzymes above is to prevent the interaction of superoxide, hydrogen peroxide, and transitional metals, thus preventing the subsequent formation of highly toxic hydroxyl radicals (Maxwell, 1995). Superoxide dismutase, catalase, and glutathione peroxidase exist in the intracellular environment, whereas extracellular space is not armed with enzymatic scavengers of ROM (Weiss, 1986).

**SCAVENGING (“CHAIN-BREAKING”, “SACRIFICIAL”) ANTIOXIDANTS**

Scavenging antioxidants are powerful electron donors. They are oxydized by toxic free radicals with the formation of relatively inactive ones before more vital structures are damaged. Scavenging antioxidants are often divided into the water soluble, such as ascorbic (vitamin C) and uric acids, bilirubin, and thiols, and the lipid-soluble, such as tocopherol, β-carotene, and ubiquinol-10 (Maxwell, 1995).
II. LUMINOL-DEPENDENT CHEMILUMINESCENCE AS AN INSTRUMENT FOR MEASUREMENT OF PRODUCTION OF REACTIVE OXYGEN METABOLITES BY PHAGOCYTES

The respiratory burst of phagocytes is accompanied by emission of small, but measurable amounts of photons of light (chemiluminescence) (Allen et al., 1972; Bellavite, 1988). The cyclic hydrazide luminol is an easily oxidizable ("chemilumigenic") substance which produces light of high intensity in contact with activated phagocytes, indicating the activity of the respiratory burst (Allen and Loose, 1976). Luminol has been used in a chemiluminescence assay for the hypochlorite produced in the myeloperoxidase-catalyzed formation of hydrogen peroxide (Reaction 9) (DeChatelet et al., 1982). However, in phagocytes, hydrogen peroxide is formed in the dismutation reaction of the superoxide radical (Reaction 8). Indeed, it has been shown earlier that luminol-dependent chemiluminescence from human mononuclear phagocytes is completely inhibited by superoxide dismutase (Nyberg and Klockars, 1990). This suggests that luminol-dependent chemiluminescence in fact measures the superoxide production dependent on the NADPH oxidase – myeloperoxidase system of phagocytes, and thus is phagocyte-specific.

To facilitate phagocyte reactions, phagocyte-stimulation has been used in most experiments. Phorbol esters, such as phorbol myristate acetate (PMA), potently activate NADPH oxidase directly through an interaction with the signal transduction system with no phagocytosis needed (in contrast to phagocytosable particles, e.g., opsonized zymozane), which leads to progressive and irreversible activation of the respiratory burst Bellavite, 1988).
### APPENDIX 2. Main results of studies I – V, mean (I, II, and III) or median (IV and V) (range)

<table>
<thead>
<tr>
<th>Study I. Neuroleptic-resistant schizophrenia, Out-patients on CLO for up to 15 years (n = 39). A retrospective follow-up</th>
<th>Helephrenic schizophrenia (n = 27)</th>
<th>Non-helephrenic schizophrenia (n = 12)</th>
<th>Statistics, helephrenic vs. non-helephrenic</th>
<th>Duration of hospitalization during CLO and social improvement (positive correlations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical improvement</td>
<td>3.6 (2 – 54)</td>
<td>3.1 (2 – 4)</td>
<td>U = 226.0, p = 0.032*</td>
<td>r = 0.372, p = 0.020</td>
</tr>
<tr>
<td>Social improvement</td>
<td>3.2 (1 – 4)</td>
<td>2.5 (1 – 4)</td>
<td>U = 233.0, p = 0.024*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study II. Early neuroleptic-resistant schizophrenia, a prospective 26-week CLO trial (n = 10)</th>
<th>Week 0 (baseline)</th>
<th>Week 26 (end-point)</th>
<th>Change from baseline, %</th>
<th>Duration of illness (DI) and reduction of positive PANSS (inverse correlations); r = -0.639, p = 0.047; DI (years) was longer in fair than in good responders; 3.25 (3.0 – 3.8) (fair) 1.5 (0.9 – 1.0) (good)</th>
</tr>
</thead>
<tbody>
<tr>
<td>total PANSS</td>
<td>54.5 (38 – 65)</td>
<td>37.6 (25 – 49)</td>
<td>31.0 (21.0 - 51.0) %</td>
<td></td>
</tr>
<tr>
<td>Quality of Life Scale</td>
<td>37.1 (21 – 79)</td>
<td>62.7 (33 – 89)</td>
<td>81.7 (181.0 – 6.3) %</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study III. Neuroleptic-treated schizophrenia with mainly negative symptoms, a prospective 26 week add-on nefazodone trial (n = 8)</th>
<th>Week 0</th>
<th>Week 6</th>
<th>Week 6 vs. week 0, %</th>
<th>Positive symptoms, observed in 3 patients and panic attacks in 2 patients entirely disappeared in all cases.</th>
</tr>
</thead>
<tbody>
<tr>
<td>total PANSS</td>
<td>64 (48 – 89)</td>
<td>45 (33 – 63)</td>
<td>29 (13 – 60)% (*)</td>
<td>Doses of neuroleptics could have been significantly decreased in most and benzodiazepines discontinued in all cases.</td>
</tr>
<tr>
<td>MADRS</td>
<td>15 (8 – 29)</td>
<td>5 (0 – 11)</td>
<td>63 (18 – 100) % (*)</td>
<td></td>
</tr>
<tr>
<td>SARS (extrapyramidal symptoms)</td>
<td>4 (0 – 9)</td>
<td>3 (0 – 7)</td>
<td>43 (0 – 100) % (*)</td>
<td></td>
</tr>
<tr>
<td>UKU (sensory dysfunctions)</td>
<td>4 (0 – 11)</td>
<td>3 (0 – 11)</td>
<td>38 (0 – 100) % (NS)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Studies IV and V. Neuroleptic-resistant schizophrenia, a prospective 10 week CLO trial (n = 8)</th>
<th>Week 0</th>
<th>Week 3</th>
<th>Statistics; week 3 vs. week 0</th>
<th>IV B. Concentrations of CLO and changes in ROM production, (correlations at week 3) CLO: r = 0.092, p = 0.085 MOn: r = 0.761, p = 0.047*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV A. Non-stimulated monocytes (MOns), in vivo</td>
<td>311 (93 – 1438)</td>
<td>560 (140 – 1445)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PMA-stimulated monocytes (MOns), in vivo</td>
<td>518 (150 – 2228)</td>
<td>771 (198 – 1576)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

| IV C. Changes in ROM production and in total PANSS, correlations at week 3 CLO: r = 0.743, p = 0.035* MOn: r = 0.838, p = 0.009** |
|---|---|---|---|---|
| V A. MOn + solvent (in vitro) | 291 (89 – 1342) | 569 (126 – 1348) | CLO vs. CLO + solvent, week 0 and 3, respectively 0.017*, 0.012* |
| MOn + solvent + CLO (in vitro) | 242 (67 – 1156) | 433 (100 – 1226) | |

** = significance level < 0.05
* = significance level < 0.01

1 Longer duration of hospitalization corresponded with a more pronounced improvement
2 Longer duration of illness corresponded with less pronounced improvement
3 Higher concentrations of CLO corresponded with a clear-cut decrease, but lower with an increase in ROM production
4 Decrease in ROM production was associated with a more, but an increase with a less noticeable reduction in PANSS scores
5 More pronounced decrease in ROM production was associated with a more noticeable reduction in PANSS scores
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ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:


III. Grigori Joffe, Björn Appelberg, Ranan Rimón. ADJUNCTIVE NEFAZODONE IN NEUROLEPTIC-TREATED SCHIZOPHRENIC PATIENTS WITH PREDOMINANTLY NEGATIVE SYMPTOMS: AN OPEN PROSPECTIVE PILOT STUDY. International Clinical Psychopharmacology, in press


V. Grigori Joffe, Peter Nyberg, Andres Gross, Björn Appelberg. CLOZAPINE-INDUCED DECREASE IN THE PRODUCTION OF REACTIVE OXYGEN METABOLITES BY MONOCYTES IN VITRO MAY PREDICT CLINICAL RESPONSE TO CLOZAPINE IN TREATMENT-RESISTANT SCHIZOPHRENIA. Human Psychopharmacology 1999;14:203-209
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>BPRS</td>
<td>Brief Psychiatric Rating Scale</td>
</tr>
<tr>
<td>CAT</td>
<td>catalase</td>
</tr>
<tr>
<td>CGI</td>
<td>Clinical Global Impression</td>
</tr>
<tr>
<td>CLO</td>
<td>clozapine</td>
</tr>
<tr>
<td>CPRS</td>
<td>Comprehensive Psychiatric Rating Scale</td>
</tr>
<tr>
<td>CYP</td>
<td>cytochrome P450</td>
</tr>
<tr>
<td>D2</td>
<td>dopamine receptors 2</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSM-III-R</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, revised</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, 4th edition</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>glutathione peroxidase</td>
</tr>
<tr>
<td>SHT</td>
<td>5-hydroxytryptamine (serotonin)</td>
</tr>
<tr>
<td>IL</td>
<td>interleukine</td>
</tr>
<tr>
<td>MADRS</td>
<td>Montgomery-Åsberg Depression Rating Scale</td>
</tr>
<tr>
<td>MGG</td>
<td>May-Grünwald-Giemsa</td>
</tr>
<tr>
<td>MO</td>
<td>monocytes</td>
</tr>
<tr>
<td>MOn</td>
<td>non-stimulated monocytes</td>
</tr>
<tr>
<td>MOs</td>
<td>monocytes, stimulated with phorbol myristate acetate</td>
</tr>
<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>PANSS</td>
<td>Positive and Negative Syndrome Scale</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PMA</td>
<td>phorbol myristate acetate</td>
</tr>
<tr>
<td>PMNL</td>
<td>polymorphonuclear phagocytes</td>
</tr>
<tr>
<td>PMNLn</td>
<td>non-stimulated polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>PMNLs</td>
<td>polymorphonuclear leukocytes, stimulated with phorbol myristate acetate</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acids</td>
</tr>
<tr>
<td>QLS</td>
<td>Quality of Life Scale</td>
</tr>
<tr>
<td>ROM</td>
<td>reactive oxygen metabolites</td>
</tr>
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<td>SAS</td>
<td>Simpson-Angus Scale</td>
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<tr>
<td>sILR</td>
<td>soluble interleukine receptors</td>
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<td>SOD</td>
<td>superoxide dismutase</td>
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<td>TNF-α</td>
<td>tumor necrosis factor alfa</td>
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<td>UKU</td>
<td>Committee for Clinical Investigations (Udvalg for kliniske undersøgelser) Side Effect Rating Scale</td>
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1. INTRODUCTION

1.1. Schizophrenia

Schizophrenia is a chronic and disabling desintegrative psychotic illness. Symptoms of schizophrenia fall into three clusters: positive symptoms, which include delusions and hallucinations; disorganized thought, speech, and behavior; and negative, or deficit symptoms, such as reduced thought and speech, blunted affect, and decreased initiation of goal-directed behavior. Although presenting with somewhat similar clinical features, schizophrenia is likely a group of disorders with heterogenous prognosis and causes.

The worldwide prevalence of schizophrenia is about 1%, with somewhat higher figures (1.5%) reported in Finland (Lehtinen, 1996). Being long-lasting and incapacitating, schizophrenia exacts disastrous costs from patients, their families, and society. Despite tremendous progress in understanding schizophrenia and the rapid development of biological and psychosocial therapeutic interventions during the last decades, the fight against this illness is still far from its victorious completion.

1.2. Treatment-resistant schizophrenia

Between one-fifth and one-third of patients with schizophrenia do not show an adequate response to neuroleptic medication (Prien and Cole, 1968; Essock et al., 1996). These "treatment-resistant" patients create a serious public health problem due to their extensive hospitalization needs (McGlashan, 1988) and consequent high costs of the treatment (Revicki et al., 1990).

Treatment-resistance has been defined in different ways. The most restrictive criteria have been introduced by Kane et al. (1988):

1. Persistent positive psychotic symptoms: Item score ≥ 4 (moderate) on at least two of four positive symptom items (rated on a 1 – 7 scale) on the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) – hallucinatory behavior, suspiciousness, unusual thought content, and conceptual disorganization.

2. Current presence of at least moderately severe illness: Total BPRS score ≥ 45 on the 18-item scale and a score ≥ 4 (moderate) on the Clinical Global Impression scale (CGI) (Guy, 1976).

3. Persistence of illness: No period of good social or occupational functioning within the last 5 years.

4. Drug-refractory condition: At least three periods in the preceding 5 years of treatment with conventional antipsychotics from at least two chemical classes at doses ≥ 1000 mg per day of chlorpromazine equivalents for 6 weeks, each without
significant symptom relief, and failure to improve by at least 20 percent as measured by total BPRS score or intolerance of haloperidol at 10 to 60 mg per day during a 6-week prospective trial.

Different broader definitions of treatment-resistance have been used by others (Breier et al., 1994; Juarez-Reyes et al., 1995), and today treatment-, or neuroleptic-resistance is often defined as a failure to respond to the usual drug treatment (Wahlbeck et al., 1998). For the term “treatment-resistant schizophrenia” has frequently been substituted the term “difficult-to-treat schizophrenia”, especially when used in a broader sense.

