Journal of Psychiatric Research xxx (2013) 1–9

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Impact of mood stabilizers and antiepileptic drugs on cytokine production in-vitro

Hubertus Himmerich [a,*,1], Stefanie Bartsch [a,1], Hajo Hamer [b], Roland Mergl [a], Jeremias Schönherr [a], Charlotte Petersein [a], Alexander Munzer [a], Kenneth Clifford Kirkby [c], Katrin Bauer [d], Ulrich Sack [d]

[a] Department of Psychiatry, University of Leipzig, Semmelweisstr. 10, 04103 Leipzig, Germany
[b] Epilepsy Center, Department of Neurology, University Hospital Erlangen, 91054 Erlangen, Germany
[c] Department of Psychiatry, University of Tasmania, Hobart, Tasmania, Australia
[d] Institute of Immunology, University of Leipzig, Johannisallee 30, 04103 Leipzig, Germany

ARTICLE INFO

Article history:
Received 11 February 2013
Received in revised form
19 June 2013
Accepted 26 July 2013

Keywords:
Cytokines
Bipolar disorder
Epilepsy
Mood stabilizer
Antiepileptic drugs

ABSTRACT

Changes within the immune system have been reported to contribute to the pathophysiology of bipolar disorder and epilepsy. Interestingly, overlapping results regarding the cytokine system have been found for both diseases, namely alterations of interleukins IL-1β, IL-2, IL-4, IL-6, and tumor necrosis factor-α (TNF-α). However, the effect of mood stabilizers and antiepileptic drugs (AEDs) on these cytokines has not been systematically evaluated, and their effect on IL-17 and IL-22, other immunologically important cytokines, has not been reported. Therefore, we systematically measured levels of IL-1β, IL-2, IL-4, IL-6, IL-17, IL-22 and TNF-α in stimulated blood of 14 healthy female subjects in a whole blood assay using the toxic shock syndrome toxin TSST-1 as stimulant. Blood was supplemented with the mood stabilizers or antiepileptic drugs primidone (PRM), carbamazepine (CBZ), levetiracetam (LEV), lamotrigine (LTG), valproic acid (VPA), oxcarbazepine (OXC), topiramate (TPM), phenobarbital (PB), lithium, or no drug. IL-1β production was significantly decreased by PRM, CBZ, LEV, LTG, OXC, PB and lithium. IL-2 significantly decreased by PRM, CBZ, LEV, LTG, VPA, OXC, TPM and PB. IL-22 significantly increased by PRM, CBZ, LEV, OXC, TPM and lithium and decreased by VPA. TNF-α production significantly decreased under all applied drugs. The mechanism of action and side effects of mood stabilizers and AEDs may involve modulation of IL-1β, IL-2, IL-22 and TNF-α signaling pathways. IL-22 may be a research target for specific therapeutic effects of mood stabilizers and AEDs. These drugs might influence cytokine production by modulating ion channels and γ-aminobutyric acid (GABA) receptors of immune cells.

1. Introduction

In recent years the pathophysiology of bipolar disorder has been shown to include changes in both the cerebral and peripheral immune system leading to altered cytokine production in the brain and other parts of the body, and preliminary studies have pointed to the efficacy of immunomodulatory therapies in bipolar disorder (Stertz et al., 2013).

An increasing body of evidence indicates a possible causal role for the immune system in epilepsy, too. Cytokines seem to contribute to the development and course of epilepsy (Li et al., 2011; Vezzani et al., 2002).

Inflammatory processes play a role in the pathophysiology of a variety of brain disorders and diseases such as infections and autoimmune diseases of the brain, for example multiple sclerosis (Nylander and Hafler, 2012) or narcolepsy (Himmerich et al., 2006). But also in neurodegenerative diseases such as Alzheimer's disease (Rubio-Perez and Morillas-Ruiz, 2012) and Parkinson's disease (Tufekci et al., 2012). In other affective disorders, such as depression (Himmerich et al., 2008), and in schizophrenic disorders (Himmerich et al., 2012; Müller et al., 2012) the immune system is pivotally involved.

