Translocator Protein (TSPO) Expression in Platelets of Depressed Patients Decreases during Antidepressant Therapy

Authors

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C. Nothdurfter, MD Department of Psychiatry and Psychotherapy University of Regensburg Universitätsstrasse 84 D-93053 Regensburg Germany Caroline.Nothdurfter@medbo.de Abstract

Introduction: A promising candidate in the field of pharmacological treatment options regarding major depressive disorder (MDD) is the mitochondrial translocator protein (18 kDa) (TSPO). TSPO is crucial for neurosteroid synthesis, which is in turn important for the regulation of emotions. It has already been shown that TSPO expression in platelets of depressed patients is reduced compared to healthy subjects.

Methods: We measured TSPO levels in platelets of 37 depressed patients before and after 6 weeks of pharmacological treatment to test the hypotheses that i) such treatment would increase TSPO expression and ii) that this increase would be correlated with therapeutic response.

Results: Surprisingly, TSPO levels in platelets of all patients were significantly reduced after 6 weeks of treatment (p=0.044). Within the responder group, a non-significant trend towards greater TSPO level reduction could be observed. **Discussion:** These results challenge our hypotheses that TSPO levels might increase during antidepressant therapy along with a decrease in depressive symptoms. Thus, we assume that TSPO expression in platelets is not a suitable state marker for MDD.

Introduction

Mental disorders are widespread and highly disabling with considerable socioeconomic impact. Among these, major depressive disorder (MDD) is one of the most prevalent and is predicted by the World Health Organization to become the disease resulting in the largest global burden of disease within the next 2 decades [1,2]. The etiology of depression is complex; genetic, psychological, and psychosocial factors are supposed to be involved and also to interact. This multifactorial genesis of depression makes the treatment a challenge, which is also reflected by the fact that currently approved pharmacological agents are "dirty drugs" with multiple mechanisms of action. These compounds also have a delayed onset of action of several weeks and many side effects. Therefore, it is obvious that novel pathophysiological mechanisms underlying depression have to be discovered and characterized in order to create the basis for innovative and more beneficial drugs for the treatment of these disorders.

One candidate in this field might be the translocator protein (18 kDa) (TSPO). TSPO is a 5-helical transmembrane protein located in the outer mitochondrial membrane [3]. It is supposed to play an important role in neurosteroid synthesis and systemic endocrine regulation, with implications for the pathophysiology of immune, inflammatory, neurodegenerative, and neoplastic as well as psychiatric diseases [4]. More precisely, TSPO is an important component of a multiprotein complex, which promotes the transport of cholesterol into the mitochondrial matrix, which is a crucial step in neurosteroid synthesis [5]. Neurosteroids, such as allopregnanolone and 3α , 5α-THDOC (3alpha-, 5alpha-tetrahydrodeoxycorticosterone), are potent modulators of GABA_A receptor function. Thus, alterations in TSPO activity could affect the regulation of emotions via changes in GABAergic tone, and indeed, some TSPO ligands have already been shown to display efficacy towards the treatment of anxiety disorders [6]. To collect further evidence as to whether TSPO ligands may also be useful in the treatment of depression, there is a need to deepen our understanding of the functional role of TSPO in the pathophysiology of this disease [7].

TSPO is expressed in many organs, with the highest expression levels in steroid-synthesizing cells,

eg, adrenal, gonad, and brain cells [8]. In the central nervous system (CNS), TSPO is mainly expressed in microglia [9] and reactive astrocytes [10]. However, TSPO is also expressed in peripheral blood cells, such as platelets. While evidence exists purporting that TSPO is reduced in platelets of patients suffering from depression compared to healthy probands [11,12], no studies to date have determined whether antidepressant treatment, effective or otherwise, can reverse this deficit. Therefore, the aim of this study was to investigate the effect of antidepressant treatment on TSPO expression levels in platelets. We assessed TSPO expression in the platelets of 37 patients suffering from MDD before and 6 weeks after pharmacological treatment and correlated it to treatment response. Taking into consideration previous data on reduced platelet TSPO levels in depression, we hypothesized that, in cases of TSPO being a state marker, TSPO levels would increase during antidepressant therapy concomitant with a decrease in depressive symptoms.