Although most definitions of treatment resistance consider positive symptoms, there has been an awareness of the problem of persistent deficit symptoms. Negative symptoms of schizophrenia (lack of normal mental activities such as thought, speech and motivation) are well known as especially resistant to treatment interventions (McPhillips and Barnes, 1997). In addition, depression and extrapyramidal side-effects (EPS) of neuroleptics, which are often indistinguishable from each other and from the negative symptoms of schizophrenia (Coffey, 1994; Sax et al., 1996) can make schizophrenic patients treatment-resistant (Kane, 1996) – either directly or via EPS-related incomppliance with antipsychotic medication. To distinguish enduring (“primary”, “deficit”, or “core”) negative symptoms from less stable secondary negative features is theoretically and prognostically important, but difficult due to the lack of valid measures (McPhillips and Barnes, 1997). Negative symptoms scores correlate strongly with the duration of initially untreated psychosis (Waddington, 1996).

1.3. Active brain process as plausible background of schizophrenia and treatment-resistance

Gradual deterioration in schizophrenic patients was observed by Kraepelin before the neuroleptic era. However, the convincing evidence for neurodevelopmental pathology in schizophrenia has since the 1980s led to general abandonment of the classical chronic deterioration disease concept. It is interesting, however, that while many patients recover almost completely from their first psychotic episode, the majority subsequently experience additional episodes of psychosis with an increasing, often persistent morbidity. In contrast to Alzheimer’s disease, which is characterized by a continuing inexorable progression, the progression in schizophrenia occurs mostly during the initial years of the disease. McGlashan and Fenton (1993) found an instability of clinical picture with a drift toward disorganized, non-specified, and deficit subtypes of schizophrenia and significant worsening of negative symptoms during the first 5 years of active psychosis with a plateau later on. These changes were associated with poor functional outcome 15 years later. Negative symptoms, although
most variable at the initial phase of the illness increased in severity, stability, and adverse prognostic weight during the later course of the disease. Consistent with the view that the decline in schizophrenia occurs early in the illness and then plateaus, Harrison et al. (1996) found that the illness course over the 2 years after onset is strongly associated with the course over the ensuing 10 years. Progression of negative symptoms, accompanied by relatively stable positive symptoms was also found by Peralta et al., 1995. After the first years, the patients tend to reach a relatively stable, symptomatic treatment-resistant phase. Although the etiology of this process is obscure, timely effective intervention at the early stage is essential for the outcome (Wyatt, 1991; Loebel et al., 1992; McGlashan and Fenton, 1993; Lieberman et al., 1993; Waddington et al., 1996; Wyatt et al., 1997). This evidence proposes that although the disease may be developmental, a neurodegenerative component is involved in treatment resistance.

A convincing body of evidence indicates that the treatment response and the level of recovery in early stages of the disease are improved despite only low doses of neuroleptics (McEvoy et al., 1991; Lieberman et al., 1996). In addition, the intervention in this stage is beneficial also in the long term (Schooley et al., 1997; Lieberman et al., 1992). A long duration of untreated psychosis as a predictor of poor outcome has been reported in numerous studies (Loebel et al., 1992; Lieberman et al., 1992; Waddington et al., 1995; Szymanski et al., 1996). Also several modern neuroimaging studies have demonstrated a more rapid loss of brain volume in schizophrenic patients than in controls during the first years of the disease (DeLisi et al., 1997; Gur et al., 1998). Nair et al. (1997) have found two different groups of patients – with or without progression of such brain volume loss, possibly due to at least two etiologically distinct processes. Loss of volume was associated with neurobehavioral decline (Gur et al., 1998). It has been concluded that periods of active psychosis involve neurodegenerative and/or neurotoxic processes (Wyatt, 1995; Nair et al., 1997; Lieberman et al., 1997), which, if not ameliorated by neuroleptics, may result in negative symptoms and treatment refractoriness.

It has been shown that along with a prolonged untreated psychosis tardive dyskinesia is a predictor of poor outcome in first-episode schizophrenia, and that subsequent non-responders are more liable to develop it (Lieberman et al., 1992). In addition, an association between tardive dyskinesia and cognitive deterioration has been observed (Waddington et al., 1997). Tardive dyskinesia therefore has been proposed to be a phenotypic marker of the illness, associated with treatment resistance (Lieberman et al., 1992). The neurobehavioral decline associated with negative symptoms seems to
be interrelated with tardive dyskinesia (Lieberman et al., 1992) and with the loss of brain volume during the course of the disease, manifesting the neurotoxicity of the schizophrenic process (Gur et al., 1998).

Consequently, it can be postulated that the most effective treatment modes available with beneficial effects on positive and (even more important) negative symptoms and tardive dyskinesia should be used aggressively at the very beginning of the disease in order to protect the brain and to avoid the development of treatment-resistance. In this respect, clozapine (CLO) and the other “atypical” neuroleptics ought to be discussed.

1.4. Clozapine

CLO, a prototypic “atypical” neuroleptic, is a tricyclic dibenzazepine compound which was developed in 1957 by Wander, a small Swiss pharmaceutic company. Initially designed as an antidepressant, CLO surprisingly showed a potent antipsychotic action without noticeable extrapyramidal side-effects. Since CLO did not have cataleptic and amphetamine antagonism properties (believed at that time to be necessary for an antipsychotic), it was initially not introduced to clinical practice, and the first clinical studies were published only in the late 60s (Berzewski et al., 1969; Gross and Kaltenbäk, 1970; Angst et al., 1971). In Finland CLO was registered in 1975. However, when, during a relatively short period, 8 out of 16 Finnish patients developing leukopenia during CLO treatment died because of agranulocytosis (Amsler et al., 1977; Iäänäen-Heikillä et al., 1977), the drug was withdrawn from the market in Finland and most other countries. Nevertheless, re-introduction of CLO was shortly thereafter permitted for many patients who did not respond to any other drug. Hence, considerable clinical experience was obtained until the middle 80s, when CLO was shown to be more effective than chlorpromazine in a controlled double-blind study by Honigfeld et al. (1984). However, only when a methodologically more restrictive study by Kane et al. (1988) revealed the superiority of CLO over haloperidol in a group of 286 patients with treatment-resistant schizophrenia, was CLO launched in the United States and the UK - in 1990. This manifested a new breakthrough in the pharmacotherapy of schizophrenia. Nevertheless, due to the 1 – 3% risk of agranulocytosis (Fritton and Heel, 1990; Krupp and Barnes, 1992; Alvir et al., 1993; Owens, 1996), CLO in most countries was reserved for treatment-resistant cases only - a situation requiring a clear definition of this condition. Hereby, the whole concept of treatment-resistance gained a new meaning, relevant to clinical practice.

CLO differs from conventional neuroleptics in many ways. It is claimed to be a more potent antipsychotic with a degree of response of 30 to 60% in schizophrenia resistant
to conventional neuroleptics (Kane et al., 1988; Christison et al., 1991; Szymanski et al., 1994; Breier et al., 1994; Schooler et al., 1994; Barnes and McEvety, 1996; Wahlbeck et al., 1998). It is effective against both positive and negative symptoms of schizophrenia (Kane et al., 1988), including the primary negative symptoms (Miller et al., 1994; Brar et al., 1997), it postpones relapses (Wahlbeck and Cheine, 1998), and has beneficial effects on neurocognition in schizophrenia (Lee et al., 1994). CLO appears to be a potent mood stabilizer (Zarate et al., 1995; Vestergaard, 1997) with beneficial effects on impulsive, aggressive (Garmendia et al., 1992; Mallya et al., 1992), and suicidal (Okayli et al., 1992; Meltzer and Okayli, 1995) behaviors. Treatment with CLO seems to improve the quality of life of schizophrenic patients (Meltzer, 1992a) and is cost-effective despite expensive mandatory hospitalization at the beginning of the treatment and frequent white blood cell count monitoring (Honigfeld and Patin, 1990; Morris et al., 1998).

The side-effect profile of CLO differs as well from that of conventional compounds. In addition to agranulocytosis, CLO more frequently causes troublesome hypersalivation (Lieberman et al., 1989), weight gain (Leadbetter et al., 1992), and seizures (Devinsky et al., 1991), but it is almost free from extrapyramidal side-effects, tardive dyskinesia, and hyperprolactinemia (Meltzer, 1992b; Tamminga et al., 1994; Owens, 1996).

To summarize, the superior efficacy of CLO, primarily with regard to negative symptoms and tardive dyskinesia could be an argument for its early use in schizophrenia in terms of overall clinical outcome and prevention of treatment-resistance (Lieberman, 1996; Edwards et al., 1998). However, the CLO-related risk of a life-threatening agranulocytosis makes it a drug of reserve and thus exerts pressure toward creating safer alternative compounds or drug strategies with at least equal efficacy, or both.

1.5. Serotonin-dopamine antagonism and the atypicality of neuroleptics

The antipsychotic potency of the conventional neuroleptics has been related to their ability to inhibit the dopamine D2 receptors (Connell, 1958; Randrup and Munkvad, 1972; Carlsson, 1977; Kapur et al., 1996). This observation has led to the development of the dopamine theory of schizophrenia. Numerous D2 inhibitors of different chemical classes have been developed since the invention of chlorpromazine in 1952. Since they are of equal antipsychotic efficacy and differ from each other mainly in terms of side-effects, the change from one D2 blocking neuroleptic to another usually offers no additional gain in treatment outcome (Kane et al., 1988;
Kinon et al., 1993). In addition, chronic treatment with conventional dopaminergic drugs may result in persistent neural dysfunction (Lieberman et al., 1990) and poor clinical outcome (Chouinard, 1991; McEvoy, 1991; Lieberman et al., 1993).

The remarkable properties of CLO have been mostly attributed to its specific receptor affinity spectrum, different from that of conventional neuroleptics. CLO shows higher affinity to D1 (Farde and Nordstrom, 1992; Farde et al., 1992) and D4 (Van Tol et al., 1991) receptors than to D2 receptors. It antagonizes also 5HT3 serotonin receptors, ε1 and ε2 adrenoreceptors, H1 histamine receptors, and muscarinic acetylcholine receptors (Beerpoot et al., 1996; Wirshing et al., 1997) (with the exception of M4 receptors, which are stimulated by CLO) (Zorn et al., 1994), and shows a partial agonism in 5HT1A receptors (Meltzer and Roth, 1998). However, the higher affinity of CLO to serotonin 5HT2a and 5HT2c than to D2 receptors (Meltzer, 1989a; Roth et al., 1992; Meltzer, 1994; Nordström et al., 1995; Kapur and Remington, 1996; Schotte et al., 1996; Meltzer and Roth, 1998) as an explanation of its unique efficacy has accumulated the most convincing empirical support. Indeed, the novel atypical neuroleptics risperidone, olanzapine, and sertrindole, designed on the basis of this CLO-like serotonin-dopamine antagonism (SDA) concept, have each shown in double-blind clinical trials an efficacy superior to conventional neuroleptics in reducing negative symptoms (Marder and Meibach, 1994; Beasley et al., 1996; Zimbroff et al., 1997). These drugs may also prove to be more effective in producing remission of psychosis and have been promoted as first-line antipsychotics. However, their role in treatment-resistant schizophrenia has yet to be established. Today CLO remains the only neuroleptic with established efficacy in rigorously defined treatment-resistant schizophrenia (Christison et al., 1991; Barnes and McEvdy, 1996; Fleischhacker, 1999). Despite the markedly well-developed knowledge of the receptor-level effects of CLO, the exact mechanism of its novel action is still far from clear (Barnes and Kane, 1996). Neither is it known whether the desirable clinical effects of CLO and its blood dyscrasia-provoking property are interrelated.

1.6. Serotonin-dopamine antagonism concept and antidepressants in schizophrenia with negative and depressive components

Both the antidepressants (Siris et al., 1987; Silver and Nassar, 1991; Delle Chiaie et al., 1994; Goff et al., 1990; Goff et al., 1995; Salokangas et al., 1996) and the inhibitors of postsynaptic 5HT2 serotonin receptors (Strauss and Kliesser, 1991; Silver et al, 1991; Duinkerke et al., 1993; Lee et al., 1995; Meltzer et al., 1996) seem to be useful adjuncts to conventional neuroleptics in some schizophrenic patients with negative and depressive symptoms. In theory, these two strategies could be used
simultaneously in a complementary fashion by means of combining conventional neuroleptics with novel antidepressants (e.g., mirtazapine or nefazodone), which are able to inhibit the post-synaptic 5HT2 receptors. In addition to enhanced efficacy, such a combination could also diminish side-effects, since, like neuroleptics, antidepressants may cause EPS (Leonard and Faherty, 1996) and sexual dysfunction (DeVane, 1995), whereas the 5HT2 blockade can counter both conditions. Moreover, the sufficient antipsychotic level of the D2 blockade by the SDA neuroleptics is lower than that by conventional neuroleptics (Farde et al., 1994; Goyer et al., 1996).