Interestingly, overlapping results regarding the cytokine system have been reported for epilepsy and bipolar disorder, namely alterations of interleukin (IL)-1β, IL-2, IL-4, IL-6, and tumor necrosis

* Corresponding author. Tel.: +49 0 341 97 24490; fax: +49 0 341 97 24559.
E-mail address: Hubertus.Himmerich@medizin.uni-leipzig.de (H. Himmerich).
[1] Both authors contributed equally to the manuscript.

0022-3956/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jpsychires.2013.07.026

factor-α (TNF-α) (Li et al., 2011; Hope et al., 2011; Drexhage et al., 2010; Nelson, 2004; Nowak et al., 2011). However, data regarding IL-2 and IL-4 is limited and the few studies do not show consistent results.

Involvement of IL-17 and IL-22 in the pathogenesis of epilepsy or bipolar disorder has not been investigated. This is of note, because T helper type 17 (TH17) cells which produce IL-17 are implicated in numerous immune and inflammatory processes (Hemdan et al., 2010; Park et al., 2005; Murdaca et al., 2011). IL-17 may play a significant role in autoimmune diseases of the brain such as multiple sclerosis (Park et al., 2005). Another cytokine produced by TH17 cells is IL-22. Studies have indicated the importance of IL-22 in host defense and in the development and pathogenesis of several autoimmune diseases (Pan et al., 2013). Therefore, from a neurological, psychiatric and immunological point of view, IL-1β, IL-2, IL-4, IL-6, IL-17, IL-22 and TNF-α are interesting, suitable and promising research targets in bipolar disorder and epilepsy. Beyond their involvement in the pathophysiology of bipolar disorder and epilepsy, exploring the effects of antiepileptic drugs (AEDs) and lithium on these cytokines may assist in elucidating mechanisms by which these drugs exert their therapeutic effects.

Valproic acid (VPA), carbamazepine (CBZ), and lamotrigine (LTG) are evidence-based treatments for bipolar disorder. There are also indications of therapeutic potential for oxcarbazepine (OXC), topiramate (TPM) and levetiracetam (LEV) in bipolar disorder (Grunze et al., 2013).

In-vitro and in-vivo experiments show that AEDs can affect cytokine levels. In patients with epilepsy, CBZ, VPA and phenytoin were reported to lead to elevated levels of IL-1β, IL-2, IL-5, IL-6 and TNF-α (Andrzejczak, 2011; Basta-Kaim et al., 2008). In-vitro, however, CBZ, VPA and phenobarbital (PB) were reported to inhibit the production of IL-2, IL-4, IL-6 and TNF-α (Andrzejczak, 2011; Yang et al., 1992). In patients with affective disorders, CBZ and lithium led to increased plasma concentrations of TNF-α and its soluble receptors sTNF-R p55 and p75 (Himmerich et al., 2005a). However, in this study, the patients gained weight during treatment, and therefore, the activation of the TNF-α system may be due to activation of macrophages in adipose tissue (Himmerich et al., 2009), or activation of Kupffer cells by inflammatory processes within the liver, caused by nutritional overload from increased appetite caused by these neuropsychopharmacological drugs (Himmerich et al., 2005b).

The discrepancy of results of in-vitro vs. in-vivo experiments enjoins us to interpret the results of in-vitro experiments with caution. Nevertheless, for a deep understanding of the mechanisms how psychopharmacological agents work and lead to side effects, it is important to know their effects on different tissues such as blood, liver or brain tissue. Moreover, in depression and bipolar disorder, the stimulated in-vitro production of cytokines has been shown to differ in patients vs. controls and to change during successful therapy (Krause et al., 2012; Knijff et al., 2007; Seidel et al., 1995). Therefore, stimulated in-vitro production of cytokines may be a biomarker for disorders of the brain. For the correct interpretation of data concerning the in-vitro production of cytokines in patients treated with psychopharmacological agents, it is necessary to know the effects on blood cells alone without the influence of a brain disorder and its improvement during therapy.

