

LETTERS TO THE EDITOR**Differential gene expression in peripheral blood of patients suffering from post-traumatic stress disorder**

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Post-traumatic stress disorder (PTSD) may develop as a failure of the body to reverse the acute stress response. Because of the sustained stress of the sympathetic hyperarousal state in PTSD, also immune functioning has early been hypothesized to be affected. Therefore, in this study the notion has been favored that genes involved in stress and immune responses are differentially transcribed in PTSD patients.

Two approaches have been pursued simultaneously: (1) re-evaluation of metabolic parameters tentatively associated with PTSD, and (2) cDNA microarray investigations using ‘stress/immune chips’. Recently, similar experiments have been carried out in patients suffering from acute traumatic events, using Affymetrix chips.¹ In our study, considerable efforts have been made to accurately standardize any condition possibly interfering with measurements. Whole blood was used and, very importantly, the group of PTSD patients was homogeneous in that all of them experienced the same traumatic event (Ramstein air show catastrophe, 1989). Despite the long-time interval between the traumatic event and the time of this study, in these individuals’ features characteristic for PTSD in the questionnaire interviews and typical psychosomatic symptoms still persist. However, on the metabolic level no differences between the patients and healthy controls could be detected with cortisol, adrenaline, noradrenaline, vanillylmandelic acid, homovanillic acid, and the cytokines interleukin (IL)-6, IL-1 β and tumor necrosis factor- α (Supplementary Information 1).

The genes represented on our home-made ‘stress/immune chips’ had been selected based on up-to-date knowledge about their role in inflammation, apoptosis, stress response and related pathways. We included eight patients and eight controls (Supplementary Information 2). The chip experiments were carried out according to Zieker *et al.*² After normalization of the data, we applied three statistical methods, the paired *t*-test, the Wilcoxon’s test and the rank product method³ (Supplementary Information 3). Four up- and 14 downregulated genes (altogether ~5% of total valid transcripts compared) have been obtained that showed statistical significance for at least one of the three statistical methods (Table 1).

In the present investigation, we have been focusing on the downregulated genes. Seven of those genes and one additional gene, the xc(-) glutamate–cystine–antiporter were selected for confirmations by quantitative (real-time) polymerase chain reaction (PCR), because they appeared to be physiologically connected. Real-time PCR results confirmed regulation of all of them except 3-phosphoglycerate dehydrogenase (Supplementary Information 4).

Most downregulated transcripts are associated with immune functions or with reactive oxygen species (ROS). The first set of transcripts encompasses thioredoxin reductase (TXR), superoxide dismutase (SOD) and the xc(-) transporter. Selenocysteine is the essential component for the catalytic activity of selenoenzymes,⁴ like TXR. There might be a connection between the availability of selenium in PTSD patients and the amount of TXR in their blood. It has been shown that SOD reduces the O₂ radical to H₂O₂. In this context, we assume that H₂O₂ production is decreased owing to the downregulation of SOD. Therefore, temporarily reduced availability of antioxidative enzymes like SOD and TXR may lead to repetitive bursts of OH-radical overload,⁵ which cannot be efficiently compensated for. Hence, ROS accumulation may be one reason for the higher risk of PTSD patients to develop autoimmune diseases like insulin-dependent diabetes⁶ or cardiovascular diseases.⁷

Glutathione (GSH) is the major antioxidative intracellular redox buffer and cysteine (Cys) is a rate-limiting precursor for GSH synthesis. The primary uptake mechanism of cystine (Cys₂) is mediated by the chloride-dependent xc(-) cystine–glutamate exchanger.⁸ Its downregulation as reported here may influence GSH replenishment through reduced Cys₂ uptake. In this way, xc(-) transporter connects the oxidative pathway with SOD as a key enzyme and the GSH system (Figure 1). This exchanger also plays a crucial role in the maintenance of extracellular glutamate in brain and has been implicated in relapse to drugs.⁹ Interestingly, relapse can be attenuated by treatment of animals with *N*-acetyl cysteine (NAC), an xc(-) exchanger agonist.¹⁰ We, therefore, hypothesize that treatment of PTSD patients with NAC may alleviate stress burden by increasing the efficiency of Cys₂/glutamate exchange.