Thus, the 5HT2 blockade by the add-on nefazodone or mirtazapine might allow a reduction in neuroleptic doses with enhanced efficacy, a further lessening of side-effects, better compliance, and improved quality of life. Two recent preclinical experiments with mirtazapine, performed in rodents on the basis of this assumption, support this hypothesis, since co-treatment with mirtazapine enhanced the antipsychotic-like effect and reduced extrapyramidal side-effects of conventional neuroleptics (Berendsen et al., 1998; Pinder et al., 1998).

To our knowledge, clinical trials with such combinations are lacking. Nevertheless, combinations with theoretically similar pre- and postsynaptic effects, i.e., CLO and selective serotonin reuptake inhibitors, may be useful in some patients. For example, the improved outcome for schizophrenic patients in the study of Szegedi et al. (1995) was attributed to the pharmacodynamic benefits of co-administration of CLO and fluvoxamine. Pharmacokinetic interactions between study drugs might, however, contaminate these results (Koponen et al., 1995).

1.7. Cellular immune mechanisms and free radicals as a plausible background for schizophrenia and its neurotoxicity

1.7.1. MONONUCLEAR PHAGOCYTES AND SCHIZOPHRENIA

Immune mechanisms in schizophrenia have been extensively investigated throughout the decades. An increasing number of studies indicate that immune mechanisms, e.g., an autoimmune process (Heath et al., 1967; Kirch, 1993; Ganguli et al., 1993) or a viral infection (DeLisi and Crow, 1986; Kirch, 1993) underlie the pathophysiology of schizophrenia, at least as a contributing factor. One of the most consistent findings in this field has been a defect in interleukin (IL)-2. Increased (O’Donnell et al., 1996) or decreased (Ganguli et al., 1995) levels of IL-2, increased soluble IL-2 receptors (sIL-2R) in peripheral blood (Rapaport et al., 1989; Ganguli and Rabin, 1989), and increased IL-2 in CSF (Licinio et al., 1993; McAllister et al., 1995) have been reported in schizophrenia. IL-2, a natural stimulant of mononuclear phagocytes (e.g., microglia in the brain and their precursor, peripheral blood MO) is
produced by T-lymphocytes. Microglia and its activated form, brain macrophages, are also capable of production of cytokines, some of which (e.g., IL-1 and transforming growth factor-beta) can (respectively) stimulate or suppress T-lymphocytes. Thus, macrophages and T-lymphocytes are functionally entangled (Roitt et al., 1993). IL-2 from leukocytes has been shown to induce positive schizophreniform symptoms, such as hallucinations, delusions, paranoia, agitation, and irritability in healthy subjects (Denicoff et al., 1987). In addition, negative symptoms may relate to an immune defect in schizophrenia, since low IL-2 production is associated with an earlier age of onset and more severe negative symptoms (Ganguli et al., 1995). Furthermore, macrophage-produced cytokines (e.g., alpha-interferon) may have behavioral effects resembling prodromal and/or negative symptoms of schizophrenia, i.e., fatigue, depression, signs of frontal lobe pathology, slowing of behavior, and motor perseveration (Adams et al., 1984; Renault et al., 1987; Niiranen et al., 1988); this evidence, however, is inconsistent (Katila et al., 1993). These observations have generated the macrophage-T-lymphocyte theory of schizophrenia (Smith, 1992). According to this theory, an activation of macrophages (via consequent hyperactivation of T-lymphocytes, who thereafter wrench themselves free of the control of macrophages) initiates the schizophrenic process. An acute phase response, as well, has been shown in schizophrenia (Smidt et al., 1988; Wong et al., 1996). Based on the model for acute phase response in liver (Heinrich et al., 1990), this observation also in schizophrenia would prescribe a role for monocyte (MO)/macrophage-produced cytokines, such as IL-1, IL-6, tumor necrosis factor, and perhaps nerve growth factor, and hence the involvement of MO/macrophages or their products also in the pathogenesis of schizophrenia. Indeed, enhanced levels of IL-6 and sIL-6R receptors have been measured in the plasma of young schizophrenic patients (Maes et al., 1994), giving support to this monocyte/macrophage theory. Moreover, elevated proportions of macrophages in the CSF (Nikkilä, 1997) and an increase in microglia in the frontal and temporal cortex of schizophrenic patients (Radewicz et al., 1998) indicate an involvement of microglia/macrophages in the schizophrenic process.

1.7.2 FREE RADICALS AND SCHIZOPHRENIA

1.7.2.1 Free radicals and the brain

Free radicals are species that contain one or more unpaired electrons and are capable of independent existence (Halliwell and Gutteridge, 1989). Such species - in the human mainly reactive oxygen metabolites (ROM) (see APPENDIX 1) - are unstable and highly reactive, and they achieve stability by the annexing of electrons from, meaning oxidation of surrounding molecules. These molecules in turn become free
radicals and may thus initiate a chain ("redox") reaction (Maxwell, 1995). ROM function as intra- and extracellular signaling molecules and can directly affect the cellular signaling apparatus and control of gene expression (Palmer and Paulson, 1997). ROM in turn are controlled by the antioxidant defense systems. Although ROM are generated under physiological conditions, their excessive amount, due to either ROM hyperproduction or to the insufficiency of antioxidant defense systems, may lead to oxidative stress (Mahadik and Scheffer, 1996). Oxidative stress due to such an excess can initiate apoptosis in some cell types (Sugaya et al., 1997). Polyunsaturated fatty acids (PUFA) are especially sensitive to oxidative stress, and their long chain reactions can rapidly lead to cell membrane dysfunctions: in addition to their role in the transport of ions and nutrients, PUFAs serve as second messengers in neuronal transduction (Mahadik and Scheffer, 1996). Proteins, as well, when attacked by ROM, may lose their normal structure with consequent functional disturbances in, for example, ion channels or receptors. ROM-induced damage in PUFAs, proteins, and deoxyribonucleic acid (DNA) can cause cell dysfunctions and even death. ROM have a pathogenic impact in a variety of human diseases. The nervous system is particularly vulnerable to the ROM attack due to its special biochemistry, anatomy, and physiology; it has:

1) a high rate of oxidative metabolism
2) high concentrations of membrane lipid PUFAs, which are highly oxidizable
3) low levels of antioxidant enzymes catalase (CAT) and glutathione peroxidase (GSH-Px)
4) high endogenic ROM generation in, for example, monoamine oxidase-catalyzed oxidation of catecholamines, metabolism of prostaglandines, or active production by macrophage-type microglia cells
5) highly specialized neuronal signal transduction dependent on faultless membrane function
6) a high surface area/cytoplasmic volume ratio
7) long axons endangered by peripheral injury
8) a disruption-sensitive neuronal network
9) a lack of cell turn-over (Evans, 1993)
10) a high content of iron and the inability of CSF to bind released iron ions (Halliwell, 1992)

Evidence is mounting that ROM are involved in the central nervous system (CNS) membrane pathology, e.g., in Parkinson’s and Alzheimer’s diseases, Down’s syndrome, multiple sclerosis, trauma, and ischemia (Evans 1993).
1.7.2.2. Free radicals in schizophrenia

The theory of a pathological role for free radicals in schizophrenia, proposed originally in the mid 1950s by Hoffer et al. (1954), has recently been supported by a rapidly growing body of findings (Reddy and Yao, 1996):

1. There is evidence (although not unequivocal, Katila et al., 1997) for peroxidative damage of membranes, e.g., increased levels of malondialdehyde, pentane, and phospholipase A2 in medicated (Prilipko, 1984; Phillips et al., 1993; Gattaz et al., 1987; McCreadie et al., 1995) and drug-naïve (Scheffer et al., 1995) patients, especially those with tardive dyskinesia (Lohr et al., 1990). Interestingly, negative symptoms are associated with high levels of saturated and low levels of long-chain unsaturated fatty acids, whereas the picture with regard to positive symptoms is the opposite (Glen et al., 1994). Altered antioxidant defence has been demonstrated: decreased or increased superoxide dismutase (SOD) and GSH-Px activity in neuroleptic-treated and drug-free patients, as well as decreased CAT activity (Abdalla et al., 1986; Reddy et al., 1991; Mukherjee et al., 1994; Zhang et al., 1998) and decreased E-vitamin cholesterol rate in schizophrenia (McCreadie et al., 1995) and tardive dyskinesia (Cadet and Kahler, 1994).

2. Promising results of clinical efficacy trials with supplementation of:
   a) E-vitamin (a dietary lipid-soluble chain-breaking antioxidant) in schizophrenia (Sram and Blinkova, 1992) and (especially when used early, according to Reddy and Yao, 1996) in tardive dyskinesia (Adler et al., 1993; Peet et al., 1998) or
   b) essential fatty acids (EFAs) (eicosanoids) in schizophrenia (Vaddadi, 1992; Puri and Richardson, 1998) and tardive dyskinesia (Vaddadi et al., 1989) have been reported.

3. Prognosis of schizophrenia in developing countries is better despite the lack of maintenance treatment, possibly due to a low consumption of animal fats (which is associated with increased oxidative tone) (Christensen and Christensen, 1988).

1.7.2.3. Free radicals and conventional neuroleptics

Some conventional antipsychotics are able to exert pro- (e.g., haloperidol) or antioxidant (e.g., chlorpromazine and prochlorperazine) activity in vitro and possibly in vivo (Jeding et al., 1995). In a recent study by Dalla Libera et al. (1998), the conventional neuroleptics chlorpromazine and trifluoperazine acted as good antioxidants, as did serotonin and CLO. Interestingly, CLO was the most potent of all four substances in a hydrophobic environment of the type present in biological membranes. Most data, however, suggest that the conventional neuroleptics themselves are a direct source of free radicals (Chignell et al., 1985) and correspondingly are a cause of enhanced lipid peroxidation (Pall et al., 1987).
1.7.3. MONOCYTES AND MICROGLIA/MACROPHAGES AS A POSSIBLE LINK BETWEEN IMMUNE AND FREE RADICAL MECHANISMS IN SCHIZOPHRENIA

One of the major sources of ROM in human are phagocytes: neutrophils (polymorphonuclear leukocytes, PMNL) and MO in the peripheral blood, and macrophages in organs. For instance, in the brain ROM are modified (along with the processes of incomplete reduction of oxygen in mitochondria, monoamine oxidase catalyzed oxidation of dopamine and noradrenaline, and metabolism of prostaglandins) (Evans, 1993) by microglia or their activated state, called brain macrophages. The ability to produce ROM is an important function of phagocytes, necessary for destroying microbes, parasites, and altered cells (Babior, 1978; Karnovsky and Badwely, 1983). The ability of microglia/brain macrophages to produce ROM might provide a link between the free radical and immune theories of schizophrenia: the phagocyte-produced ROM may be neurotoxic and seem to participate in diverse brain pathology, accompanied by behavior disturbances (Fisher, 1988; Thery et al., 1993; Banati et al., 1993; Oken, 1995; Benveniste, 1997). It has been hypothesized that brain damage by activated microglia-produced ROM is a common pathophysiological mechanism for various brain diseases characterized by behavioral disturbances, including schizophrenia (Oken, 1995). While microglia from a living person are difficult to obtain for experiments, the peripheral blood phagocytes are easily available, MO are of particular interest in this respect. First, they constitute a precursor of tissue macrophages, including microglia, and share with the latter numerous functions, e.g., ROM production (Lydyard and Grossi, 1993; Langermans et al., 1994). Second, they are capable of penetrating the blood-brain barrier (BBB) under normal conditions (Lassmann et al., 1993), and especially so when the BBB is altered, as it is in about one-third of patients with schizophrenia (Müller and Ackenheil, 1995). “Altered” MO have been proposed even as an etiologic factor in schizophrenia (Smith, 1991).

Despite these considerations, the ROM production by phagocytes in schizophrenia has been insufficiently explored. Two depression studies from Ireland, comprising small control groups of schizophrenic patients, are the only available reports on this issue (O’Neill and Leonard, 1990; McAdmas and Leonard, 1992). In the first study, the ROM production by PMNL from six drug-free schizophrenic patients was measured cross-sectionally by luminol-dependent chemiluminescence with no differences evident between patients and controls. The ROM production by MO was not assessed. In the second study, the ROM production by MO and PMNL of 10 drug-free schizophrenic patients (3 females and 7 males) was assessed longitudinally before and after successful treatment with conventional neuroleptics. The ROM production by both cell populations increased during follow-up. However,
during the time that the initially low values for PMNL normalized, those for MO, normal before the neuroleptic treatment, increased significantly to levels twice as high as in the controls. The investigators, however, found no changes in the ROM production by blood phagocytes from their patients after incubation of the cells in vitro with the patients’ current neuroleptics. In both studies the cells were challenged by opsonized zymosan. No data for non-stimulated cells were reported.

1.7.4. NEUROLEPTICS, FREE RADICALS, AND CELL IMMUNITY

1.7.4.1 Conventional neuroleptics

The antipsychotic activity of neuroleptics, although associated with receptor-level phenomena, can not be attributed exclusively to them. For instance, the delay between receptor occupancy and clinical response calls for clarification. One possible explanation may be an immunosuppressive effect of neuroleptics that may differ between conventional compounds and CLO (Leykin et al., 1997).

