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Venlafaxine and sertraline does not affect the expression of genes regulating stress response in female MDD patients

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Summary

Aim. The aim of this study was to analyze the expression of 3 genes involved in the regulation of HPA axis: GR, HSP90 and FKBP5, in patients with major depressive disorder (MDD) before antidepressant treatment and after 8 weeks of pharmacotherapy. Additionally, we analyzed the level of glucocorticoid receptor isoforms before and after treatment.

Methods. The study included 30 female patients (aged 18–60 years), with major depressive disorder diagnosed on the basis of the Structured Clinical Interview for DSM-IV (SCID). Antidepressant treatment included use of sertraline or venlafaxine. The assessment of patients' mental state (severity of depression) was checked by the Hamilton Depression Rating Scale (HDRS). After 8 weeks of treatment, the same clinical and molecular tests were performed. All of the patients underwent dexamethasone suppression test (DST). MRNA was isolated from the peripheral blood to evaluate the expression of the studied genes using real-time PCR with TaqMan probes. The concentration of GR isoforms (α and β) in serum was also determined using ELISA. Statistical analysis was performed using Statistica v.12.0 software.

Results. The abnormal cortisol level was only seen in 20% of patients. Dysregulation on HPA axis was observed in 10% of patients. We observed significant clinical improvement after 8 weeks of pharmacotherapy in all patients. Almost the whole group of patients (except one patient) showed full remission of symptoms. We observed significant moderate correlation between cortisol level after DST before treatment and after 8 weeks of pharmacotherapy ($r^2 = 0.44$). The results showed no significant difference in the expression of 3 analyzed genes

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compared before and after 8 weeks of therapy. The results of ELISA showed decreased level of α isoform after pharmacotherapy, independent of drug.

Conclusions. The results showed no significant changes in the expression of genes involved in the stress axis activity during antidepressant therapy.

Key words: depression, stress axis, dexamethasone suppression test

Introduction

One of the endophenotypes of major depressive disorder (MDD) associated with lower mood during depressive episode is aberrant regulation of the hypothalamic-pituitary-adrenal axis (HPA) [1–4]. Dysregulated function of the HPA axis contributes to incorrect response of glucocorticoid receptor (GR) to chronically elevated cortisol level associated with chronic stress [2, 5]. Disorders of the HPA axis regulation can be demonstrated in the dexamethasone suppression test (DST), as 50–70% of patients with depression show no inhibition of cortisol secretion after administration of dexamethasone [6].

Glucocorticoids (GC) regulate numerous biological processes in the body, including stress response, lipid and glucose metabolism as well as immunological reactions. Glucocorticoid receptor is located in the cytoplasm as part of a large multiprotein complex that comprises of chaperones (HSP90, HSP70) and immunophilins (FKBP5) [7]. After GC binding with the receptor complex, GR dissociates from the protein complex and undergoes translocation to the nucleus, where it modifies transcription processes by direct interaction with the target gene promoter sequence (Glucocorticoid Response Element – GRE) or by protein-protein interaction with transcription factors such as NF-κB, AP-1, jun/fos, STAT, and NFAT [5].

Alternative splicing of the GR gene (*NR3C1*) in humans enables the regulation of the amount of mRNA molecules of the receptor to GC concentration. Alternative splicing results in 5 GR isoforms: GRα, GRβ, GRγ, GR-A, and GR-P. The predominant isoform, GRα, increases receptor sensitivity to GC, while GRβ is an isoform less sensitive to the presence of GC [7]. GR activity and bioavailability depends significantly on the α to β isoform ratio. In patients with depression decreased level of GRα isoform (but not GRβ) was demonstrated, thus suggesting that alternative splicing of GR receptor may be aberrant in depression [7].

Animal model studies showed that antidepressants increased expression of GR mRNA in rat hippocampus [8, 9]. Prenatal stress in depression model in rats was correlated with increased GR expression in the hippocampus and decreased FKB51 expression in the frontal cortex, whereas administration of antidepressants (fluoxetine, imipramine, mirtazapine, or tianeptine) normalized biochemical alterations observed in this model of depression without any changes in the control group [10]. Xing et al. investigated a novel antidepressant candidate (RO-05) and observed that its chronic administration normalized the HPA axis function [11]. Incubation of peripheral blood mononuclear cells with mirtazapine upregulated expression of GR mRNA in human leukocytes and monocytes after 2.5 hrs and downregulated mRNA expression after 4, 24 and 48 hrs [12].

Our recent studies showed that polymorphisms in genes involved in stress axis regulation (*NR3C1, FKBP5*) play a significant role in MDD predisposition [13–15]. Based on the studies mentioned above, we hypothesized that antidepressants may influence expression of genes involved in the HPA axis regulation in female depressive patients. The aim was analysis of the expression of three genes involved in the regulation of the HPA axis: *GR, HSP90* and *FKBP5,* as well as analysis of GRα and GRβ isoforms level in MDD patients before antidepressant treatment and after 8 weeks of pharmacotherapy, taking into account the result of dexamethasone suppression test.