The major transcripts of the second group of genes revealed by the microarray studies are IL-18, IL-16 and endothelial differentiation sphingolipid

Table 1 Microarray results

Gene	Acc. no.	P-value (RP)	P-value (t-test)	P-value (Wilcoxon)
<i>Upregulated genes^a</i>				
Insulin-like growth factor 2	NM_000612	0.0008	0.0049	0.0078
IL-8 receptor alpha	NM_000634	0.0040	0.2751	0.1875
Integrin beta 4	NM_002212	0.0211	0.0011	0.0039
High-affinity aspartate/glutamate transporter, m 6	NM_005071	0.0326	0.0052	0.0039
EDG4	NM_004720	0.0857	0.0133	0.0234
<i>Downregulated genes^b</i>				
Colony-stimulating factor 2 receptor, beta	NM_000395	0.0003	0.0058	0.0391
TXR 1	NM_003330	0.0003	0.1794	0.1875
IL-18	NM_001562	0.0011	0.0494	0.0039
Chemokine receptor 1	NM_001295	0.001	0.0092	0.0039
3-PGDH	NM_006623	0.0021	0.1315	0.1563
Casein kinase 1, gamma 3	NM_004384	0.007	0.0457	0.0547
Caspase 2	NM_001224	0.0067	0.0125	0.0391
SOD 1	NM_000454	0.0097	0.0003	0.0039
CD 3Z	NM_000734	0.0108	0.1779	0.1484
IL-16	NM_004513	0.0282	0.1736	0.2305
EDG1	NM_001400	0.0266	0.0593	0.0781
G-protein-coupled receptor 65	NM_003608	0.0306	0.0129	0.0234
Calnexin	NM_001746	0.0328	0.0991	0.1094
CD 81	NM_004356	0.0474	0.0814	0.1250

Abbreviations: EDG, endothelial differentiation sphingolipid; G-protein-coupled receptor; IL, interleukin; 3-PGDH, 3-phosphoglycerate dehydrogenase; SOD 1, superoxide dismutase 1; TXR, thioredoxin reductase 1.

^aUpregulated genes with a *P*-value <0.05 in at least one of the three statistical methods used. RP=rank product method.³

^bDownregulated genes with a *P*-value <0.05 in at least one of the three statistical methods used. Transcripts in bold have been confirmed by quantitative (real-time) PCR (Supplementary Information 4).

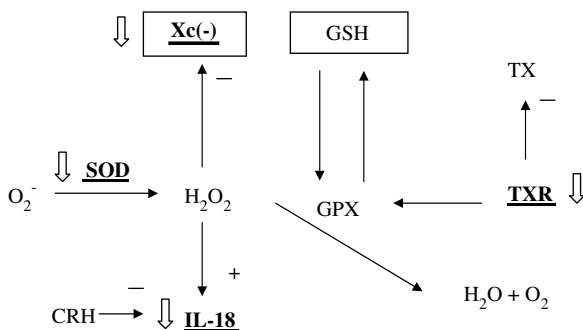


Figure 1 Inefficient radical inactivation in PTSD patients. Interactions of the genes differentially regulated in PTSD patients. All genes marked with are downregulated in our study both in real-time PCR and microarray analysis. The xc(-) transporter connects the GSH system with the antioxidative enzyme system via H_2O_2 . GSH reductases and peroxidases (GPX) closely interact with thioredoxins (TX) and TXR. Both GPX and TXR are seleno-dependent enzymes. Reduced expression of SOD and TXR likely results in accumulation of O_2^- and OH-radicals. IL-18 has been shown to be upregulated by H_2O_2 and downregulated by CRH. Reduced levels of H_2O_2 and hyperactivity of CRH may, therefore, result in impaired immune functions in PTSD patients. (-) Reduction; (+) increase.