Some authors have reported suppressive (Ruutu, 1972a; Ruutu, 1972b; Salovera et al., 1987; Boukhris, 1988; Bessler et al., 1995) or stimulatory (Ferguson et al., 1978; Goldstein et al., 1980) effects of conventional neuroleptics on cell immunity, while others have failed to demonstrate any significant effects (Pollmacher et al., 1997). Conventional neuroleptics have been shown to inhibit in vitro various types of phagocytes from the human and other species (Ruutu, 1972a; Ruutu 1972b; Horwitz et al., 1981; Pfister et al., 1984; Baciu et al., 1988; Brewton and MacCabe, 1988; Watanabe et al., 1988; Krumholz et al., 1995). Little is, however, known about their effects on the ROM production by phagocytes, although the sparse data published give some evidence for an inhibitory effect of phenothiazines on the ROM production by animal alveolar and peritoneal macrophages in vitro (Chang et al., 1983; Traykov et al., 1997).

Few patient data exist on effects of neuroleptics on macrophages in schizophrenia. Nikkilä (1997) found that elevated proportions of macrophages in CSF from schizophrenic patients tend to normalize following neuroleptic treatment. The direct in vivo effect of neuroleptics on the ROM production by phagocytes in schizophrenia remains to be explored, although treatment with conventional antipsychotics seems to increase ROM production (O’Neill and Leonard, 1992).

1.7.4.2. Clozapine, cell immunity, and free radicals

Side-effects of CLO such as hyperthermia, transient leukocytosis, eosinophilia, and agranulocytosis, along with its unique efficacy, have raised particular interest in its possible immunological effects. In the study of Pollmacher et al. (1996), CLO was
found to enhance tumor necrosis factor-alpha (TNF-alpha), soluble TNF receptors p55 and p75, and sIL-2R in schizophrenic patients, while haloperidol showed no such effects (Pollmacher et al., 1997). Another group reported CLO in vivo to elevate sIL-2R (which may facilitate immunosuppressive effects) while not affecting IL-6 levels (Maes et al., 1994). The same authors found an increase in plasma sCD8 antigen, IL-1R antagonist, IL-6, and Clara cell protein at different phases of CLO treatment (Maes et al., 1997). Spener-Unterweger et al. (1993) demonstrated a suppression of granulocyte-macrophage colony-stimulating factor by CLO in vitro and interpreted this finding as an indicator of a mediator role for cytokines in CLO-induced agranulocytosis. Pollmacher et al. (1997) observed a transient increase in granulocyte colony-stimulating factor at the second week of a CLO trial in 55% of their patients, accompanied by increases in MO and PMNL counts, in rectal temperature, and in plasma levels of cytokines. CLO showed a significant immunosuppressive effect, equal to that of haloperidol - an effect assessed by the production in vitro of IL-2, IL-4, and interferon-gamma by healthy subjects’ lymphocytes stimulated with phytohemagglutinin (Leykin et al. 1997). Thus, although no exact knowledge yet exists as to immune effects of CLO, the conclusion is warranted that in schizophrenia CLO may have complex immunomodulatory effects.

The direct effects of CLO on human phagocytes have been studied mainly from the point of view of agranulocytosis and therefore apply mostly to PMNL (Pisciota et al., 1992; Liu and Uetrecht, 1995), without simultaneous analysis of clinical modalities. In these studies it has been found (and consistently replicated) that CLO is oxidized by the myeloperoxidase and the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system of PMNL to a free radical (Fish et al., 1991; Mason and Fisher, 1992; Liu and Uetrecht, 1995; Uetrecht, 1995), presumably a relatively stable nitrenium ion. These findings, supported by preliminary data on changes in the antioxidant defense (low plasma and red blood cells’ GSH-Px and selenium as factors predisposing to agranulocytosis) in CLO-induced agranulocytosis survivors (Linday et al., 1995) suppose an involvement of free radical mechanisms in this complication. The authors did not study or discuss the metabolism of CLO to a free radical in regard to the clinical effects of CLO. It is known, however, that reactive metabolites of such substances as aryamines formed, via the same mechanism by PMNL, can inhibit phagocyte function and mediate some of the therapeutic effects of these drugs (Uetrecht, 1995). Furthermore, the chain-breaking, or “sacrificial” antioxidants, in contact with toxic free radicals, become themselves relatively inactive free radicals; through this, the toxic ones are detoxified (Maxwell, 1995). The exact role of the CLO-derived free radicals in the interplay with the phagocyte-produced ROM remains to be investigated.
1.7.5. CLOZAPINE AND THE PRODUCTION OF REACTIVE OXYGEN METABOLITES BY PHAGOCYTES

If a link between the phagocyte and free radicals mechanisms does exist and underlie the pathophysiology of schizophrenia and/or tardive dyskinesia, this could be revealed by longitudinal intraindividual studies with parallel monitoring of changes in clinical picture and in the phagocyte-produced ROM status during the course of a neuroleptic-treated schizophrenic psychosis. To the best of our knowledge, virtually no such studies have been published thus far. CLO seems to be a convenient drug for a study of this kind. It is effective in treatment-resistant schizophrenia and against negative symptoms, while not causing tardive dyskinesia - all of these being conditions in which free radicals may play a role (Cadet and Kahler, 1994; Horrobin et al., 1994). Some authors have observed a complex free-radical interplay between CLO and PMNL in their agranulocytosis-focused studies (Fisher, 1991; Lindsay, 1995; Liu and Uetrecht, 1995).
2. AIMS OF THE STUDY

The aims of the present research, study by study, were:
I. To investigate retrospectively the effects of long-term CLO treatment in out-patients with treatment-resistant schizophrenia
II. To examine prospectively the clinical effects of CLO in schizophrenic patients with early signs of developing treatment resistance
III. To study prospectively whether a combination of conventional neuroleptics with nefazodone leads to CLO-like beneficial effects in difficult-to-treat schizophrenic patients with prominent negative or depressive symptoms or both
IV and V. To explore the in vivo (IV) and in vitro (V) effects of CLO on the ROM production by blood phagocytes in treatment-resistant schizophrenia
3. PATIENTS AND METHODS

3.1. Patients

The series of studies included 71 patients with difficult-to-treat schizophrenia, whose general characteristics are presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age and duration of illness are expressed as median/mean (range)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Study I (n = 44)</td>
</tr>
<tr>
<td>Study II (n = 11)</td>
</tr>
<tr>
<td>Study III (n = 8)</td>
</tr>
<tr>
<td>Studies IV and V (n = 8)</td>
</tr>
<tr>
<td>Entire population (n = 71)</td>
</tr>
</tbody>
</table>

Study I

Patients with DSM-III-R (APA, 1987) schizophrenia (n = 43) or schizo-affective disorder (n = 1) fulfilled the inclusion criteria (duration of illness at least 2 years and duration of CLO treatment at least 1 year) and were enrolled into the study. Five of these 44 were in-patients with disorganized schizophrenia (further referred to as hebephrenic, according to the Finnish version of DSM-III-R). They had a history of continuous or almost continuous long-term hospital treatment before and after the initiation of CLO, and their CLO medication was continued due to a slight improvement despite a lack of optimal treatment response. The other 39 were out-patients.

The main focus of the study was on these 39 out-patients: 24 men and 15 women, mean/median age 41.3/42 (range 24 – 61) years, of whom 27 had hebephrenic, 10 paranoid, one undifferentiated schizophrenia, and one schizo-affective disorder. These patients were treated with CLO for 8.0/7.8 (3 – 14.8) years, CLO daily doses at the endpoint were 436/400 (200 – 700) mg. In these 39 patients duration of illness prior to CLO was 13.1/11.4 (3 – 33) years and duration of hospitalization 4.1/3.2 (0.08 – 11) years before and 2.5/2.2 (0.04 – 9.2) years after introduction of CLO.
Study II
This study comprised 11 schizophrenic (DSM-III-R) (APA, 1987) patients suffering from overt psychotic illness with a total score of at least 18 on the 18-item Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) (each item rated from 0 to 6), despite routine treatment with one or two conventional neuroleptics (haloperidol, sulpiride, trifluoperazine, or chlorpromazine) for 2 - 12 months. These 11 patients were shifted to a CLO trial due to their lack of response to adequate conventional neuroleptic medication (7 patients had, in addition, disturbing side-effects). The current episode of illness had to be the first or second one, with a duration of less than one year. Established therapy-resistant patients were excluded, specifically those who had failed to show any significant clinical response to two neuroleptics of two different classes at a daily dose of 800 mg chlorpromazine equivalents (Kaplan and Sadock, 1993) or more for at least three weeks each.

One patient soon withdrew his informed consent. The remaining 10 patients (six men and four women, four in- and six out-patients) had a mean/median age of 25/24 (18 – 44) years. Their duration of illness was 2.2/2.3 (0.9 – 3.8) years, duration of the current episode was 0.61/0.7 (0.25 – 0.9) years, and duration of the last conventional neuroleptic trial was 0.42/0.4 (0.25 – 0.75) years. Seven patients had paranoid, one catatonic, and two undifferentiated schizophrenia. Four patients had previously received one and six patients consecutively two conventional neuroleptics. At the screening phase all patients were on either haloperidol (15 to 35 mg per day) or trifluoperazine (12.5 to 25 mg per day).

Study III
Eight schizophrenic (DSM-IV) (APA, 1994) patients (four males and four females, two in- and six out-patients) were enrolled in the study. They exhibited a remission into either a non-psychotic or a residually psychotic state after their last episode of schizophrenia and, despite an adequate conventional neuroleptic medication, suffered from long-lasting disabling negative and/or depressive symptoms. Treatment with any antidepressant within the last weeks prior to the trial was forbidden. Five patients had residual, two patients undifferentiated, and one patient paranoid schizophrenia. These patients had previously suffered 3.5/2 (0 – 13) episodes of the disease, and at the screening phase were on conventional neuroleptics at doses of 415/175 (30 – 1700) mg chlorpromazine equivalents.

Studies IV and V
Eight in-patients (one male and seven females) with chronic schizophrenia (DSM-III-R) (APA, 1987), who suffered from an active psychosis despite standard conventional
medication, were enrolled in a CLO trial. Seven patients had undifferentiated, and one patient disorganized schizophrenia. The patients had previously received 5.5/5 (2 - 9) conventional neuroleptics for 8.9/7.5 (1 - 22) years. Each patient had received at least one neuroleptic at doses of 500 mg chlorpromazine equivalents (six patients over 700 mg and one patient over 600) or more for at least two months. Allergic states, acute infection, hyperthermia, or steroid and/or antimicrobial medication formed the exclusion criteria.

3.2. Methods

3.2.1. CLINICAL ASSESSMENTS

Psychopathology was assessed by the 12-item version of the Comprehensive Psychiatric Rating Scale (CPRS) modified for patients with schizophrenia (Montgomery et al., 1978) (Study I), the 18-item Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham., 1962) (II), and the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) (II – V). In addition, the deficit syndrome (impaired intrapsychic, interpersonal, and instrumental role functioning) was assessed with the Quality of Life Scale (QLS) (Heinrichs et al., 1984) (II, III).

The Montgomery-Åsberg Depression Rating Scale (MADRS) (Montgomery and Åsberg, 1979) was used for a more thorough evaluation of the depressive symptoms (II – V).

A retrospective analysis of improvement in terms of clinical symptoms (clinical improvement) and social functioning (social improvement) during clozapine treatment was performed with a 6-point global assessment rating scale, derived from the item for global improvement of the CGI (Guy, 1976) with two additional intermediate stages. The eventual scale was as follows: 0. no change; 1. minimally improved; 2. much/minimally improved; 3. much improved; 4. very much/much improved; 5. very much improved (I). The retrospective assessment was based on available data on the need for psychiatric hospitalization and other types of health care, and on occupational, educational, and marital status, as well as social and intimate relations.

The Simpson-Angus Scale (the Neurological Rating Scale for Extrapyramidal Side Effects) (Simpson and Angus, 1970) was used for assessment of extrapyramidal side-effects of medication (III). Furthermore, the overall clinical severity of illness was rated by the Clinical Global Impression (CGI) severity item (Guy, 1976) (III). Five items for sexual functioning (Increased Sexual Desire, Diminished Sexual Desire, Erectile Dysfunction, Orgastic Dysfunction, and for the male participants also

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1 Item 10 (salivation), although rated, was not included in the statistical calculations and is reported separately for the only patient with a disturbance related to this item (see below).
Ejaculatory Dysfunction) derived from the Committee on Clinical Investigations (Udvalg for kliniske undersøgelser, UKU) Side Effect Rating Scale (Lingjaerde et al., 1987) served for assessment of sexual dysfunctions (III).

The patient’s subjective impression of the medication was evaluated by the Patient Global Impression (PGI) (Guy, 1976) (II)

The scores for the last observations were carried forward for statistical analysis (III).

3.2.2. EXPERIMENTAL ASSESSMENTS (STUDIES IV AND V)
3.2.2.1. Blood sampling
Overnight fasting blood samples were taken at every visit at 07:45 - 08:00 h. From smokers the samples were drawn immediately after their first cigarette of the day.

