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SPECIAL ISSUE

Norbert Müller · Markus Ulmschneider · Constanze Scheppach · Markus J. Schwarz · Manfred Ackenheil · Hans-Jürgen Möller · Rudolf Gruber · Michael Riedel

COX-2 inhibition as a treatment approach in schizophrenia: Immunological considerations and clinical effects of celecoxib add-on therapy

Abstract Recent advances in immunological research regarding the differentiation between the type-1 and type-2 immune response are discussed. Increased levels of Interleukin-6 (IL-6) and the activation of the IL-6 system in schizophrenia might be the result of the activation of type-2 monocytes/macrophages, too. On the contrary, several parameters of the specific cellular immune system are blunted, e. g. the decreased type-1 related immune parameters in schizophrenic patients. This study was performed as a double-blind, placebo-controlled, randomized evaluation of risperidone and celecoxib versus risperidone and placebo. Fifty schizophrenic patients were included in the study: 25 patients received risperidone and placebo, and 25 patients received risperidone and celecoxib for 5 weeks after the wash-out period. The treatment effect was calculated by ANCOVA. In parallel, serum levels of sTNF-R1 and sIL-2R, and the percentages of $CD3^+$-, $CD4^+$-, and $CD19^+$ lymphocytes were estimated. As expected, both groups of schizophrenic patients showed significant improvement. However, the celecoxib add-on therapy group showed a significant group effect in the PANSS total score. The cytokines and lymphocytes reflected the type-1/type-2 balancing effects of COX-2 inhibitors. Additional treatment with celecoxib has significant positive effects on the therapeutic action of risperidone with regard to the total schizophrenia psychopathology. Moreover, the fact that treatment with an immunomodulatory drug shows beneficial effects on the symptomatology of schizophrenia indicates that immune dysfunction in schizophrenia is not just an epiphenomenon, but related to the pathomechanism of the disorder.

Key words COX-2 · immunology · schizophrenia · psychosis · inflammation

Introduction

Inflammation is a phenomenon of the immune response. The immune system recognizes foreign antigens and differentiates between self and non-self. During an inflammatory reaction normally non-self is eliminated by means of the immune system. In certain cases inflammation changes from a clearing reaction of the immune system providing homeostasis to an organism-injuring process. Such an injurious process can result from an acute inflammation, a chronic inflammation or an autoimmune reaction.

Psychiatric symptoms, especially schizophreniform and depressive symptoms, have been described both during inflammation and during different types of autoimmune disorders involving the CNS. These observations led to the suggestion that inflammation may be an important pathogenetic mechanism underlying schizophrenia. Our own investigations showed an inflammatory process in at least a subgroup of schizophrenic patients: signs of inflammation have been found in the cerebrospinal fluid as well as in postmortem CNS tissue of schizophrenic patients (Wildenauer et al. 1990; Körschenhausen et al. 1996).

Prof. Dr. med. Dipl.-Psych. N. Müller (✉) · M. Ulmschneider · C. Scheppach · M. J. Schwarz · M. Ackenheil · H. J. Möller · M. Riedel
Hospital for Psychiatry and Psychotherapy
Ludwig-Maximilians-Universität Nußbaumstr. 7
80336 München, Germany
Tel.: +49-89/51603397
Fax: +49-89/51604548
E-Mail: nmueller@psy.med.uni-muenchen.de

R. Gruber
Hospital for Psychiatry and Psychotherapy
and Institute of Immunology
Ludwig-Maximilians-Universität
München, Germany

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CNS development, infection, and cytokines in the CNS

Findings from animal studies elucidate the relationship between the increased risk for schizophrenia after infection and the disturbed development of schizophrenic brains. It has been shown that, e. g., prenatal influenza infection leads to a disturbed migration of cortico-hippocampal neurons. This might be due to a decreased expression of Reelin caused by the infection (Cotter et al. 1995). The reduction of Reelin expressing neurons in the brain of schizophrenic patients has been shown by various groups of researchers (Impagnatiello et al. 1998). Further indications for a disturbed neuronal migration can be seen in experimental pre- or peri-natal infections with the murine cytomegalovirus (Kosugi et al. 2000), mumps virus (Rubin et al. 1998), and the Parvo-Virus (Ramirez et al. 1996). Furthermore, a direct influence of infections on the balance of neurotransmitters could be shown. Already in 1975 it was observed that a viral infection in new born mice can lead to a disturbance of the catecholaminergic metabolism in the brain (Lycke and Roos 1975). It is known today that several different viruses, such as the influenza virus, can alter the monoaminergic balance within the brain after experimental infection. In particular, an activation of the serotonergic and noradrenergic system has been described.