Subjects and Methods

Study overview

52 depressed inpatients without antidepressive pharmacological pretreatment, treated at the Department of Psychiatry and Psychotherapy, University of Regensburg, Germany, were included in this open-label 6-week trial. All patients were recruited between December 2012 and May 2015. The study was designed to investigate the relationship between clinical response (defined as a reduction of at least 50% in the Hamilton Depression Rating Scale [HAMD-21] sum score [13] after 6 weeks of treatment) and the impact on TSPO levels in platelets. Therefore HAMD-21 sum scores (response/non-response) and TSPO values were pre-defined as primary outcome measures. All raters were experienced psychiatrists and blind to TSPO measurements. Raters were not changed in a single patient throughout the whole study period. Full blood samples for the assessment of TSPO levels in platelets (9 ml citrate blood collection tubes) were collected before antidepressant treatment started (PRE = day - 1) and after 6 weeks of treatment (POST = after 42 days). Patients had to be psychopharmacologically untreated at baseline investigation (with the exception of zopiclone up to 7.5 mg per day in case of sleep difficulties, and lorazepam up to 2 mg per day in case of inner tension and anxiety). The study protocol also allowed antidepressant pretreatment for patients who needed a change of medication due to non-response but required a wash-out period of 3 days before baseline investigation in such a case. However, all patients in this sample had no pretreatment. Patients were then treated for 6 weeks with different antidepressant medication. Medication was chosen according to clinical judgement at the discretion of the psychiatrist in attendance. In detail, 12 patients received selective serotonin reuptake inhibitors (SSRIs) (32.4%), 12 patients had mirtazapine (32.4%), 7 patients (18.9%) received dual antidepressants (venlafaxine or duloxetine), and 6 patients got agomelatine (16.3%). 18 patients additionally took atypical antipsychotics (48.6%). 2 patients also received lithium (5.4%). 8 patients dropped out of the study because they were discharged before day 42, 2 patients committed suicide, 2 patients withdrew their consent form, 2 patients refused further blood samples due to thin blood vessels, and 1 patient already started antidepressant medication before baseline measurement.

The study was carried out according to the Declaration of Helsinki (http://www.wma.net) and had been approved by a local ethics committee (intramural review panel of the University Regensburg, Faculty of Medicine).

Eligibility

52 inpatients (between 19 and 57 years of age) with a MDD episode according to DSM-IV criteria [14] were included in the study after the procedures had been fully explained and written, informed consent had been obtained. The structured Clinical Interview for DSM-IV, German version [15], was used to diagnose the participants. The patients had to show clear signs of a MDD episode with HAMD-21≥18. All participants were free of comorbid psychiatric and somatic disorders; regular laboratory parameters, blood pressure, electrocardiogram and encephalogram were required. Female patients could not enter the study in case of pregnancy. Patients did not receive payment or other rewards for study participation. 37 (20 males and 17 females) out of 52 patients completed the full study period up to day 42 including 2 blood withdrawals for TSPO-determinations and 2 HAMD-21 ratings (t 0 and t 1, respectively). Clinical and demographic characteristics of all patients who completed the study are given in **• Table 1**.

Protein lysates of platelets

Full blood samples were centrifuged at 1 300 g for 2 min at room temperature (RT). Supernatants were collected and mixed with citrate dextrose solution (ACD; Sigma-Aldrich, Taufkirchen, Germany) (6 ml plasma plus 1 ml ACD). After another centrifugation at 2 100 g for 2 min at RT, the supernatant of this second centrifugation step was wasted and pellets containing platelets were stored at -80 °C. Later, platelets were solved in 1 ml HEPES buffer (20 mM HEPES, 5 mM EDTA, 1 M NaCl) supplemented with a protease inhibitor cocktail (Sigma-Aldrich) and subsequent sonication. Lysates were stored at -20 °C until further use.

Western blotting

Protein concentrations of lysates were quantified by means of the Bradford method [16] with the Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Munich, Germany). 10µg of protein lysates were separated by sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) with 15% polyacrylamide gels before transfer onto a nitrocellulose membrane. All samples were measured as duplicates on one the same gel. Membranes were blocked with 5% non-fat dry milk in TBST (10 mM TRIS, 150 mM NaCl, 0.1% Tween 20; pH 7.4). TSPO was detected with a rabbit-monoclonal-anti-TSPO antibody (Abcam, Cambridge, UK) and ß-actin with a rabbit-anti-ß-actin (Sigma-Aldrich) overnight at 4°C. Blots were washed with TBST and incubated with secondary horseradish peroxidase (HRP)-conjugated antibodies for 2 h at RT. Bands were detected with the Chemiluminescence Substrate Super Signal West Pico (Fisher Scientific, Schwerte, Germany) and visualized with a digital imaging system (Image Quant LAS 4000, GE Healthcare Europe, Freiburg, Germany). Densitometric analyses were performed with Image] Software (Wayne Rasband, National Institute of Health, USA). TSPO values were normalized to ß-actin values of the same sample and to a control sample of a healthy control which was applied on every gel.