3.2.2.2. Quantitation of serum clozapine
Serum clozapine concentrations were determined with a high performance liquid chromatographic assay (Lovdahl et al., 1991). The intra- and interassay variation (1000 μmol/l) was 3.7 and 6.2, respectively.

3.2.2.3. Cell counts
Determination of the total leukocyte count from blood anticoagulated with EDTA was performed with a Counter Coulter (Counter Electronics Ltd, UK) within one hour after blood sampling. At the same time blood smears were made, dried in room air, and later stained with May-Grünwald-Giemsa (MGG) for differential counts.

3.2.2.4. Isolation of polymorphonuclear leukocytes (PMNL) and mononuclear leukocytes
Ten ml of heparinized venous blood was layered on 7 ml Ficoll-Paque (Pharmacia, Sweden) in one hour after blood sampling and centrifuged at 450 g for 20 min at 20°C. The band in the interface between the plasma and the Ficoll layer, containing mononuclear cells (i.e., lymphocytes and MO), was aspirated and washed once in phosphate buffered saline (PBS). The leukocyte count was determined with the Counter Coulter, and the cell suspension was diluted to 5 x 10⁶ mononuclear cells/ml. Cytocentrifuge preparations of the cell suspension were prepared immediately, dried in room air, and stained with MGG for determination of the MO proportion.

The pellet from the density centrifugation, containing PMNL and erythrocytes, was collected and hemolyzed during continuous mixing in a hypotonic solution for 10 min at +4°C. The cells were washed at 450 g for 5 min in PBS, the hemolysis was repeated once, and the cells were washed twice at 450 g for 5 min in PBS. The cell
count was determined with the Counter Coulter, and the suspension was diluted to 5 x 10⁶ PMNL/ml.

3.2.2.5. Chemiluminescence assay

Fifty µl of the mononuclear cell or PMNL suspension was added to a solution containing 5.6 mM luminol (Bio-Orbit, Finland). Half of the samples were stimulated with 0.1 µg/ml phorbol myristate acetate (PMA) (Sigma, USA). The light emission caused by the production of ROM was recorded for 30 min at 2-min intervals with a Bio-Orbit 1251 luminometer (Bio-Orbit, Finland) operated by the Bio-Orbit Phagocytosis Program 1251-124. The results are expressed as the areas under the light emission curves per 100,000 cells. The MO results were corrected for the actual MO count on the basis of the differential count obtained from the cytocentrifuge preparations and are further referred to as the ROM production by MO.

Prior to the chemiluminescence assay, the patients’ isolated (i.e., free from serum clozapine) MO and PMNL, both non-stimulated (MO and PMNL) and stimulated with PMA (MOs and PMNLs) were incubated for 1 hour in 37°C in suspensions.
- with PBS only (IV, V)
- or with CLO (5 mg/L) dissolved in water with 1 equivalent HCl for one hour at 37°C (V)
- or with the solvent (HCl) only (V)

3.3. Design, arrangements, and regulations

Study 1

The study was performed as a chart review with a supplementary cross-sectional clinical evaluation with CPRS. The observation period comprised the time from the onset of the disease (first admission for psychosis) until discontinuation of CLO medication, death of the patient, or the end-point of the study (end of 1994). The following information was obtained from hospital records and clinical interviews: sex, age, diagnosis, duration of the disease before CLO administration, duration of hospitalization per year before and during CLO treatment. In addition, data were registered on the best level of working capacity during any period of life and at the end-point of the study (the end of 1994), number of conventional antipsychotics used prior to introduction of CLO, CLO doses at the end-point of the study, and the need for concomitant psychotropic medication at the end-point of the study. Global assessment of improvement in terms of clinical symptoms (clinical improvement) and
social functioning (social improvement) during CLO treatment, need for psychiatric hospitalization and other types of health care, occupational, educational, and marital status, as well as social and intimate relations were recorded as well.

A mirror design was used when comparing the duration of hospitalization per year before and during CLO treatment. This meant that equal numbers of complete years before and during CLO treatment, adjusted according to the shorter of these periods, were taken into consideration. For example, if the patient had been ill for 10 years before clozapine treatment and had received clozapine for more than 3 years, all the months of hospital treatment year by year within the last 3 complete years before, and correspondingly within the first 3 complete years during clozapine treatment, were summarized and compared to each other.

Study II
This study was an open prospective 26-week clinical CLO trial. The following information was obtained from hospital records and clinical interviews: sex, age, age at onset of the first psychotic symptoms, duration of illness before CLO, and number of conventional antipsychotics prior to CLO. At the screening visit, pertinent physical and psychiatric examinations were performed. A 1- to 9-day wash-out from conventional orally used neuroleptic was conducted before the base-line (week 0) evaluation (none of the patients had received depot-neuroleptics).

The recommended CLO daily dose regimen was as follows: 12.5 - 25 mg once or twice on the first day with stepwise increases of 25 to 50 mg during 7 to 14 days until 300 to 450 mg (up to 600 mg) was reached. Individual adjustment of the doses was allowed, and the use of the lowest effective dose during the maintenance period was recommended. Concomitant use of medication with primarily central nervous system activity besides short/middle-acting benzodiazepines was forbidden.

Assessments of treatment efficacy were performed weekly for the first 8 weeks and thereafter bimonthly until the end-point at week 26 (for the CGI also prior to wash-out, and for QLS at base-line and end-point). The PANSS and BPRS scores were adjusted by distracting 1 point from each item (ratings from 0 to 6). The BPRS scores and their changes were additionally counted before the adjustment for the purpose of comparability with some other studies.

Study III
This study was an open prospective add-on nefazodone trial with no wash-out period. Nefazodone was used orally at an initial daily dose of 100 mg with subsequent upward titration through 200 mg on day 3 to 300 mg on day 7 of medication. A further weekly increase of the daily dose by 100 mg up to the maximum of 600 mg was applied if possible. No concomitant use of psychotropic drugs was allowed besides the pre-
existing neuroleptic, or lithium, or long-term benzodiazepine medication. Any changes in medication were prohibited during at least the last 4 weeks prior to and the first 6 weeks of the trial. Thereafter, a reduction in the doses of concomitant drugs was allowed if clinically justified.

Studies IV and V

CLINICAL CONSIDERATIONS

The studies were conducted as two (in vivo and in vitro) aspects of an open naturalistic prospective 10-week CLO trial with no prescribed study drug dose regimen. A washout period of 36 to 48 hours preceded the study medication. Use of concomitant neuroleptics was prohibited. Clinical and experimental assessments were performed simultaneously at weeks 0, 3, and 10 of the trial.

EXPERIMENTAL CONSIDERATIONS

STUDY IV was designed to examine longitudinal in vivo changes in the ROM production during CLO treatment

IV.A. The ROM production by MOn, M0s, PMNLn, and PMNLs at weeks 3 and 10 was compared to that at base-line.

IV.B. Correlations were calculated between the changes in the ROM production by the cells during the trial (the values at base-line minus those at week 3 or week 10) (Δ ROM) and serum concentrations of CLO at weeks 3 and 10.

IV.C. Correlations were calculated between Δ ROM at weeks 3 and 10 and clinical changes (the PANSS or the MADRS scores at base-line minus those at week 3 or week 10) (Δ PANSS and Δ MADRS).

STUDY V was a separate experiment designed to examine the in vitro effects of CLO on the ROM production, including the possible predictive value of these effects at baseline on the clinical outcome of CLO medication.

V.A. The ROM production by the CLO-incubated MOn, M0s, PMNLn, and PMNLs was compared to that of their solvent-incubated counterparts at weeks 0, 3, and 10 of the trial. PBS-only incubated cells served as a second control.

V.B. Correlations were calculated between the in vitro CLO-induced changes in ROM production at baseline (values for the solvent-incubated minus those for the CLO-incubated cells at week 0) and Δ PANSS and Δ MADRS at weeks 3 and 10.

3.4. Safety

A thorough clinical examination preceding the start of the study drugs was performed at baseline in all clinical trials. The mandatory hematological follow-up was
conducted in the trials with CLO. In Study III also out-patients were hospitalized for at least the 2 initial weeks of nefazodone medication for safety reasons. Rating scales for assessment of safety have been described (see 3.2.1.)

3.5. Ethics
All the studies were performed in accordance with the principles of the Helsinki and Madrid Declarations and Good Clinical Practice. The patients had a level of understanding sufficient to communicate intelligently with the investigators and gave their written (Studies III - V) or oral (Studies I and II) consent. It was underlined that the patients could withdraw at any time from the trial with no negative consequences for their treatments. The study protocols of the clinical trials were approved by the ethics committees of the pertinent institutions.

3.6. Statistics
Descriptive statistical data are expressed as means/medians with the ranges in parenthesis. For non-completers the scores for the last observations were carried forward (LOCF) (III). Due to the small or disproportional sizes of the groups studied, non-parametric tests were chosen for statistical calculations - Kruskal-Wallis one-way ANOVA (I and II), Wilcoxon’s non-parametric test for matched pairs (III - V), and Spearman’s correlation coefficient ($r_s$) (I, II, IV,V). General Linear Modeling (Dillon and Goldstein, 1984) was used as the means for studying interactions between the cross-sectional in vitro effects of CLO and longitudinal clinical aspects and localization of significant differences (V).
4. RESULTS

The main results of the series of studies are presented in APPENDIX 2.

Study I

The duration of illness and duration of hospitalization (but not the duration of illness prior to CLO) before and after start of CLO were significantly longer in the in-patients (U = 32.0, p = 0.015*; U = 21.5, p = 0.005**, respectively) than in the out-patients. The in-patients were, as expected, more severely ill, as assessed by their total CPRS scores (U = 7.00, p = 0.001**).

While for the in-patient group clinical improvement during CLO was only minimal and no social improvement was observed, in all out-patients social improvement was at least minimal and the clinical improvement more than minimal.

OUT-PATIENTS

Of the 39 out-patients the clinical improvement in 35, and the social improvement in 26 were rated as much, very much/much, or very much. The age at the onset of the disease showed a negative correlation with the level of clinical (r = - 0.434, p = 0.006) and social (r = - 0.399, p = 0.012) improvement. The social improvement correlated positively with the duration of CLO treatment (r = 0.384, p = 0.016) and with the duration of hospitalization after the initiation of CLO (r = 0.372, p = 0.020). The duration of hospitalization per year showed a peak within the period from 1 year before to 1 year after the start of CLO with a subsequent continuous and significant decline during the following 10 years. Although 33 patients had been capable of work during some period of their lives, at the time of the study only one patient was capable of work comparable to work at pre-illness level and seven patients were in sheltered work, while the remaining 31 patients were disabled.

OUT-PATIENTS WITH HEBEPHRENIC SCHIZOPHRENIA

Despite an earlier age at the onset of schizophrenia and a longer duration of illness prior to, and duration of hospitalization prior to and after the initiation of CLO, the patients with hebephrenic schizophrenia improved significantly more than their non-hebephrenic counterparts in both clinical (U = 226.0, p = 0.032*) and social (U = 233.0, p = 0.024*) terms.

Study II

During the wash-out period no patient improved or deteriorated. Due to disturbing sedation and hypersalivation and, on the other hand, rapid improvement of the
symptoms, the mean/median daily doses of CLO of 192.5/200 (range 100.0 - 350.0) mg at week 8 and 225.0/250 (50.0 - 450.0) mg at the end-point were lower and their ascent slower than initially recommended. All 10 patients exhibited hypersalivation, and nine patients additionally complained of sleepiness, sedation and/or fatigue, while minor extrapyramidal symptoms, observed in all patients at baseline, abated. During the trial none of the out-patients needed hospitalization, and five of the six in-patients could be discharged from the hospital. In all the patients, clinical improvement was seen as assessed by all rating scales, with the exception of slight deterioration on the negative PANSS score in two patients. The BPRS scores (before adjustment) showed improvement of 21/21% (13 – 36) at week 8 and 24/23% (19 – 31) at the end-point. Only one patient improved less (19%) than 20% - the degree of response proposed as clinically significant (Baldessarini and Frankenburg, 1991). In six patients (defined below as good responders) the decrease in total PANSS scores (30 - 50%) exceeded 30%. The remaining four patients (fair responders) showed 21 - 26% improvement. The clinical improvement was observed mostly within the first 8 weeks with only minimal changes thereafter. The QLS assessments also showed a marked improvement of 81.7/71% (6.3 – 181.0).

The duration of illness correlated negatively ($r_s = -0.639, p = 0.047^*$) with the reduction in the positive PANSS scores. Duration of illness also showed a tendency towards a negative correlation with improvement in total PANSS ($r_s = -0.555, p = 0.096$) and a positive correlation with improvement in total QLS ($r_s = 0.612, p = 0.060$) scores. Since improvement corresponds with decrease in PANSS and increase in QLS scores, a shorter duration of illness tended to correlate with better results on both scales.

The mean duration of illness in the fair responders was 3.25/3 (3.0 – 3.8) years, which was twice as long as the 1.5/1.3 (0.9 – 1.6) years in the good responders.