Cytokines are known to act on the noradrenergic, serotonergic, and dopaminergic neurotransmission. For example, animal experiments show that IL-6 increases the dopaminergic neurotransmission in the hippocampus, but also IL-2 increases the hippocampal serotonergic and dopaminergic neurotransmission (Zalcman 1994). IL-1 and IL-6 both stimulate hormones of the HPA-axis through stimulation of hypothalamic structures in the CNS. These effects of the cytokines may contribute to the effects of (viral) infection and inflammation to the serotonergic, noradrenergic, and dopaminergic neurotransmission and to clinical symptoms of psychiatric disorders such as schizophrenia and depression. Moreover, these cytokines mediate so-called "sickness behavior" in animal experiments, which is associated with depressed mood, lack of drive, anergia, anorexia, and fever (Dantzer 2001).

Cytokines also play a crucial role in the development of the brain. The effect of interferons and IL-6 on neurons starts very early during brain development, where they regulate neuronal migration and differentiation (Bakhiet et al. 2001; Gadient and Otten 1997). A disturbance of the cytokine production and balance due to infection may thus not only have implications for neurodevelopmental processes but also for the distribution of CNS cells with immunological functions and for the immunological polarization and priming of these cells. Microglial cells and astrocytes in particular have several immunological functions in the CNS and contribute to the type-1 and type-2 immune response in the CNS in parallel to the polarized type-1 and type-2 immune response in the peripheral immune system. Microglia progressively acquire a clear-cut macrophage phenotype in response to CNS injuries (Kreutzberg 1996) and can induce the production of the type-1 cytokine IL-12 (Krakowski and Owens 1997; Stalder et al. 1997) and that of type-2 cytokines such as IL-10 and TGF-β (Aloisi et al. 2000). Astrocytes are also potential sources of TGF-β, which inhibits MHC II and ICAM-1 expression in macrophage/microglia (Hailer et al. 1998). Microglia and astrocytes also secrete chemokines that may affect the recruitment of Th1 and Th2 cells. In the peripheral immune systems, the T-helper cells and macrophages have polarizing and balancing functions, which contribute to activation and down-regulation of the immune system and to different types of inflammation.

Immunomodulation of psychotropic drugs

Pharmacological down-regulation of activating cytokines in the CNS using anti-inflammatory therapy may possibly have favorable effects in some schizophrenic patients. This view is supported by the fact that antipsychotics (Müller et al. 1997a) and possibly atypical antipsychotics in particular have immunomodulatory properties (Maes et al. 1995; Lin et al. 1998) that may lead to a down-regulation of the immune response in the CNS. The effects of clozapine to the peripheral immune system have been studied repeatedly (Pollmächer et al. 2000), while other antipsychotics, including other atypical antipsychotics have not been examined specifically. Moreover, only limited conclusions can be drawn from the peripheral immune system and from in vitro studies to immunological drug effects in the CNS regarding interfering variables such as the blood-brain barrier, the fact that immunocompetence in the CNS is mainly carried by other cells than in the peripheral immune system, etc.

Vaccination treatment in psychosis

One of the pioniers of psychoneuroimmunology was Ritter Julius Wagner von Jauregg, the Nobel prize laureate for medicine in 1927. He developed malaria therapy for syphilis. The immunological mechanism of this therapy is the activation of the immune system by attenuated antigens from strains of malaria in order to recognize and eliminate foreign antigens as non-self of the organism. Long before the development of the malaria therapy for syphilis, Wagner von Jauregg studied the effects of fever therapy in psychosis. The development of the fever therapy was based on observations of the effects of typhus infections – which occurred recurrently as epidemics during the 19th century – on mental illness. In 1887 Wagner von Jauregg published a type of meta-analysis, where he compared the observations of other psychiatrists from various countries during typhus epidemics. These authors published the in-

teresting observation that during typhus epidemics, the mentally ill (psychiatric patients) developed a much lower rate of infections than the "guards" – the nurses during earlier times – did. On average, only 17 % of the mentally ill subjects showed signs of an infection, while signs of an infection were observed in 39 % of the guards (Wagner von Jauregg 1887). That means the guards developed a rate of infection that was twice as high as observed in psychiatric patients.

An even more interesting observation of several authors was that after recovering from the typhus infection some of the patients had also improved with respect to their mental illness. Up to 75 % improved and up to 48 % became mentally healthy after the infection. On average, 48 % of the patients were unchanged after infection, while 32 % were improved and 20 % free from the mental illness. This observation of Wagner von Jauregg and other authors let to the suggestion that certain infections might help with respect to mental illness. Consequently, this became the starting point for the further development of fever therapy. Wagner von Jauregg followed the subject of fever therapy and immune-mediated therapeutic mechanisms in psychosis during his further scientific life and published a paper in 1926 suggesting vaccination therapy in psychosis (Wagner von Jauregg 1926). For fever therapy, attenuated strains of salmonella typhii, of plasmodium malariae and of mycobacterium tuberculosis were used. However, due to the clinical side-effects of vaccination with typhus, malaria, or tuberculosis, in particular to the often uncalculable course of the infection and fever, and later on to the introduction of therapy with neuroleptics, the focus of therapeutic and basic research changed from vaccination and the immune system, to dopaminergic neurotransmission and to neuroleptic drugs. Nevertheless, the common immunological mechanisms of these therapies are of particular interest, since these three infectious agents induce the activation of the type 1 immune response, as we know today (Ramarathinam et al. 1993; Mastroeni et al. 1992; Winkler et al. 1998; Flesch et al. 1995).