G-protein-coupled receptor 1 (EDG1) mRNAs. It has been shown that IL-18 is regulated by different mechanisms, one of which is its induction by H_2O_2 .¹¹ As suggested above, H_2O_2 might be decreased

in PTSD patients owing to downregulated SOD. Further, IL-18 expression in keratinocytes reportedly is downregulated by corticotropin-releasing hormone (CRH).¹² This downregulation may also occur in PTSD patients who typically express hyperactive central CRH.¹³ We believe that our data showing downregulation of IL-18 mRNA may result from decreased levels of H_2O_2 and increased levels of CRH. Pro-IL16 is cleaved to its mature bioactive form by caspase-3,¹⁴ which interacts with caspase-2. These enzymes are regulated by sphingosine 1-phosphate via EDG1. Downregulation of EDG1 leads to activation of caspase-3, which may be part of a mechanism to convert more pro-IL16 into its mature form.

In summary, using cDNA microarrays, we clearly see changes on the mRNA level in PTSD patients even 16 years after the traumatic event. The transcripts identified here have not been associated with PTSD yet. The majority of the molecular targets can be grouped around ROS-related metabolism. Even cytokines, like IL-18, can be viewed in this context. Additional transcripts on the gene lists may also be important in PTSD, but in a context distinct from ROS-related events. Whether or not the gene products are more suitable targets for drug therapy can only be tested on the protein level. Nonetheless, the transcripts identified in this investigation are promising candidates and may support the notion of temporary perturbances in the oxidative (stress) system and in specific immune parameters of PTSD patients.

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Hypothalamic oxytocin mRNA expression and melancholic depression

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One of the main hypotheses about the pathogenesis of depression concerns hyperactivity of the hypothalamo-

pituitary-adrenal (HPA) axis. This axis is driven by corticotropin-releasing hormone release of neurons located in the paraventricular nucleus (PVN) of the hypothalamus, that causes adrenocorticotrophic hormone (ACTH) release at the level of the pituitary. ACTH release is potentiated by hypothalamic vasopressin (AVP).¹ AVP differs only two amino acids from oxytocin (OXT), a neuropeptide with many effects in social interactions.² Both neuropeptides are released from the hypothalamic paraventricular and supraoptic nucleus (SON).¹ Whereas AVP potentiates HPA-axis activity,³ animal experiments have shown that OXT attenuates the stress-induced activity of the HPA-axis in various species, including humans,⁴ and that OXT inhibits basal HPA-axis activity.⁵ Van Londen *et al.*⁶ found elevated AVP plasma levels in depressed patients and normal OXT levels, but described a larger variability in these levels compared to controls. Plasma OXT does not readily cross the blood–brain barrier, and there is no direct relationship between the release of OXT into the blood by the neurohypophysis and the variations in OXT levels in the cerebrospinal fluid.^{1,2} We therefore previously determined the number of OXT-expressing neurons in the PVN of depressed patients and this number turned out to be increased.⁷

Recently, we found in a post-mortem sample of depressed subjects a significant increase of AVP mRNA expression in the SON, and in both the SON and PVN when only the more severe, melancholic subgroup was taken into account.⁸ In the same group of depressed patients, with one control added, we performed a quantitative OXT mRNA *in situ* hybridization using the same technique.^{8,9} Briefly, hypothalami of depressed subjects (six melancholic type, three non-melancholic type) and nine control subjects matched for age and sex were obtained from the Netherlands Brain Bank in accordance with the formal protocols for use of human brain material and clinical information for research purposes.⁸ Differences among groups were evaluated by the non-parametric Kruskal–Wallis test and Mann–Whitney *U*-test. Correlations were evaluated by Spearman's rho. Statistical significance was set at $P < 0.05$.

A significant increase of OXT mRNA in melancholic type patients compared to non-melancholic type patients existed in the PVN ($Z = -2.074$, $P = 0.038$), whereas melancholic type patients compared to controls showed a trend ($P = 0.099$) towards higher OXT mRNA in the PVN (Figure 1). There was no difference in OXT mRNA in either the PVN or SON when comparing the entire group of depressed patients with control subjects. The group of depressed patients did not differ significantly from the control subjects concerning gender, post-mortem delay and fixation time.

This is the first report in which OXT mRNA has been quantified in the SON and PVN in depressed patients and control subjects. In the PVN, we found an increased OXT mRNA expression in the melan-