Table 1 Characteristics and course of psychopathology of PTSD and/or BPD patients treated with doxazosin.

	All patients (n=37)	Responders (n = 24)	Non-Responders (n=13)	Statistical evaluation		
Chi-square test (qualitative variables)						p-value
Sex [M/F]	20/17	14/10	6/7			0.071 ª
T-test (quantitative variables)				т	df	p-value
HAMD-21, PRE	25.89 ± 4.58	25.92±4.25	25.85±4.25	0.44	35	0.965
HAMD-21, POST	10.51±6.77	6.96 ± 5.03	17.08 ± 4.11	-6.201	35	0.000 *
TSPO, PRE	0.79 ± 0.38	0.78 ± 0.35	0.82 ± 0.46	-0.350	35	0.729
TSPO, POST	0.70 ± 0.36	0.64 ± 0.30	0.82 ± 0.43	-1.449	35	0.156
Age [years]	39.54±11.51	38.21±12.12	42.00±10.29	-0.956	35	0.346
Age of onset [years]	35.16±11.12	34.75±11.95	35.92±9.81	-0.302	35	0.764
Number of depressive episodes	01.03 ± 2.44	0.67 ± 2.06	1.69 ± 3.01	-1.227	35	0.228
Duration of index episode [weeks]	33.05±47.82	43.00±56.57	14.69 ± 12.81	1.769	35	0.027 *
Weight [kg]	77.10±16.87	77.80±17.02	75.81±17.20	0.339	35	0.737
Height [cm]	174.32±8.62	174.67±8.33	173.69±9.47	0.324	35	0.748

^aFisher's exact test (two-sided). * Significant at α = 0.05

Statistical analyses

Due to duplicate determination of TSPO levels, the arithmetic mean of each TSPO value per single sample was determined. After this step, possible deviations from normal distribution of the data were checked using the Kolmogorov-Smirnov test.

To compare TSPO levels at PRE and POST, a one-tailed, paired *t*-test was conducted, according to our hypothesis that TSPO levels would increase during antidepressant treatment in all patients. For statistical comparison of TSPO levels regarding response vs. non-response (measured with HAMD-21 sum scores), a repeated measures analysis of variance (rmANOVA) was conducted using "time" (TSPO levels: PRE, POST) and "group" (HAMD-21: response vs. non-response) as within-subjects and between-subjects factors.

In addition, we investigated potential differences of TSPO levels within response and non-response groups (PRE and POST) with one-tailed *t*-tests. As well, we included a bar diagram to illustrate the tendency of response with regard to the different medication groups. If the sphericity test (Mauchly-W test) was significant, a correction was applied to the degrees of freedom (Huynh-Feldt correction). Statistical analyses were performed using SPSS for Windows (Release 20, SPSS Inc., Chicago, IL 60606, USA). As a nominal level of significance, alpha=0.05 was accepted.

Results

Clinical characteristics

At baseline (PRE), TSPO levels between responders and nonresponders did not differ significantly (**• Fig. 1**). Also, there were no marked differences between the groups of responders and non-responders with regard to clinical or demographic variables (p > 0.05 for all comparisons; see **• Table 1**), with the exception of the duration of the index episode. However, the duration of the index episode seems not to be decisive for the probability of remission [17].

Responders vs. non-responders

Regarding HAMD-21 sum scores at PRE compared to POST, 24 patients were responsive to antidepressive treatment, while 13 patients were non-responders (see **• Table 1** and **• Fig. 1**). The response rate of 64.86% in our sample was comparable to other studies [18, 19].

TSPO protein expression over time

TSPO levels in platelets of depressed patients were assessed before (PRE) and after 6 weeks of antidepressive treatment (POST). Within the complete sample of patients with MDD a paired *t*-test (variable 1: TSPO level PRE; variable 2: TSPO level POST) revealed that there was a significant change in TSPO levels over 6 weeks of treatment (*t* = 1.756; *p* = 0.044). To identify the direction of this change in TSPO levels of all patients at PRE and POST (**• Table 1**), the descriptive values revealed that over the time of 6 weeks, TSPO levels significantly declined (TSPO level PRE:.79; TSPO level POST:.70). Interestingly, responders descriptively showed a greater reduction in TSPO levels compared to non-responders (**• Table 1** and **• Fig. 2a**). In summary, within the complete sample of patients with MDD a significant reduction of TSPO levels after 6 weeks of treatment could be found.