Recent immunological studies point to a dysbalance between the type-1 and type-2 immune response in schizophrenia with an over-activation of the type-2 response and a lack of type-1 response. In conclusion, the distinct activation of the type-1 response without causing an infectious disorder would be expected to show advantageous effects in schizophrenia.

One class of modern drugs is well known to induce a shift from the type-1 like to a type-2 dominated immune response: the selective cyclooxygenase-2 inhibitors. Several studies demonstrated the type-2 inducing effect of PGE2 – the major product of COX-2, while inhibition of COX-2 is accompanied by inhibition of type-2 cytokines and induction of type-1 cytokines (Stolina et al. 2000; Pyeon et al. 2000).

Cyclooxygenase-2 inhibition in schizophrenia

Therefore, it seemed meaningful to study the effects of COX-2 inhibition using an add-on design together with a well-proven neuroleptic medication in schizophrenic patients (Müller et al. 2002). Celecoxib is a selective cyclooxygenase-2 (COX-2) inhibitor, which accesses the CNS easily and has few adverse side effects. Risperidone was selected because it is an atypical neuroleptic with high efficacy in the therapy for both positive and negative symptoms of schizophrenia. In addition a wealth of experience with risperidone treatment has been collected to date (Marder and Maibach 1994; Möller et al. 1998).

This study was performed as a prospective single-center, double-blind, placebo-controlled, randomized, add-on, parallel group evaluation of risperidone and celecoxib versus risperidone and placebo. Fifty schizophrenic patients were included in the study; 25 (11 f, 14m) were randomly assigned to the risperidone and celecoxib treatment group, and 25 (14 f, 11m) to the risperidone and placebo group. All patients were hospitalized inpatients due to an acute exacerbation of their schizophrenic psychoses. Sixteen of the patients had been hospitalized for the first time, eight in the celecoxib group and eight in the placebo group.

Clinical outcome of Celecoxib add-on therapy in schizophrenia

The celecoxib add-on therapy had a significant effect on the mean improvement in total PANSS score (between subjects factor *celecoxib* $F = 3.8$, $df = 1;47$, $p = 0.05$). The difference between the two treatment groups was not homogeneous across time (multivariate *celecoxib* by *time* interaction $F = 3.91$, $df = 4;44$, $p = 0.008$). The main effects of celecoxib were seen in the middle of the treatment period (quadratic interaction component $F = 12.5$, $df = 1;47$, $p = 0.001$). In simple posthoc t-tests the difference between the two treatment groups is significant from week 2 ($t = 2.06$, $df = 48$, $p = 0.05$) to week 4 (week 3 $t = 2.64$, $df = 48$, $p = 0.01$, week 4 $t = 2.54$, $df = 48$, $p = 0.01$). Regarding the mean improvement, none of the subscales showed a significant effect (positive symptoms: *celecoxib* $F = 1.74$, $df = 1;47$, $p = 0.19$, negative symptoms: *celecoxib* $F = 2.82$, $df = 1;47$, $p = 0.10$, global subscale: *celecoxib* $F = 3.19$, $df = 1;47$, $p = 0.08$). The quadratic trend in the *celecoxib-time* interaction, however, was present in all subscales (positive symptoms: $F = 4.77$, $df = 1;47$, $p = 0.03$, negative symptoms: $F = 8.86$, $df = 1;47$, $p = 0.005$, global subscale: $F = 6.16$, $df = 1;47$, $p = 0.02$). The celecoxib add-on treatment resulted in an earlier improvement in all subscales (Fig. 1).