Material

The study included 30 patients diagnosed with a depressive episode in MDD, aged between 18 and 60 years (mean age 38±10). The patients were recruited at the Department of Adult Psychiatry, Poznan University of Medical Sciences. The diagnosis was based on DSM-IV diagnostic criteria using a structured clinical questionnaire (SCID) [16]. Mental condition of the patients was assessed using the 17-item Hamilton Depression Rating Scale (HDRS) [17]. Patients with at least moderate depression (HDRS score > 18) were included in the study. The exclusion criteria were: the use of oral contraceptives or hormone replacement therapy, the diagnosis of severe somatic and neurological diseases, the coexistence of other mental disorders or addictions. Patients enrolled in the study were patients who had never been treated with antidepressants or who had not been treated with antidepressants within 6 months before inclusion in the study – so-called drug free.

Antidepressants used to treat depressive episode were sertraline or venlafaxine for the doctor's choice, according to his best medical knowledge and experience based on the clinical evaluation of symptoms. The doses used in the study were in the range: 50–150 mg for sertraline and 75–225 mg for venlafaxine. Patients were treated in monotherapy only, hypnotic drugs (such as zolpidem, benzodiazepines) were used on demand with no less than 2 days "wash out" before blood collection on the day 0 and after 8 weeks of therapy.

Before the implementation of treatment, basic laboratory tests and dexamethasone suppression test (DST) were performed for all the patients. Blood collection for gene expression analysis and cortisol measurement was performed before DST at 8 a.m. The same laboratory and clinical tests were performed after 8 weeks of therapy in accordance with the protocol previously used in GENDEP study.

Patient recruitment and study protocol were accepted by the Bioethics Committee of the Poznan University of Medical Sciences.

Methods

Dexamethasone suppression test (DST)

On the day before the tests each patient took 1 mg of dexamethasone at 11:00 p.m. To determine the serum cortisol concentration, the blood was collected next day (day

"1_0") at 8:00 a.m. This procedure was repeated after 8 weeks of antidepressant treatment (day "1_8") according to the scheme (Figure 1). When the HPA axis functions properly, inhibition of cortisol secretion is observed the next morning after dexamethasone administration. The normal value is cortisol level < 50 nmol/l (< 1.8 μg/dl).

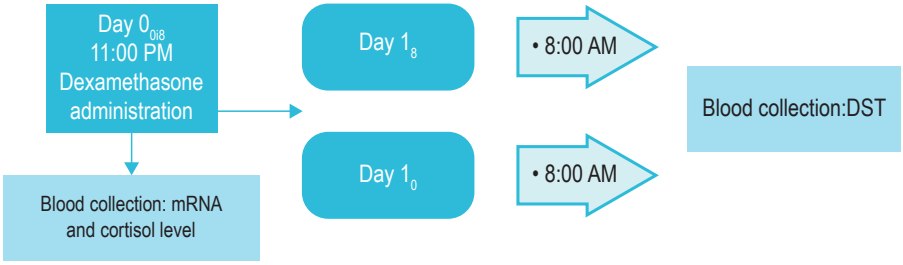

Figure 1. **Procedure of the dexamethasone suppression test (DST). Day 0_0 – inclusion in the study during depressive episode; day 0_8 – the day before DST after 8 weeks of treatment; day 1_0 – the day after the inclusion into the study, the day when DST was performed; day 1_8 – the day after 8 weeks of treatment**

The cortisol level was determined in the laboratory of the Karol Jonscher Clinical Hospital, Poznan University of Medical Sciences, using radioimmunoassay (RIA).

Molecular analysis

We analyzed the expression of 3 genes at the mRNA level: *GR* (Hs00353740_m1), *HSP90* (Hs00743767_sH) and *FKBP5* (Hs01561006_m1), involved in the regulation of the activity and bioavailability of GR receptor. Total RNA was isolated from leukocytes of peripheral blood of the patients at inclusion into the study (week 0) and after 8 weeks of pharmacotherapy (week 8). Reverse transcription was done with the use of SuperScript III First-Strand Synthesis (Invitrogen). Quantitative PCR (qPCR) analysis was performed using TaqMan expression assays on Abi Prism7900HT Sequence Detection System (Thermo Fisher Scientific). The expression level analysis was performed using ddCt method using 18S rRNA as a reference gene. Results were analyzed in SDS RQ Manager and Expression Suite v.1.0.3 software (Thermo Fisher Scientific)

ELISA

Blood collected into tubes without anticoagulant was centrifuged after one hour to obtain serum and frozen at – 80°C. Concentration of GRα and GRβ isoforms in serum samples in week 0. and 8. was analyzed using ready-made ELISA kits (Cloud-Clone Corp.) according to the manufacturer's protocol.

Statistical analysis

The differences in gene expression in the same patients before and after antidepressant therapy were compared with the use of non-parametric Wilcoxon signed-rank test. The analysis of the correlation between the severity of depression and the level of gene expression was performed using the Spearman's rank correlation test. The analyzes were performed using Statistica v.12 software.