**LONG-TERM FOLLOW-UP**
All those six patients who continued the treatment with the Swiss-made CLO were asymptomatic at follow-up (6 – 15 months after the end-point), and five of them were capable of work. Substitution of the original medication by Ukrainian-produced CLO, tried in two patients, failed in both.

**Study III**
Nefazodone in daily doses of 537.5/600 (300 – 600) mg at week 6 and 575/600 (300 – 600) mg at endpoint was well tolerated by all five patients who completed the study.

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2 The sixth in-patient had been sentenced to hospital treatment by a court and therefore regardless of his psychiatric condition could not be discharged.
protocol. Of the remaining three patients (drop-outs), only one had an adverse event likely to be related to nefazodone. These eight patients (LOCF) showed statistically significant (p < 0.05) clinical improvement as assessed by their total PANSS and all PANSS subscales, MADRS (mainly items 6, 7, and 8 - concentration difficulties, lassitude, and inability to feel), and CGI, mostly within the first 6 weeks with only modest changes thereafter. While significant favorable changes in the Quality of Life Scale scores continued throughout the study, the decrease in the SAS scores, significant at week 6, lost its significance later on due to the deterioration shown by patient 5.

Delusions, observed initially in three patients and panic attacks in two patients, vanished entirely in all cases. The sadness and pessimism expressed by two patients (in one of them also suicidality) abated rapidly. After the first 6 weeks (the phase of stable neuroleptic doses required by the study protocol) doses of neuroleptics could be significantly reduced. No patient needed benzodiazepines at the end-point. Sexual dysfunctions initially seen in five patients improved or remained unchanged, although this finding was not statistically significant.

Studies IV and V

CLINICAL RESULTS

All eight patients completed the study protocol without hematologic complications. CLO was prescribed at daily doses of 185/163 (15 – 250) mg and 400/400 (200 – 600) mg, and the serum concentration increased from 509/275 (150 – 1225) ng/ml to 886/900 (435 – 1200) ng/mol at weeks 3 and 10, respectively. The clinical improvement (mean 29% on the total PANSS scores) was observed mainly within the first 3 weeks. Five patients showed a more than 30% and one additional patient a more than 20% improvement on the total PANSS scores. The decrease in the MADRS and all the PANSS subscale (including total) scores at the end-point was statistically significant (data not shown).

EXPERIMENTAL RESULTS

IN VIVO

IV.A. ROM production by the phagocytes

Considerable inter- and intraindividual variations were observed, but no statistically significant longitudinal trends in the ROM production by M0n, M0s, PMLn, or PMNLs.
IV.B. Serum concentrations of CLO and longitudinal changes in the ROM production

The serum concentrations of CLO at week 3 correlated positively ($r_t = 0.761, p = 0.047\ast$) with changes (post- minus pre-treatment values) in the ROM production ($\Delta$ROM) for MOs (with a similar trend, $r_t = 0.692, p = 0.085$ for MON) at week 3. The CLO concentrations at week 3 also correlated positively with $\Delta$ROM for MOs ($r_t = 0.985, p < 0.001^{***}$) and MON ($r_t = 0.903, p = 0.005^{**}$) at week 10.

IV.C. Longitudinal changes in ROM production and in clinical rating scale scores

$\Delta$ROM for MON and MOs correlated positively with $\Delta$PANSS total and negative scores at week 3, and $\Delta$ROM for MOs with $\Delta$PANSS positive scores at week 10 (see Table 2). Although other correlations for MO were not statistically significant, all of them were positive, whereas those for PMNL showed no any consistent trend, and at no point did they reach statistical significance.

Table 2. Correlations between changes in ROM ($\Delta$ ROM) production by MON and MOs, and changes in PANSS ($\Delta$ PANSS) scores during the CLO trial

<table>
<thead>
<tr>
<th></th>
<th>week 0 vs. week 3</th>
<th>week 0 vs. week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta$ ROM, MON</td>
<td>$\Delta$ ROM, MOs</td>
</tr>
<tr>
<td>$\Delta$ PANSS positive</td>
<td>$r_t = 0.590$</td>
<td>$r_t = 0.627$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.123$</td>
<td>$p = 0.096$</td>
</tr>
<tr>
<td>$\Delta$ PANSS negative</td>
<td>$r_t = 0.719$</td>
<td>$r_t = 0.766$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.045 \ast$</td>
<td>$p = 0.027 \ast$</td>
</tr>
<tr>
<td>$\Delta$ PANSS general</td>
<td>$r_t = 0.476$</td>
<td>$r_t = 0.548$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.233$</td>
<td>$p = 0.160$</td>
</tr>
<tr>
<td>$\Delta$ PANSS total</td>
<td>$r_t = 0.743$</td>
<td>$r_t = 0.838$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.035 \ast$</td>
<td>$p = 0.009^{**}$</td>
</tr>
</tbody>
</table>

IN VITRO

V.A. Effect of clozapine on ROM production

The overall effect of CLO on the production of ROM by the cells was significant ($p = 0.012$) only for MON, with a similar trend ($p = 0.093$) for the PMNL. The ROM production by the CLO-incubated MON was systematically lower than that for the solvent-incubated MON at all three time-points. The difference was statistically significant at weeks 0 and 3 with the same trend at week 10. The ROM production by the solvent- and by the PBS-incubated cells did not significantly differ from each other. No statistically significant differences appeared between the three time-points for either CLO- or solvent-incubated cells.
V.B. In vitro effect of clozapine on ROM production by monocytes and changes in clinical rating scale scores

Spearman’s correlation coefficient showed significant positive correlations for in vitro CLO-induced changes in the ROM production by MOn at baseline with ΔPANSS total and with almost all subscale scores at weeks 3 and 10. ΔPANSS positive scores at week 3 and ΔPANSS negative scores at week 10 (with a similar trend for the latter) were the only exceptions. All the correlations were positive, indicating that the degree of the CLO-induced decrease in the ROM production by MOn in vitro at baseline was associated with the degree of the subsequent longitudinal decrease in the PANSS scores (i.e., clinical improvement) during CLO treatment. General Linear Modeling found ΔPANSS negative scores at week 3 (multiple R² = 0.789, p = 0.003) to be the most significant single explanatory variable for the changes in the ROM production by MOn. No other variables were needed.
5. DISCUSSION

5.1. Methodological limitations of own study
The results of all five studies should be viewed with some caution due to methodological limitations. While the first part of the work was a retrospective study, the other four parts were clinical trials, performed on small patient samples without control groups. More extensive studies are needed before conclusions can be made with more confidence.

5.2. Long-term effects of CLO in out-patients with treatment-resistant schizophrenia
Our naturalistic study on CLO-treated out-patients with chronic schizophrenia represents possibly one of the longest (up to 15 years) published observations on this kind of patient population. Of the patients treated with CLO for more than one year, nearly 80% were able to be discharged from the hospital in 2.3/2.2 (0.1 – 9.0) years, which is in agreement with a previous Finnish report (Kuoppasalmi et al., 1993). The shift to CLO led in the present study to a desirable turning point in the previously unfavorable course of the disease, as illustrated by increased yearly hospitalization time prior to the shift and its gradual decrease after one year of CLO treatment. For those eight patients who continued on CLO for 10 years or more, no re-hospitalizations were any longer needed. These findings are in line with those from a retrospective study by Connelly and Fullick (1998), whose CLO-treated out-patients’ hospital days dropped from 7.9 to 1.8 for comparable time periods. It is unlikely that the higher proportion of at least moderately improved patients in our study (almost 90%, versus the 63% of Connelly and Fullick, 1998) was due to our longer follow-up, since the duration of CLO treatment in our patients did not correlate with their clinical outcome. Possible explanations may involve different patient samples and schizophrenia subtypes. Indeed, Leppig et al. (1989) reported marked or almost complete improvement in 78% of their retrospectively studied 69 out-patients with schizophrenia, which corresponds relatively closely with our results.

While all our patients with paranoid schizophrenia after the transfer to CLO improved enough to be discharged from the hospital, the hebephrenic patients fell in two groups with clearly different courses. One group of 27 patients evidenced – unexpectedly, due to the notoriously poor prognosis of this type of schizophrenia – even a more pronounced clinical and social improvement than did the non-hebephrenic patients, while another five patients remained chronically psychotic in-patients. Thus, CLO seems to make it possible to distinguish two subtypes of chronic schizophrenia – one to whom CLO was especially beneficial, and another, possibly
the so-called devastating subtype (Kaplan and Sadock, 1995), showing no response to any medication. The clinical or theoretical relevance of this finding is thus far unclear.

Prolonged hospitalization for out-patients after the shift to CLO was, in the present study, associated with better social outcome - perhaps because a more intensive rehabilitation effort became possible in this patient subgroup. This explanation is supported by a recent report by Rosenheck et al. (1998); their CLO-treated patients were more likely to participate in psychosocial treatment, which augmented independently the pharmaocotherapeutic benefit of CLO at 12 months both in reduced symptoms and in improved quality of life.

Our finding of a more pronounced clinical and social improvement in patients of a younger age at the onset of the disease shows a discrepancy with the generally known age-outcome predictor (Frangou and Murray, 1996). In this sample this discrepancy was statistically explained by the hebephrenic patients, who experienced a more noticeable improvement. However, Breier et al. (1994) also found an association of younger age at first psychotic symptoms with good response to CLO in out-patients. Hence, those authors might have dealt with a similar patient subgroup.

Unlike clinical outcome, social outcome was directly associated with the duration of CLO treatment. This supports the suggestion of Meltzer (1989b) as to the necessity of a prolonged (up to one year) CLO trial for each individual patient, and that of the Swedish group (Lindström, 1988; Lindström & Lundberg, 1995) as to the need for a separate follow-up of social outcome, which chronologically may be delayed after clinical outcome.

Duration of illness before CLO in our study was not associated with clinical outcome. This was not surprising in this set of patients with a mean duration of illness of 11.8 years (in the out-patient group, 13.1 years), i.e., far longer than the proposed period of an active brain process (McGlashan and Fenton, 1993). However, the patients in the Swedish studies (Lindström, 1988; Lindström and Lundberg, 1995), for whom CLO was started earlier in the course of the disease (8.7 and 9.6 years), had a much higher rate of employment (up to 40%) than our patients. This indirectly speaks in favor of an earlier introduction of CLO, proposed by some authors (Lieberman, 1996; Edwards, 1998).

5.3. CLO in early treatment-resistant schizophrenia

In contrast to earlier CLO studies on treatment-resistant schizophrenia, including our own (I), duration of illness in our 10 patients with early signs of treatment-resistance showed inverse correlations with clinical improvement (r = -0.639, p = 0.047* for the positive PANSS scores, with a similar trend for the total PANSS scores). It is plausible that such a correlation can be observed only within the first years of
psychosis, which is in line with the theory of the neurotoxicity of the initial period of the schizophrenic process. While the proportion of CLO-responders in treatment-resistant schizophrenia remains between 30 and 60% (see 1.4.), nine of ten patients in this study responded to CLO medication. The effects of CLO in our patients resembled those of conventional neuroleptics in drug-naïve patients in terms of both lower sufficient antipsychotic doses and of enhanced incidence of side-effects.

The minimum standard for an unsatisfactory response to neuroleptic drugs has not been established. It has been proposed that if even moderate social impairment, persistent negative symptoms, and mild-to-moderate positive symptoms are present despite treatment with neuroleptics, CLO should be considered, because CLO should produce clinically significant benefits in the majority of such patients (Meltzer, 1998). This study was focused on the duration of active psychosis and number of previous neuroleptic trials rather than on the number of previous episodes. This is in contrast to a growing body of research on first-episode schizophrenia, in which duration of illness has not been delimited. Indeed, CLO-treated first-episode patients studied until now have been ill for many years (e.g., 4.4 years in the study of Szymanski et al., 1994) and have undergone numerous conventional neuroleptic trials, missing possibly the optimal period of the disease, when CLO might interrupt the development of treatment-resistance. There are few CLO studies with a duration of psychosis of less than 3.0 years. The patients of Claghorn et al. (1987) were ill for 2.0 years but were neuroleptic-intolerant rather than neuroleptic-resistant. The patients of Singer and Law (1974) with a duration of illness of 2.5 years were acute patients, not selected as non-responders to other medications. Thus, our patients may represent a population not investigated earlier. The new term “early treatment-resistance” is here introduced, although it needs further clarification and validation before it can be used in clinical practice for the initiation of treatment with CLO.