According to our hypothesis, the celecoxib add-on therapy group had a significantly better effect on the PANSS total scores. The largest improvements were seen between weeks 2 and 4. In practice this means an earlier

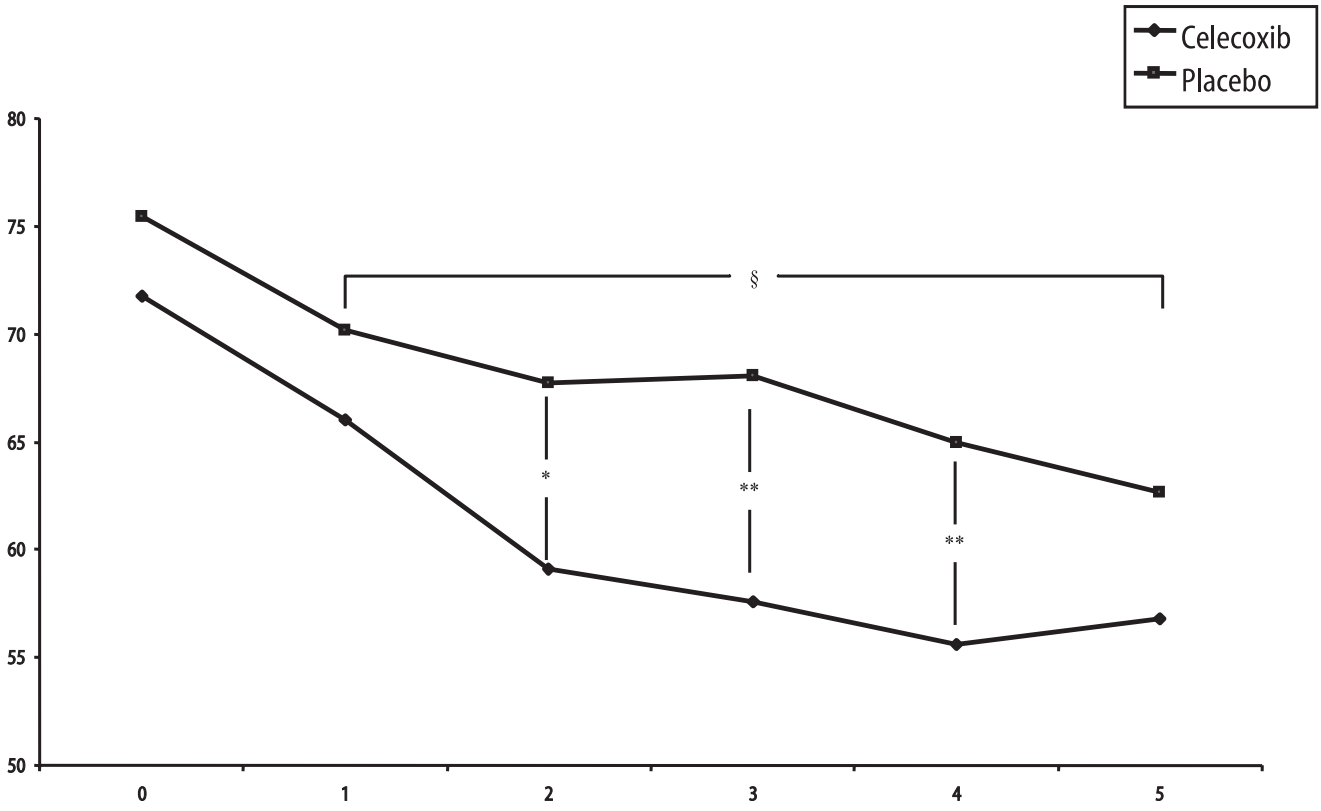

Fig. 1 PANSS-total scale during therapy with risperidone add-on celecoxib or placebo (§ ANCOVA Group difference $p \leq 0.05$; * t-test $p \leq 0.05$; ** t-test $p \leq 0.01$

treatment response under the add-on therapy. The acceleration of the treatment response was seen in a similar way in all subscales. These results show that the additional treatment with celecoxib has significant positive effects on the psychopathology of schizophrenia. Therapy with 400 mg celecoxib was well tolerated, and no clinically important side effects were observed.

Immune effects of celecoxib add-on therapy in schizophrenia

In order to evaluate the effects of the celecoxib therapy to the immune system in schizophrenia, markers of the peripheral immune system were measured in the blood of schizophrenic patients before the start of the therapy, weekly during therapy and at the end of the study.

As immunological parameters, the serum levels of soluble IL-2 receptors (sIL-2R) and of the soluble tumor-necrose-factor-α receptor-I (sTNF-R1) as the markers of the type-1 immune response and the $CD19^+$ lymphocytes as the marker of the (immune-response type-2 related) B-lymphocytes in the blood of both groups of patients were measured. Moreover, the percentages of $CD4^+$ and $CD8^+$ T-lymphocytes were estimated. The hypothesis was that COX-2 inhibition promotes an activation of the type-1 immune response and a decrease of the type-2 response.

Laboratory methods

After centrifugation, serum samples were frozen (–80 °C) and stored until analysis. IL-2R were estimated by using a commercially available double-sandwich ELISA system (R&D Systems, T-Cell Sciences, Cambridge, MA, USA). Levels of sIL-2R, sICAM-1, and sTNF-R1 were determined according to the manufacturer's instructions. Each concentration was measured in duplicate.