TSPO protein expression and clinical response vs. non-response

To explore potential differences between the groups of responders and non-responders concerning TSPO levels during antidepressive treatment, rmANOVA was conducted using "time" (between-subject-factor: TSPO levels PRE, POST) and "group" (within-subject-factor: HAMD-21 response vs. non-response) variables. The results indicated no significant effect for TSPO levels over time (rmANOVA Huynh-Feldt correction: F=1.759; p=0.193). As well, no significant difference between the group of responders and the group of non-responders could be observed (rmANOVA: F=0.906; p=0.348). Furthermore, the interaction "TSPO levels over time × responders vs. non-responders" showed no significant effect (rmANOVA Huynh-Feldt correction: F=1.475; p=0.117).

However, further statistical analyses of the 2 subgroups (responders vs. non-responders), showed a significant change in TSPO levels within the response group (paired-sample *t*-test within the response group; variable 1: TSPO level PRE; variable 2: TSPO level POST t=1.94; p=0.032). A paired *t*-test within the non-response group did not reveal a significant change (t=0.09; p=0.464). Taken together, the above presented results indicated no significant impact of response/non-response on TSPO levels over time and vice versa.

Regarding group of medication, a bar diagram shows that patients who received mirtazapine had the highest probability to be in the responder group (**©** Fig. 3).



Fig. 1 Clinical response upon antidepressive treatment. Changes in Hamilton depression rating scale sum scores, 21-item version (HAMD-21), of depressed patients at PRE (time at admission) compared to POST (after 42 days of antidepressive treatment). Data represent mean ± SEM. Clinical response (responders) was defined by a reduction of at least 50% of the HAMD-21 score after 42 days of treatment.

Discussion

The aim of this study was based on previous data showing reduced platelet TSPO levels in depression [11,12] to assess whether TSPO levels in platelets of depressed patients increase during antidepressant therapy concomitant with a decrease in their depressive symptoms. To our knowledge, this is the first study that has repeatedly analyzed TSPO expression in peripheral blood cells in MDD, in order to test the assumption that TSPO levels may be a state marker for this disease.

We could show that TSPO levels were significantly reduced following a 6-week period of antidepressant treatment. However, this reduction was not markedly associated with the clinical response to the treatment; although we observed a non-significant tendency towards more pronounced TSPO reduction in the group of responders compared to non-responders in rmANOVA. This lack of statistical association is possibly due to the small sample size and, therefore, limited power for statistical significance. The generalizability of our results is limited by several issues concerning the composition of the sample: it cannot be ruled out that the unequal number of patients in the response (n=24) und non-response group (n=13) may somewhat affect the observed findings. It also has to be mentioned that regarding descriptive characteristics the index episode of responders was significantly longer than in the group of non-responders. However, Gilmer and colleagues investigated possible associations between the duration of MDD and remission [17] and concluded that longer MDD duration seems not to be decisive for the probability of remission. Unfortunately, no other study examining



Fig. 2 TSPO protein expression in platelets of depressed patients. Full blood samples underwent a 2-step centrifugation to isolate platelets. Protein levels of lysates were determined by means of the Bradford assay. Equal amounts of protein were separated by SDS-PAGE. Specific antibody binding against TSPO and ß-actin was detected by chemiluminescence. Afterwards, densitometrical analyses were performed. All samples were measured as duplicate determination. TSPO values were normalized to ß-actin of the same sample and to a control sample applied on every gel. a Relative changes in TSPO levels of depressed patients at PRE (time at admission) compared to POST (after 42 days of antidepressive treatment) are shown in relation to therapy response. TSPO = translocator protein (18 kDa), PRE = time at admission, and POST = after 42 days of treatment. Data represent mean ± SEM. Clinical response (responders) was defined by a reduction of at least 50% of the HAMD-21 (Hamilton depression rating scale sum scores, 21-item version) score after 42 days of treatment. **b** One representative Western blot panel is shown. ctrl = control, PRE = time at admission, POST = after 42 days of treatment, R = responder, and N-R = non-responder.

the relationship between the duration of MDD and clinical response is known to the authors. Hence, we may only state that responders in our study showed significantly longer durations of MDD, while the replicability of this finding should be investigated in a more balanced sample.

Regarding TSPO levels and antidepressant medication, our results strongly challenge our hypotheses that TSPO levels in platelets may serve as a state marker of depression and correlate with clinical recovery. Nevertheless, the definition of clinical response is complex. Cut-offs on depression rating scales, such as the HAMD-21, cannot claim to cover all facets of patients suffering from MDD [20]. There was, of course, also a certain improvement of depressive symptoms within the group of non-responders.