To our knowledge, no studies exist focused on the effects of PRM and OXC on cytokine production. The modulation of cytokine production by LTG, TPM and LEV has been subject to small exploratory studies, but not systematically in-vitro or in-vivo (Neuman et al., 2012; Kim et al., 2010; Koçer et al., 2009). Systematic investigation of the modulation of cytokine production by AEDs would clarify whether AEDs with mood stabilizing properties differ from AEDs without clear mood stabilizing effects. It would also be interesting to know whether the pharmacodynamic mode of action of an antiepileptic drug (AED) is associated with its influence on cytokine expression.

Therefore, we systematically measured levels of IL-1β, IL-2, IL-4, IL-6, IL-17, IL-22 and TNF-α in the stimulated blood of 14 healthy female subjects supplemented with various mood stabilizers and AEDs, namely PRM, CBZ, LEV, LTG, VPA, OXC, TPM, PB and lithium in a whole blood assay using the toxic shock syndrome toxin 1 (TSST-1) as stimulant. To reduce inter-individual variability by reducing the influence of age and gender and therefore not disguise the immunological effect of mood stabilizer and AEDs, we only used the blood of young and healthy female subjects. One has to keep in mind while reading this article that this is a pilot study which does not claim to obtain representative data for all ages, sexes and psychiatric conditions.

We recently started to use a whole blood assay with TSST-1-stimulation as an in-vitro approach to test the immunological properties of psychopharmacological agents (Himmerich et al., 2010, 2011). TSST-1 is a staphylococcal-secreted exotoxin that is responsible for the toxic shock syndrome. TSST-1 leads to non-specific binding of major histocompatibility complex class II (MHC II) with T cell receptors, resulting in polyclonal T cell activation, stimulation of mononuclear cells and increased cytokine production (Dinges et al., 2000; Kum et al., 1993). Thus, TSST-1 does not specifically lead to T cell activation and is therefore suitable for a pilot study on overall effects of mood stabilizers and AEDs on cytokine production.

Previous in-vitro studies on immunological effects of mood stabilizers used the lectin phytohemagglutinin (PHA) and lipolysaccharide (LPS) (Maes et al., 1999). PHA has similar effects on the immune response to TSST-1 (Micusan et al., 1989). LPS primarily allows the assessment of monocyte responses.

However, these previous studies did not focus on IL-17 and IL-22. Since one innovation of our present study to investigate production of these two TH17 cytokines (Costa et al., 2012), under mood stabilizers and AEDs, we followed a methodology based on our experience using TSST-1 to measure IL-17 production under psychopharmacological agents (Himmerich et al., 2011).

2. Methods and material

2.1. Subjects

14 healthy female subjects between 22 and 47 years of age (mean: 29 ± 6.4 (SD) years). Exclusion criteria were use of illegal drugs or regular alcohol consumption, presence of any immunological, infectious or endocrinological disorder, and a history of psychiatric disorder from an interview by a psychiatrist using the Structured Clinical Interview for DSM-IV (SKID-I; German) (Wittchen et al., 1997).

2.2. Experimental procedure

The whole blood assay was performed as described previously (Kirchner et al., 1982; Seidel et al., 1996; Himmerich et al., 2010, 2011). Blood was taken from all subjects once with a heparin-monovette (Sarstedt, Nürtingen, Germany) and cultured in a whole blood assay within 1–2 h after blood collection. Cell concentration was adjusted at 3–4 × 10^9 cells/l using RPMI 1640 medium (Biochrom, Berlin, Germany). Subsequently, 100 μl of this blood plus RPMI solution was introduced into a tube and mixed with 100 μl pure psychopharmacological substance plus RPMI, resulting in a final cell concentration of 1.5–2 x 10^9 cells/l. The final concentration of each AED in this mixture was chosen as to the

Please cite this article in press as: Himmerich H, et al., Impact of mood stabilizers and antiepileptic drugs on cytokine production in-vitro, Journal of Psychiatric Research (2013), http://dx.doi.org/10.1016/j.jpsychires.2013.07.026