The lymphocyte surface molecules were measured from whole blood. Immunofluorescence flow cytometric phenotyping was performed by staining with various panels containing specific monoclonal antibodies (mAbs) directly conjugated either to fluorescein-isothiocyanate (FITC), phycoerythrin (PE), or a tandem stain with PE and Cyan5 (Tricolor®). The analysis was performed by a flow cytometer (FACScan®, BD) instrument with an air-cooled argon ion laser emitting 15 mW at 488 nm. The green fluorescence (FITC) was collected through a 530/30 nm bandpass, orange/red (PE) through 585/42 nm bandpass and red (Tricolor) through a 650 nm longpass filter. FSC and SSC signals were collected in a linear mode, and the three fluorescence signals were analyzed on a logarithmic scale.

For the analysis of the immunological parameters we did not use the method according to the criterion 'last observation carried forward' but with values which were

actually measured. Missing values were recorded as missing.

We measured the serum levels of the soluble receptor of IL-2, sIL-2R, which reflects the production of IL-2 and the serum levels of the soluble TNF-receptor 1 (TNF-receptor 55), which reflects the serum levels of TNF-α. From a (laboratory) methodological point of view, the levels of the soluble receptors are easier to estimate and more stable parameters in the serum compared to the cytokines themselves, while the sensitivity of the soluble receptors is lower. In particular for short-term effects, the cytokines are more sensitively compared to the soluble receptors.

Laboratory results

The cytokine IL-2 represents the type-1 immune response. The baseline levels of sIL-2R were 706.7 ± 395.2 ng/ml in the celecoxib group and 612.5 ± 280.3 in the placebo group. This difference was not statistically significant. During therapy, there was a slight increase of the sIL-2R levels in the celecoxib group to 794.0 ± 437.3 ng/ml after six weeks ($z = 1.75$; $p < 0.04$; one-tailed Wilcoxon-rank test). In the placebo group, there was no change in the sIL-2R levels during therapy. SIL-2R levels were 615.5 ± 252.7 ng/ml at the end of the study. The levels of the sIL-2R in the serum were significantly higher in the celecoxib group compared to the placebo group at the end of the study (day 35) ($z = 2.28$; $p < 0.01$; Mann-Whitney U-test).

The levels of the sTNF-R1 did not change significantly during the course therapy. Before the start of the therapy the sTNF-R1-levels were 1325 ± 324 pg/ml in the celecoxib group and 1280 ± 375 pg/ml in the placebo group. At the endpoint the sTNF-R1 levels were 1224 ± 303 pg/ml in the celecoxib group and 1230 ± 309 pg/ml in the placebo group. Neither within the placebo group nor within the celecoxib group were statistically significant changes observed. Accordingly, no significant differences were found between the two therapy groups.

Interestingly, when we divided the patients into responders to therapy and non-responders to therapy according to the criterion of an at least 30 % improvement on the PANS total scale, we found a statistically significant difference.

In the group of non-responders, the sTNF-R1 levels were 1414 ± 338 pg/ml before the start of the therapy while the sTNF-R1 levels were 1135 ± 193 pg/ml before the start of the therapy in the group of responders to celecoxib therapy. This difference was significant using the two-tailed t-test ($p < 0.04$). The responders to celecoxib had significantly lower sTNF-R1 levels.

No other immune parameter of the cellular immune-system ($CD4^+$, $CD8^+$, $CD19^+$), or sIL-2R, showed a statistically significant relationship to the therapy response to celecoxib (Figs. 2 and 3).

The $CD4^+$ cells were 44.3 % ± 8.6 % at start of the therapy and 40.6 % ± 16.3 % at the end of the study in the celecoxib group and 45.4 % ± 8.4 % at the start and 44.8 % ± 13.6 % in the placebo group, respectively. No significant differences were found within groups during the course of the therapy nor between the groups.

The following results were found with respect to the $CD8^+$ cells: the $CD8^+$ cells were 21.8 % ± 5.5 % at start of the therapy and 23.5 % ± 6.1 % at the end of the study in the celecoxib group and 23.3 % ± 8 % at the start and 23.9 % ± 7.7 % in the placebo group. With regard to the $CD8^+$ cells, no significant difference was found, neither within the groups nor between the groups.