Besides the controversy concerning appropriate classification of depressive symptoms, the comparability of our data with the study by Chelli and colleagues [11], who describe a reduction of platelet TSPO expression in patients suffering from depression, appears limited. In their study, some patients were also diag-



Fig. 3 Clinical response and antidepressant medication. Number of patients subdivided according to different antidepressant medication and therapy response. SSRI = selective serotonin reuptake inhibitors, and SNRI = serotonin noradrenaline reuptake inhibitors.

nosed with adult separation anxiety disorder, and it was this, rather than depressive symptoms, that were more closely associated with lower TSPO levels. Furthermore, HAMD scores of patients in the study by Chelli et al. were rather low compared to our sample, which also indicates that depressive symptoms were clinically not as prominent. In addition, some patients taking part in the Chelli et al. study were being treated with SSRIs when TSPO expression was measured. Weizman et al. also investigated TSPO in platelets of patients suffering from MDD with or without anxiety, and reported that TSPO levels in this cohort did not differ from healthy controls [21].

A very recent PET imaging study revealed that TSPO was markedly elevated in the prefrontal cortex, anterior cingulate cortex and insula of depressed patients compared to healthy subjects [22]. This study strengthens the hypothesis that neuroinflammation and TSPO specifically, plays an important role in the pathophysiology of depression. However, these results contradict the reduced TSPO expression found in platelets, thereby showing that tissue-specific characteristics limit the findings from peripheral blood cells. Another PET study suggests that TSPO binding may depend on the severity of depression [23]. However, in this sample, no statistically significant differences between depressed patients and matched controls were found. In addition, it has to be taken into account that psychopharmacological medication itself may influence the expression of TSPO. For example, it has been shown that the neuroleptic clozapine induced significant increases in TSPO binding in brain and peripheral steroidogenic tissues [24]. Moreover, in an in vivo study by Dennis et al., bulbectomy-induced TSPO binding density decrease in rats could be reversed by administration of antidepressants, such as paroxetine, suggesting that SSRIs may determine an up-regulation of TSPO expression [25]. These results from rodent studies challenge our findings of reduced TSPO levels in platelets after antidepressant treatment. However, this may again be related to tissue-specificity, as shown by Leschiner et al. [26] or species differences. To clarify the influence of specific compounds, future studies should investigate TSPO expression during the course of the respective pharmacological treatment in a larger sample.

Viewing TSPO as a modulator of microglial neuroinflammation in depression gives rise to a more complex picture of its function [27]. Overexpression of TSPO can decrease the proinflammatory response of microglia, thereby suggesting that its upregulation during neuroinflammation may be an adaptive response mechanism to facilitate recovery. In conclusion, TSPO levels may depend on the current stage of the disease, making necessary a more detailed observation of the time course of TSPO levels during antidepressant treatment. In view of such different therapy progress, a further follow-up to see if "late responders" reveal a similar TSPO expression pattern as "early responders" (after 6 weeks of treatment, as in our investigation) should be the focus of further studies.

Ultimately, neurosteroid levels rather than TSPO expression per se seem to be more important for the anxiolytic effects and recovery from depression following effective treatment [7,28]. From that point of view, some researchers support the hypothesis that TSPO expression does not necessarily correlate with neurosteroid production. In support, Costa and colleagues found that individuals suffering from depression and adult separation anxiety, who expressed normal TSPO levels, exhibited reduced pregnenolone production, while patients with normal pregnenolone production expressed lower TSPO levels. They could also associate TSPO expression with the Ala/Thr147 SNP [29]. These results could also be confirmed in a study by our group demonstrating discrepancy between TSPO ligand binding affinity, TSPO expression, and the enhancement of pregnenolone synthesis upon treatment with the TSPO ligands XBD173, etifoxine, and diazepam in 2 different cell culture models [30].

In the light of the results of the present study, we have to reject our original hypotheses, stating that TSPO levels increase during antidepressant therapy concomitant with a decrease of depressive symptoms. On the contrary, TSPO expression actually decreased during antidepressant treatment, and its expression even tended to negatively correlate with clinical response. Therefore, we have to conclude that TSPO expression in platelets is not a suitable state marker for MDD to judge the course of the disease. Nonetheless, a certain relevance of the neurosteroid pathway for the pathophysiology of affective disorders appears undoubtedly [7]. Therefore, other components of this pathway should be investigated in view of their suitability as a marker of disease, such as VDAC, as part of the multiprotein complex initiating cholesterol transport to the mitochondrial matrix [5].

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Conflict of Interest

The authors declare no conflict of interest.

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