5.4. Adjunctive nefazodone in schizophrenia with predominantly negative and depressive symptoms

In this clinical trial of add-on nefazodone in eight patients with difficult-to-treat post-psychotic schizophrenia with predominantly negative and/or depressive symptoms, clear-cut clinical improvement in terms of positive, negative, depressive, and extrapyramidal symptoms and quality of life was observed. Sexual dysfunctions also showed a tendency to improve. Whereas doses of concomitant conventional neuroleptics could be successfully decreased in most, and benzodiazepines discontinued in all cases, anxiety observed in some patients (including panic attacks in two) abated completely. Clinical improvement occurred mostly within the initial 6
weeks, the phase of a stable neuroleptic dose regimen. The complete attenuation of residual positive symptoms despite the reduced doses of neuroleptics during the trial probably indicates an enhancement of the antipsychotic effect and/or improved tolerability of conventional neuroleptics caused by adjunctive nefazodone. This is in line with the hypothetical pharmacodynamic parallel between the drug combinations studied and the CLO-like atypicality of SDA-neuroleptics, i.e., a high 5HT2/D2 inhibition ratio, with an additional increase in serotonin and noradrenaline turnover. A modest increase in the serum levels of neuroleptics as the pharmacokinetic background of the improved outcome cannot be ruled out (Barbhaiya et al., 1996). However, this seems unlikely, since nefazodone, a potent inhibitor of the cytochrome P-450 (CYP) isoenzyme 3A4, is only a weak inhibitor of the CYP2D6 isoenzyme, which is central in the metabolism of conventional neuroleptics (Owen and Nemeroff, 1998). Moreover, the attenuation of the side-effects of neuroleptics observed during co-administration of nefazodone in the present study provides support for the pharmacodynamic rather than the pharmacokinetic interpretation.

It seems that in clinical practice, add-on nefazodone can become a treatment option, e.g., when the psychiatrist and the patient are reluctant to undertake the risk of a shift to a different neuroleptic in cases with well-controlled psychosis but negative or depressive symptoms remaining. Adjunctive nefazodone can possibly be considered also in more active psychotic states. Liver function monitoring is, however, justified during nefazodone treatment, since some patients may experience idiosyncratic, potentially fatal liver damage (Aranda-Michel et al., 1999).

5.5. CLO and production of ROM by monocytes in treatment-resistant schizophrenia

CLO treatment led to in vivo concentration-dependent changes in the ROM production by MO (but not PMNL) during the first 3 weeks of medication in our eight treatment-resistant schizophrenic patients. Serum concentrations of CLO at week 3 correlated even more powerfully with subsequent (at week 10) changes in the ROM production by MO. The changes in the ROM production by MON and MOs also demonstrated several positive correlations with the decrease in clinical rating scale scores measuring psychotic (PANSS), but not depressive (MADRS) symptomatology. These correlations imply that a decrease or relatively small increase in the ROM production by MON and MOs rather than a clear-cut increase was associated both with more favorable clinical outcome, and at week 3 with higher concentrations of CLO. Interestingly, CLO concentrations exceeding at week 3 the therapeutic level of 350 ng/ml (Jann et al., 1993; Potkin et al., 1994) were associated with a tendency to decrease, while concentrations below this level corresponded instead with an increase
in the ROM production by MOs. Thus, CLO may have a biphasic concentration-dependent effect, in which relatively low serum concentrations of the drug are associated with an increase, but the high therapeutic concentrations with a decrease in ROM production. It is possible that CLO can modulate the ROM production by MO in the same fashion as melatonin modulates that of PMNL, i.e., dampens it in high but stimulates it in low concentrations (Pierl et al., 1998).

Our separate experiment revealed a clear-cut in vitro CLO-induced decrease in the ROM production by MOs in these eight patients with treatment-resistant schizophrenia throughout the CLO trial. The grade of sensitivity of MOs to this effect at the baseline of the CLO trial predicted subsequent clinical response to CLO medication. This connection was mostly due to negative symptoms.

Our findings are not directly comparable with those of McAdams and Leonard (1993) (see 1.7.3), since the patients of that Irish group were not treatment-resistant, and all their neuroleptics were of the conventional type. In addition, their experiments (also the in vitro one) considered only zymosan-stimulated cells. We failed to find in the literature any other studies on this topic.

The results of the present study support the hypothesis of an association between the ROM production by mononuclear phagocytes, neurotoxicity in schizophrenia, and the distinct efficacy of CLO against negative symptoms and treatment-resistance. It appears that the unique clinical properties of CLO may be exerted partly via modulation of the ROM production by MO.
6. CONCLUSIONS

6.1. CLO may be especially beneficial for a substantial subgroup of treatment-resistant patients with disorganized schizophrenia, especially in combination with a sufficiently long hospitalization, allowing intensive rehabilitation. Despite the high CLO cost and blood monitoring expense, CLO medication is presumably cost-saving because of the decreased need for re-hospitalization.

6.2. CLO in early treatment-resistant schizophrenia is safe and effective, and lower doses may suffice. A change in the current practice towards introduction of CLO earlier in the course of the disease may be desirable to gain all possible benefits.

6.3. Add-on nefazodone appears to enhance the antipsychotic efficacy of conventional neuroleptics and counter their side-effects – presumably due to the CLO-like postsynaptic pharmacodynamic profile of such a combination. Hence, adjunctive nefazodone may become a valuable treatment option, e.g., when the psychiatrist or patient is reluctant to undertake the risk of a shift to a different neuroleptic in a well-controlled psychosis with difficult-to-treat negative or depressive symptoms remaining.

6.4. IV. In vivo, after 3 weeks of treatment, therapeutic serum concentrations of CLO seem to dampen the production of ROM by MO, and this effect correlates positively with clinical outcome in treatment-resistant schizophrenia. Modulation of the ROM production by MO appears to have an impact in the mechanism of action of CLO.

6.5. V. In vitro, CLO reduces the ROM production by the non-stimulated MO, and the degree of this effect may be predictable for clinical outcome, a finding which may have important clinical implications.
7. SUMMARY

Treatment-resistant schizophrenia is still an unresolved problem. Today, CLO is the only drug with established efficacy in treatment-resistant schizophrenia. Unfortunately, it may cause life-threatening agranulocytosis. The mechanisms of the clinical action of CLO and the pathophysiology of CLO-induced agranulocytosis are still poorly understood; neither is it clear whether these are interrelated. Studying CLO may bring us nearer to the understanding of schizophrenia and treatment-resistance and discovery of new, better compounds or drug treatment strategies.

A series of studies was undertaken with CLO or a CLO-like drug combination in schizophrenic patients who did not respond optimally to conventional neuroleptic medication.

In a long-term (up to almost 15 years) naturalistic retrospective follow-up of 39 outpatients, with a small control group of in-patients, an earlier finding was replicated that CLO substantially decreases the need for hospitalization in treatment-resistant schizophrenia. CLO treatment was thus presumably cost-saving. Prolonged duration of hospitalization after the start of CLO was associated with better social outcome, plausibly due to the more intensive rehabilitation permitted by CLO. Furthermore, duration of CLO treatment correlated positively also with social improvement. Surprisingly, the out-patients with disorganized schizophrenia (hebephrenic schizophrenia according to the Finnish version of DSM-III-R) displayed more noticeable improvement than did those with other types of schizophrenia. All the severely ill chronic in-patients suffered also from hebephrenic schizophrenia, probably pointing to the heterogeneity of this diagnostic category.

Since schizophrenia is evidently not only a neurodevelopmental but also a neurotoxic disease, the most effective intervention, used as early as possible, is essential for a good outcome. A prospective open CLO study was performed in “early treatment-resistant schizophrenia”. Nine out of 10 patients with the first emerging signs of resistance to their conventional neuroleptics responded to CLO, in contrast to the 30 to 60% reported in earlier CLO studies of rigorously established treatment-resistant schizophrenia. Duration of psychosis prior to CLO correlated inversely with clinical improvement. This study advocates transfer to CLO earlier in the course of schizophrenia than is the general practice today.
Nefazodone is a novel antidepressant which, in addition to increasing serotonin turnover, also inhibits postsynaptic 5HT2 receptors in a CLO-like fashion. Addition of nefazodone to conventional neuroleptics in our prospective open study appeared to influence favorably the course of the disease and diminish side-effects of the neuroleptics in eight patients with mainly postpsychotic schizophrenia and prominent negative and depressive symptoms. After the acute phase of the study, during which the study protocol required the doses of neuroleptics to remain stable, adjunctive nefazodone allowed us to substantially diminish the neuroleptic dosage. Adjunctive nefazodone may become a valuable treatment option, for instance, when a shift to a different neuroleptic is risky in a patient with a well-controlled psychosis but with remaining difficult-to-treat negative or depressive symptoms.

Both the immune system, including mononuclear leukocytes, and free radicals (mainly ROM) have been proposed as underlying the pathophysiology and possibly the neurotoxicity of schizophrenia and treatment-resistance. Since human phagocytes are able to produce ROM, they may be a link between the immune and free radical theories. CLO appears to be not only the most potent neuroleptic, but also the most dangerous in terms of hematological side-effects. In contact with phagocytes, CLO is involved in a complex free radical interplay. We hypothesized that the desirable and hazardous features of CLO are interrelated and are exerted partly via modulation of the production of ROM by phagocytes. Unlike microglia/brain macrophages, mature descendants of MO, peripheral blood MO from schizophrenic patients are easily available for exploration. Eight patients with treatment-resistant schizophrenia were studied prospectively before and after their shift to CLO. The ROM production by their blood phagocytes was measured and clinical assessments were performed simultaneously before and during the CLO trial. The serum concentrations of CLO at week 3 correlated positively with the changes in the ROM production by MO, i.e., high concentrations were associated with a clear-cut decrease in the ROM production. Furthermore, this decrease in ROM production was associated with a decrease in psychotic symptoms. In addition, CLO dampened the ROM production by non-stimulated MO in vitro, and the degree of this effect at baseline predicted subsequent clinical outcome. Thus, these data support our hypothesis and may have clinical implications.

All the studies in this series have some methodological limitations, which makes additional investigations necessary. However, our work has outlined some novel theoretical and practical approaches which may be of interest to the research community.
8. ACKNOWLEDGEMENTS

First and foremost, I am thankful to all the patients who participated in our work for remaining with us throughout all the troublesome research procedures.

I wish to express my sincere gratitude to Professor Ranan Rimón, chief of our department, who introduced me not only to research in the sphere of psychopharmacology but also to the realm of modern psychiatry in its entirety. He has been generous in generating the directions of the work and has taught me much about scientific thinking and writing.

I am deeply indebted to my supervisor and co-author, Acting Professor Björn Appelberg for the most constructive and amicable guidance throughout all the phases of this work. Without him I would undoubtedly have given up many times, and this thesis would never have been completed.

I am grateful to my supervisor Docent Esa Leinonen for his comments and his attitude, both critical and warm.

I wish to express my warmest thanks to Professor Ulf-Göran Ahlfors and Docent Kimmo Kuopposalmi, the referees of this dissertation, for their most welcome constructive criticism.

Professor Brian Leonard, Dr. Cai Song and Dr. Peter Nyberg have introduced me to the exciting field of psychoneuroimmunology and taught me the laboratory techniques used in this work, for which I am deeply indebted.

Doctor Peter Nyberg has also been one of my co-authors, and I appreciate very much his invaluable contributions. I also express my deepest gratitude to all my other co-workers in Finland and Russia.

I thank Docent Heikki Katila for his very instrumental impact in ideation of the immunological branch of the study in the preliminary phase of the work.

I want also to sincerely thank Novartis OY (former Sandoz OY), which for me has been personified in Rainer Gädeke (also one of my co-authors) and Outi Wilén. Without the gift of the clozapine preparation, a substantial part of the work would have been impossible, and without financial support the realization of part of the study abroad as well as several presentations of our preliminary results in international research circles would have been difficult.
My special thanks to Bristol-Myers Squibb OY and personally to Docent Timo Muhonen for the open-handed gift of nefazodone and the financial support which made it possible to present our results to the international research community.

I express my gratitude to the foundation Liv och Hälsa and the Mjölbolsta Foundation for Medical Research for funding a substantial part of the laboratory expenses.

I want to thank sincerely pharmaceutical companies Janssen-Cilag OY, Eli Lilly Finland OY, Wyeth Lederle Finland, and Organon OY for financial support.

I wish to cordially acknowledge the hospital staff of Ekäslen hospital (Ekenäs, Finland) and Departments of Psychiatry of the Universities of Helsinki (Finland) and Petrozavodsk (Russia) for their selfless cooperation. Dr. Kaj Palmgren and Professor Mark Burkin, Chiefs of Ekäslen Hospital and the Department of Psychiatry of the University of Petrozavodsk are warmly acknowledged for their help and kind companionship.

I warmly thank librarian Anja Roilas, whose help was inestimable.

For editing of these theses in a very constructive and fascinating atmosphere I am greatly indebted to Dr. Carol Norris.

I also desire to acknowledge you, Nadezhda Bronzova, for the wonderful illustrations to this book.