The antibody-producing B-cells represent the humoral specific immune response. The CD-19 surface molecule is expressed on B-cells, which is how we estimated the $CD19^+$ lymphocytes. With respect to the $CD19^+$-B cells, the values were calculated as percentages (%) of total lymphocytes. At baseline the $CD19^+$ lymphocytes were 16.3 % ± 5.5 % of the total lymphocytes in the celecoxib group and 15.9 % ± 7.8 % in the placebo group. During therapy, there was a decrease of the $CD19^+$ lymphocytes in both groups. In the celecoxib group, the percentage of $CD19^+$-B cells decreased to 13.4 % ± 3.8 % at the endpoint after five weeks at day 35. This decrease was statistically significant using the one-tailed paired t-test ($p < 0.001$). In the placebo group,

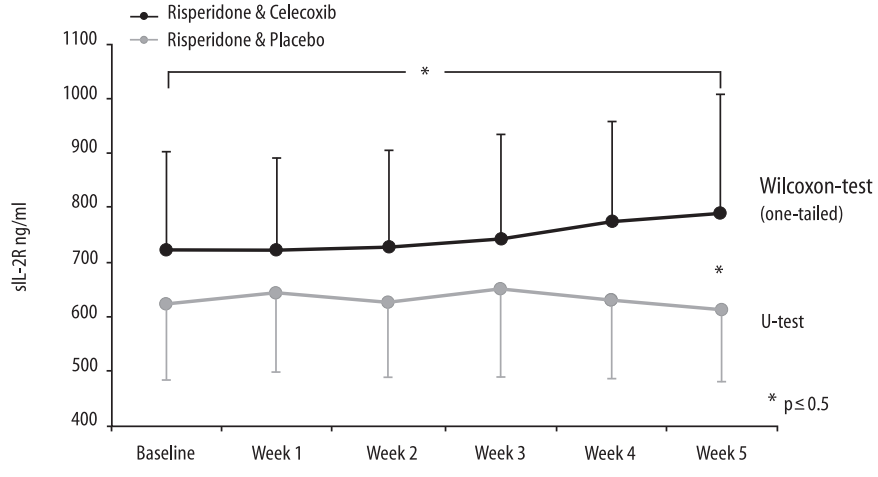

Fig. 2 Effects of celecoxib to sIL-2R in the peripheral immune system

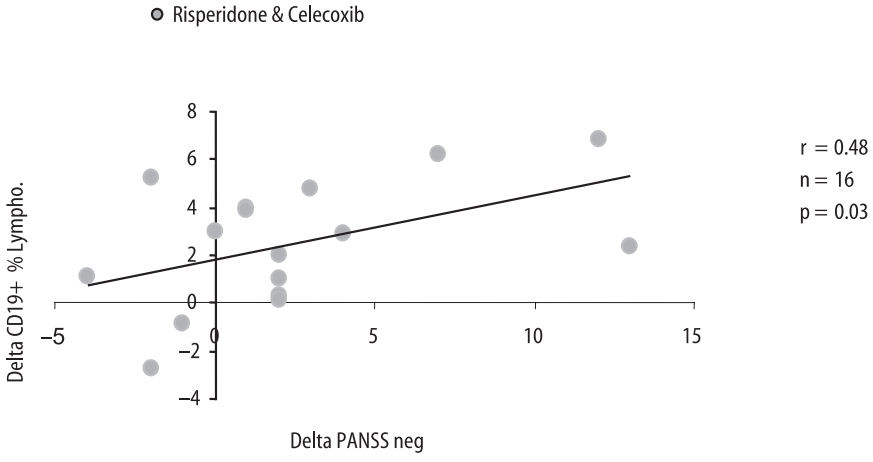

Fig. 3 Significant relationship between decrease in $CD19^+$ cells and schizophrenic negative symptoms during celecoxib treatment

however, there was also a significant decrease of the $CD19^+$ lymphocytes to 14.4 % ± 6.1 % ($p < 0.02$; paired t-test, one-tailed). Although the decrease of the B-lymphocytes was more pronounced in the celecoxib group, the $CD19^+$ lymphocytes also decreased under therapy with risperidone alone.

A result that underlines the relationship between the immunological and clinical aspects of this study was found regarding the parallels in the decrease of the PANSS negative subscale and the decrease of the $CD19^+$ lymphocytes. In the celecoxib, but not in the placebo group, we observed a significant relationship between the decrease of $CD19^+$ lymphocytes (baseline minus day 35) and the decrease of the PANSS negative-scale (baseline minus day 35) ($r = 0.48$; $n = 16$; $p < 0.03$). It could be concluded that the improvement of the schizophrenic negative symptoms seems to be related to the decrease of the $CD19^+$ B lymphocytes in the celecoxib group, but not in the placebo group (Fig. 4).

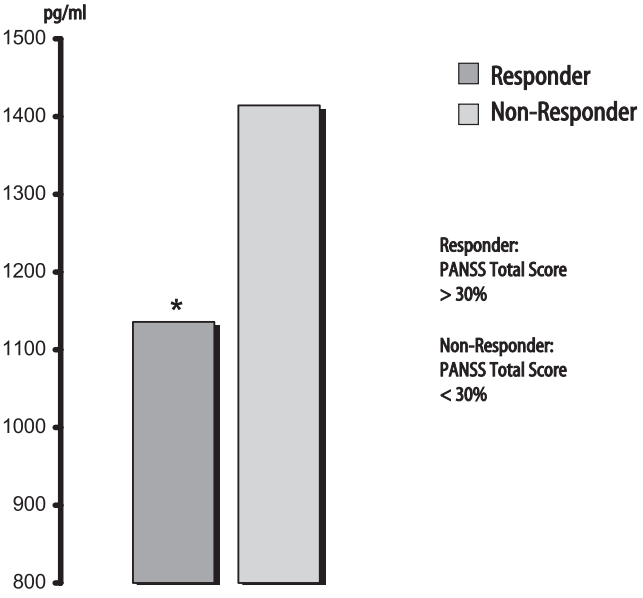

Fig. 4 Soluble TNF-α Receptor-1 (p55) and Response to COX-2 Inhibition. Low levels of TNF-R55 predict a better therapeutic response to celecoxib (*$p \leq 0.05$)

Discussion

The effects of celecoxib in the CNS have not yet been fully elucidated. There is no doubt that activation of COX-2 mediates inflammation and that COX-2 is expressed in brain tissue. COX-2 can be activated by cytokines like IL-2, IL-6, and IL-10, and cytokine-activated COX-2 expression mediates further inflammation. It is reported that IL-2 and sIL-2R (Licinio et al. 1993; McAllister et al. 1995), soluble IL-6 receptors as a functional part of the IL-6 system (Müller et al. 1997b), and IL-10 (van Kammen et al. 1997) are increased in the cerebrospinal fluid of schizophrenic patients. The increase of cytokines in the CNS compartment may be accompanied by increased COX-2 expression. We suppose that celecoxib down-regulates the cytokine-induced COX-2 activation in the CNS.

Moreover, COX-2 inhibition seems to regulate the adhesion molecule expression (Bishop-Bailey et al. 1998). Adhesion molecule regulation is impaired in schizophrenia, possibly leading to a dysbalance and a lack of communication between the peripheral and the CNS immune systems (Schwarz et al. 1998; Müller et al. 1999; Schwarz et al. 2000).

There might be a special subgroup of patients which benefits from celecoxib more than others, since even an onset of psychotic symptoms during celecoxib therapy has been described (Lantz and Giambanco 2000). On the other hand, malaria is known to induce schizophrenia-like symptoms; malaria infection was efficiently used as antipsychotic therapy by Wagner von Jauregg.

Several factors that may play a role in the effect of celecoxib in schizophrenia could not be considered because of the lack of studies and experience. Those factors are, e. g., the dose of celecoxib, the degree of CNS penetration and the duration until the onset of CNS effects of celecoxib. Thus, the ultimate therapeutic benefit of adjunctive celecoxib may require much more optimization of dose, duration of treatment, etc.

From a scientific view-point, the therapeutic effects of celecoxib without an additional neuroleptic drug would be more interesting. However, since neuroleptics are effective in antipsychotic treatment, ethics committees would not approve a study with a COX-2 inhibitor as the sole drug in acute schizophrenic patients.

This study was planned according to the psychoneuroimmunological hypothesis that a lipophilic anti-inflammatory substance may lead to therapeutic benefits in schizophrenia. The result reveals one more indication that immune dysfunction in schizophrenia may be related to the pathomechanism of schizophrenia and is not just an epiphenomenon.

The effects of the celecoxib therapy on the peripheral immune system, however, are weak. Nevertheless, they are in accordance with our hypothesis that the COX-2 inhibition activates the type-1 immune response and down-regulates the type-2 immune response given that sIL-2R levels represent the type-1 response of the cellular immune system and B-cells and activated B-cells represent the type-2 response.

An increase of sIL-2R has been described repeatedly in schizophrenic patients (Rapaport et al. 1989, 1994; Müller et al. 1997a) although the role of antipsychotic therapy has been discussed controversely as a causal contributor. It seems that certain antipsychotics including clozapine – but not risperidone – stimulate an increase of the sIL-2R (Müller et al. 1997a; Maes et al. 1995; Pollmächer et al. 1996). On the other hand, there might be a subgroup of schizophrenics showing increased levels of sIL-2R compared to healthy controls; this increase not being due to antipsychotic medication (Rapaport and Lohr 1994). From our sample, no conclusions can be drawn regarding the sIL-2R levels of controls. No change of sIL-2R levels was found in the risperidone and placebo group, while a weak, statistically significant increase was found in the celecoxib group, showing a significant difference at the end of the study and pointing to a type-1 immune response activation.