Last but not least, I am very happy to have been encouraged and lovingly supported during all these years by my patient family - my wife Marina, daughter Polina, and son Leo, as well as my mother Eva and sister Eleonora.
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APPENDIX 1. REACTIVE OXYGEN METABOLITES, ANTIOXIDANT DEFENCE SYSTEMS, AND CHEMILUMINESCENCE

1. REACTIVE OXYGEN METABOLITES AND ANTIOXIDANT DEFENCE SYSTEMS

1. BASIC CHEMISTRY

In the cell aerobic metabolism oxygen is oxidized by mitochondrial cytochrome to \( H_2O \) (Reaction 1):

\[ \text{O}_2 + 4\text{H}^+ + 4\text{e}^- \rightarrow 2\text{H}_2\text{O} \]

(Weiss, 1986)

Electrons may often escape (due to, for instance, an error in mitochondria) from the electron transport chain and react with molecular oxygen with formation of a superoxide radical (Reaction 2):

\[ \text{O}_2 + \text{e}^- \rightarrow \text{O}_2^- \]

(Mahadik and Scheffer, 1996)

The superoxide radical is rapidly converted to hydrogen peroxide by widely distributed superoxide dismutases (Reaction 3):

\[ 2\text{O}_2^- + 2\text{H} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

(Fridovich, 1983)

\( \text{H}_2\text{O}_2 \) is further reduced to a hydroxyl radical in either

1) the (very) slow Haber-Weiss reaction (Reaction 4):

\[ 2\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{HO} + \text{OH}^- + \text{O}_2 \]

(Beauchamp and Fridovich, 1970)

or

2) its rapid modification, the Fenton reaction, that occurs in the presence of transitional metals (“Fenton reagents”), such as iron or copper (Reaction 5):

\[ \text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{HO} + \text{OH}^- \]

(Weiss, 1986)

The superoxide radical can also be modified in the xantine oxidase-dependent degradation of hypoxantine to xantine and further to uric acid (Reactions 6 and 7):

hypoxantine + \( \text{H}_2\text{O}_2 \rightarrow \text{xantine} + 2\text{O}_2^- + 2\text{H}^+ \)

xantine + \( \text{H}_2\text{O}_2 \rightarrow \text{uric acid} + 2\text{O}_2^- + 2\text{H}^+ \)

(Weiss, 1986)

A membrane-bound NADPH oxidase is a phagocyte-specific enzyme complex. It is dormant in resting phagocytes, but following stimulation (with, for instance, phagocytosis or phorbol myristate acetate, PMA, a direct NADPH oxidase stimulant) it triggers a series of metabolic events resulting in a massive production of hydrogen peroxide, superoxide anion, hydroxyl radical, and singlet oxygen (Bellavite, 1988).
This phenomenon, for historical reasons referred to as the “respiratory burst” is crucial for the microbe- and tumor-cell killing by phagocytes (Babior, 1978). In this reaction NADPH serves as a donor of electrons (Reaction 8):

\[ \text{NADPH} + 2\text{O}_2 + \text{H}^+ \rightarrow \text{NADP}^+ + 2\text{O}_2^- + 2\text{H}^+ \]

(Bellavite, 1988)

In addition, phagocytes can also produce hypochlorous acid through the action of the phagocyte-derived enzyme myeloperoxidase (Reaction 9):

\[ \text{H}_2\text{O}_2 + \text{Cl}^- + \text{H}^+ \rightarrow \text{HOCl} + \text{H}_2\text{O} \]

(Weiss, 1989)

Hydrogen peroxide, myeloperoxidase, and chloride are known to constitute a most potent “cytad” system toward microbes, parasites, or tumor cells (Klebanoff and Clark, 1978). In phagocytes this reaction occurs in the extracellular space or in phagosomes (Karnovsky and Badwey, 1983), with no antioxidant enzymes available for its control. The magnitude of the ROM production by phagocytes depends on the species of animal, the cell type (e.g., neutrophil, macrophage) and state (“normal”, “elicited” i.e., inflammatory, “activated”, resting or stimulated), the nature of the stimulus, the interplay of enzymes and substrates and so forth (Karnovsky and Badwey, 1983).

ROM, if not ameliorated by antioxidant defence systems (vide infra) induce chain reactions of lipid peroxidation (Reactions 10 – 12):

- lipid-H + radical → lipid' + radical-H
- lipid' + O_2 → lipid-O_2
- lipid-O_2' + lipid-H → lipid-O_2H + lipid

(Mahadik and Scheffer, 1996)

2. ANTIOXIDANT DEFENCE SYSTEMS

The main antioxidant pathways are shown in Figure 1.

PREVENTIVE ANTIOXIDANTS

- Transferrin (lactoferrin in milk), caeruloplasmin, and albumin bind transition metals (“sequestration”) (Maxwell, 1995)

ANTIOXIDANT ENZYMES

- Superoxide dismutase is a metalloprotein that facilitates the dismutation of superoxide anion to hydrogen peroxide (vide supra, Reaction 3)
- Catalase is a hem-enzyme that catalyzes the formation of water and oxygen from hydrogen peroxide:
  \[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]
  (Weiss, 1986)
Glutathione peroxidase is a seleno-enzyme that removes \( \text{H}_2\text{O}_2 \) at the expense of reduced glutathione (GSH) with the formation of oxidized glutathione disulfide (GSSG):

\[
\text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow 2 \text{H}_2\text{O} + \text{GSSG}
\]

(Maxwell, 1995)

**Main antioxidant defense systems**

- \( \text{O}_2^- \): superoxide radical
- \( \text{H}_2\text{O}_2 \): hydrogen peroxide
- \( \text{HO} \): hydroxyl radical
- \( \text{CAT} \): catalase
- \( \text{GSH-Px} \), glutathione peroxidase
- \( \text{SOD} \): superoxide dismutase

**Preventive antioxidants**
- Bind transitional metal ions ("sequestration"), preventing their reaction with \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) to produce toxic hydroxyl radicals.
- Enzymes \( \text{SOD}, \text{CAT}, \) and \( \text{GSH-Px} \) facilitate breakdown of radicals in the intracellular space.

**Sacrificial antioxidants**
- Become oxidized in reaction with toxic radicals to become relatively inactive ones.

There are no enzymes known to control the levels of hydroxyl radicals, and the role of the three enzymes above is to prevent the interaction of superoxide, hydrogen peroxide, and transitional metals, thus preventing the subsequent formation of highly toxic hydroxyl radicals (Maxwell, 1995). Superoxide dismutase, catalase, and glutathione peroxidase exist in the intracellular environment, whereas extracellular space is not armed with enzymatic scavengers of ROM (Weiss, 1986).

**SCAVENGING ("CHAIN-BREAKING", "SACRIFICAL") ANTIOXIDANTS**

Scavenging antioxidants are powerful electron donors. They are oxidized by toxic free radicals with the formation of relatively inactive ones before more vital structures are damaged. Scavenging antioxidants are often divided into water-soluble, such as ascorbic (vitamin C) and uric acids, bilirubin, and thiols, and the lipid-soluble, such as tocopherol, \( \beta \)-carotene, and ubiquinol-10 (Maxwell, 1995).
II. LUMINOL-DEPENDENT CHEMILUMINESCENCE AS AN INSTRUMENT FOR MEASUREMENT OF PRODUCTION OF REACTIVE OXYGEN METABOLITES BY PHAGOCYTES

The respiratory burst of phagocytes is accompanied by emission of small, but measurable amounts of photons of light (chemiluminescence) (Allen et al., 1972; Bellavite, 1988). The cyclic hydrazide luminol is an easily oxidizable ("chemilumigenic") substance which produces light of high intensity in contact with activated phagocytes, indicating the activity of the respiratory burst (Allen and Loose, 1976). Luminol has been used in a chemiluminescence assay for the hypochlorite produced in the myeloperoxidase-catalyzed formation of hydrogen peroxide (Reaction 9) (DeChatelet et al., 1982). However, in phagocytes, hydrogen peroxide is formed in the dismutation reaction of the superoxide radical (Reaction 8). Indeed, it has been shown earlier that luminol-dependent chemiluminescence from human mononuclear phagocytes is completely inhibited by superoxide dismutase (Nyberg and Klockars, 1990). This suggests that luminol-dependent chemiluminescence in fact measures the superoxide production dependent on the NADPH oxidase – myeloperoxidase system of phagocytes, and thus is phagocyte-specific.

To facilitate phagocyte reactions, phagocyte-stimulation has been used in most experiments. Phorbol esters, such as phorbol myristate acetate (PMA), potently activate NADPH oxidase directly through an interaction with the signal transduction system with no phagocytosis needed (in contrast to phagocytizable particles, e.g., opsonized zymozane), which leads to progressive and irreversible activation of the respiratory burst Bellavite, 1988).
## APPENDIX 2. Main results of studies I – V, mean (I, II, and III) or median (IV and V) (range)

<table>
<thead>
<tr>
<th>Study I. Neuroleptic-resistant schizophrenia, Out-patients on CLO for up to 15 years (n = 39). A retrospective follow-up</th>
<th>Helephrenic schizophrenia (n = 27)</th>
<th>Non-helephrenic schizophrenia (n = 12)</th>
<th>Statistics, helephrenic vs. non-helephrenic</th>
<th>Duration of hospitalization during CLO and social improvement (positive correlations(^1))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical improvement</td>
<td>3.6 (2 – 54)</td>
<td>3.1 (2 – 4)</td>
<td>U = 226.0, p = 0.0032*</td>
<td>r = 0.372, p = 0.020</td>
</tr>
<tr>
<td>Social improvement</td>
<td>3.2 (1 – 4)</td>
<td>2.5 (1 – 4)</td>
<td>U = 233.4, p = 0.024*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study II. Early neuroleptic-resistant schizophrenia, a prospective 26-week CLO trial (n = 10)</th>
<th>Week 0 (baseline)</th>
<th>Week 26 (end-point)</th>
<th>Change from baseline, %</th>
<th>Duration of illness (DI) and reduction of positive PANSS (inverse correlations(^1)): r = -0.639, p = 0.047; DI (years) was longer in poor vs. good responders: 3.25 (3.0 – 3.8) (fair) vs. 1.5 (0.9 – 1.0) (good)</th>
</tr>
</thead>
<tbody>
<tr>
<td>total PANSS</td>
<td>54.5 (38 – 65)</td>
<td>37.6 (25 – 49)</td>
<td>31.0 (21.0 – 51.0) %</td>
<td></td>
</tr>
<tr>
<td>Quality of Life Scale</td>
<td>57.1 (21 – 79)</td>
<td>62.7 (33 – 89)</td>
<td>81.7 (181.0 – 6.3) %</td>
<td></td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Study III. Neuroleptic-treated schizophrenia with mainly negative symptoms, a prospective 26 week add-on nefazodone trial (n = 8)</th>
<th>Week 0</th>
<th>Week 6</th>
<th>Week 6 vs. week 6, %</th>
<th>Positive symptoms, observed in 3 patients and panic attacks in 2 patients entirely disappeared in all cases. Doses of neuroleptics could have been significantly decreased in most and benzodiazepines discontinued in all cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>total PANSS</td>
<td>64 (48 – 89)</td>
<td>45 (33 – 63)</td>
<td>29 (13 – 61) %, (*)</td>
<td></td>
</tr>
<tr>
<td>MADRS</td>
<td>15 (8 – 29)</td>
<td>5 (0 – 11)</td>
<td>63 (18 – 100) %, (*)</td>
<td></td>
</tr>
<tr>
<td>SAS (extrapyramidal symptoms)</td>
<td>4 (0 – 9)</td>
<td>3 (0 – 7)</td>
<td>43 (0 – 100) %, (*)</td>
<td></td>
</tr>
<tr>
<td>UKU (several dysfunctions)</td>
<td>4 (0 – 11)</td>
<td>3 (0 – 11)</td>
<td>38 (0 – 100) % (NS)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Studies IV and V. Neuroleptic-resistant schizophrenia, a prospective 10 week CLO trial (n = 8)</th>
<th>Week 0</th>
<th>Week 3</th>
<th>Statistics: week 3 vs. week 0</th>
<th>IV B. Concentrations of CLO and changes in ROM production, (correlations(^2) at week 3) MOn: r = 0.092, p = 0.005 MOn: r = 0.761, p = 0.007</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV A. Non-stimulated monocytes (MOn), in vivo</td>
<td>311 (93 – 1438)</td>
<td>500 (140 – 1445)</td>
<td>NS</td>
<td>IV C. Changes in ROM production and in total PANSS, correlations(^2) at week 3 MOn: r = 0.743, p = 0.035 MOn: r = 0.838, p = 0.009**</td>
</tr>
<tr>
<td>PMA-stimulated monocytes (MOn), in vivo</td>
<td>518 (150 – 2228)</td>
<td>771 (198 – 1576)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

| V A. MOn + solvent (in vitro) | 291 (89 – 1342) | 569 (126 – 1348) | CLO vs. CLO + solvent, week 0 and 3, respectively 0.017*, 0.012* |
| MOn + solvent + CLO (in vitro) | 242 (67 – 1156) | 433 (100 – 1226) |

\(^*\) = significance level < 0.05  
\(^*\) = significance level < 0.01  

\(^1\) Longer duration of hospitalization corresponded with a more pronounced improvement  
\(^2\) Longer duration of illness corresponded with less pronounced improvement  
\(^3\) Higher concentrations of CLO corresponded with a clear-cut decrease, but lower with an increase in ROM production  
\(^4\) Decrease in ROM production was associated with a more, but an increase with a less noticeable reduction in PANSS scores  
\(^5\) More pronounced decrease in ROM production was associated with a more noticeable reduction in PANSS scores