More pronounced effects were observed in our sample regarding the B cells. Increased B cells, especially $CD5^+$ B cells have been described in a subgroup of about one third of schizophrenic patients compared to controls (McAllister et al. 1989; Printz et al. 1999). An increase of B cells has also been described during therapy with antipsychotics (Rogozhnikova et al. 1993). A slightly increased mean percentage of $CD19^+$ cells has been observed in medicated schizophrenics compared to controls by the group of Rothermund (Rothermund et al. 1998). Other authors, however, did not find a difference in B cells between patients and controls, neither in medicated (Ganguli and Rabin 1993; Masserini et al. 1990) nor in unmedicated schizophrenics (Ganguli and Rabin 1993). If the observation is correct that a certain subgroup of schizophrenics shows increased B cells, then it would depend on the relative amount of those patients in the sample whether the whole sample may show increased B cells or not.

No conclusions can be drawn regarding the percentage of $CD19^+$ -lymphocytes in our patients compared to controls, but a decrease of $CD19^+$ lymphocytes was found in both groups of patients during therapy. Since in other studies unchanged or even increased B cells have been described during therapy, it has to be discussed whether therapy with atypical antipsychotics or particularly risperidone may be associated with a decrease in the B cells. The course of B cells during therapy has not yet been described using a standardized treatment protocol. However, therapeutic progress seems to be related to B cell change only in the celecoxib group, because there is a significant correlative relationship between the decrease of the schizophrenic negative symptoms and the percentage of the $CD19^+$ B cells. This decrease may reflect a type-2 immune response down-regulation.

We described the significant relationship of schizophrenic negative symptoms and the IgG content of the cerebrospinal fluid of schizophrenic patients (Müller and Ackenheil 1995) in a former study. Since IgG is the product of activated B cells, this observation corresponds well with other findings describing the relationship between the activation of components of the type-2 immune response and schizophrenic negative symptoms. An unfavorable course of schizophrenia has been described to be related to the increase of B cells (Rogozhnikova 1993). These findings suggest that long-term effects of COX-2 inhibition might be associated with improvement of the schizophrenic negative symptoms. The fact that no effects of celecoxib on the PANSS subscales have been found in our study may be due to several factors. Aside from the statistical power as one reason, a ceiling effect of risperidone on the positive symptoms and a relatively short duration of the trial with respect to therapy of the negative symptoms have to be discussed.

TNF-α is derived from various cellular sources of the immune system including activated macrophages and T-cells and has several different functions. TNF-α is called 'early response cytokine' because it coordinates pro-inflammatory signals of the early immune response in the innate cellular immune system. Due to this function, TNF-α is assigned to the type-1 cytokines. TNF-R1 is the primary signaling receptor for TNF-α (Mitzgerd et al. 2001) mediating the immunological and molecular effects of TNF-α (Alaaeddine et al. 1997). The levels of the soluble TNF-R1 – induced by shedding from the cell surface – are associated with the blood-levels of TNF-α (Alaaeddine et al. 1997). In a large study including 361 patients it was shown that sTNF-R1 levels of schizophrenic patients were slightly decreased still after taking into account the confounding factors such as age, smoking, gender, infection and medication (Haack et al. 1999). This finding of decreased sTNF-R1 serum levels in schizophrenia is in accordance with various findings pointing to a decreased type-1 immune response in schizophrenia. Several studies demonstrated the type-2 immune response inducing effect of PGE2 – the major product of COX-2, while inhibition of COX-2 is accompanied by inhibition of type-2 cytokines and induction

of type-1 cytokines (Stolina et al. 2000; Pyeon et al. 2000). The COX-2 inhibition seems to balance the type-1/type-2 immune response by inhibition of PGE_2 and by stimulating the type-1 immune response (Litherland et al. 1999). It can be speculated that low levels of sTNF-R1 reflect a low degree of type-1 immune activation and COX-2 inhibition has a high potency for the rebalancing of the type-1/type-2 immune response in patients with low levels of sTNF-R1. Whether this mechanism explains the predictive value of sTNF-R1 for the therapeutic effect of celecoxib in schizophrenia has to be elucidated in further studies.

The therapeutic effects of COX-2 inhibition are also discussed in other neuropsychiatric disorders such as Alzheimer's disease (McGeer 2000) and cerebral ischemia (Nogawa et al. 1997). The possible specific action in schizophrenia has to be explored in further studies. It has to be taken into account that the therapeutic effect of celecoxib is not only mediated by immune mechanisms but by glutamatergic mechanisms as well. COX-2 is expressed on neurons (Hewett et al. 2000) in structures critically involved in the pathology of schizophrenia such as in the hippocampus and amygdala (Yamagata et al. 1993; Breder and Saper 1996) and it is functionally related to glutamatergic receptors (Yermakova and Banion 2000).

Regardless of the mechanism(s) involved, acute add-on treatment with celecoxib appears to have a beneficial effect on schizophrenic psychopathology although this finding of a clinical effect of celecoxib needs to be replicated. Possibly, common immunological effects of COX-2 inhibition and (typical or) atypical antipsychotics can be described. Further investigations may help to understand the exact antipsychotic mechanism of COX-2 inhibition.

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