Results

Characteristics of the study group

The study group included 30 female patients with a mean age of 37.7±10.5 years. The average duration of illness was 5.1±6.0 years (spread value of 19.5). No significant correlation was observed between age of the patients and the studied hormones (cortisol, thyroid hormones). A mean baseline (week 0) HDRS score for sertraline was 21.3±2.8 and for venlafaxine – 26.9±3.7.

16 patients were treated with sertraline and 14 with venlafaxine. After 8 weeks of antidepressant treatment significant clinical improvement was observed in all studied patients regardless of the used drug (> 50% reduction in the Hamilton Depression Rating Scale, HDRS). All of them except one showed full remission of symptoms (HDRS score < 8). After 8 weeks of therapy the mean HDRS score was 3.4±3.1 for sertraline and 5.0±2.4 for venlafaxine.

Cortisol level analysis

A mean in the study group at the beginning of the study was 507.23±209.70 nmol/L, after 8 weeks of therapy the mean level of cortisol slightly decreased (mean value 476.50±200.73 nmol/L). In the study group at the beginning of the therapy, morning cortisol levels showed abnormalities in only 20% of patients (exceeding the upper limit), in the remaining ones it was within the normal range (130–690 nmol/l). The mean cortisol level did not differ significantly between the group of patients treated with sertraline and the group treated with venlafaxine – neither initially ($p = 0.381$) nor after 8 weeks of therapy ($p = 0.868$).

Dexamethasone suppression test analysis

At the beginning of the study dysregulation of the HPA axis was observed only in 3 patients (10% of the study group), and in 2 of them the HPA axis function normalized after 8 weeks of treatment. We observed a significant moderate correlation between cortisol level after DST before and after therapy ($r^2 = 0.44$). In the study group we observed higher mean cortisol level after DST at the beginning of therapy (450.83±229.88 nmol/L) as compared to the cortisol level after 8 weeks of antidepressant therapy (447.03±191.72 nmol/L), however, this difference was not statistically

significant (p = 0.944). The mean cortisol level after DST in week 0. and 8. did not differ significantly between patients receiving sertraline and venlafaxine ($p > 0.05$).

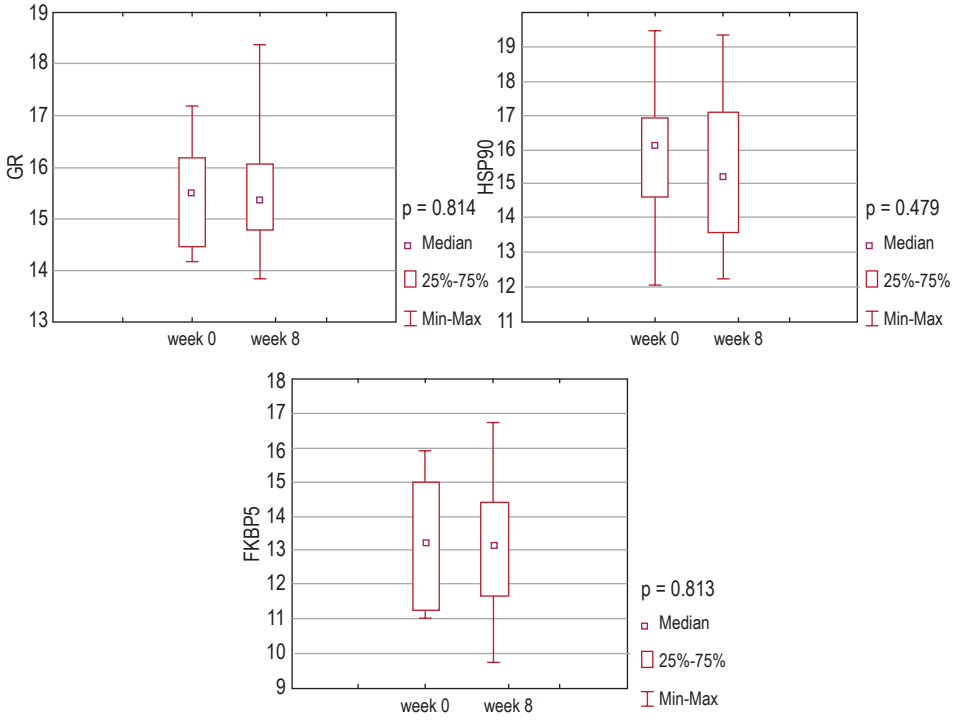

Figure 2. **The comparative analysis of mRNA expression of 3 genes (*GR*, *HSP90* and *FKBP5*) before treatment and after 8 weeks of pharmacotherapy**

Gene expression analysis

The results showed no significant differences in the expression of the three analyzed genes after 8 weeks of therapy as compared to the expression at the beginning of therapy (Figure 2). However, when we stratified gene expression results by drug we observed a tendency for decreased expression of analyzed genes after sertraline therapy, while the expression after venlafaxine therapy was increased, but these differences were not significant (Figure 3).

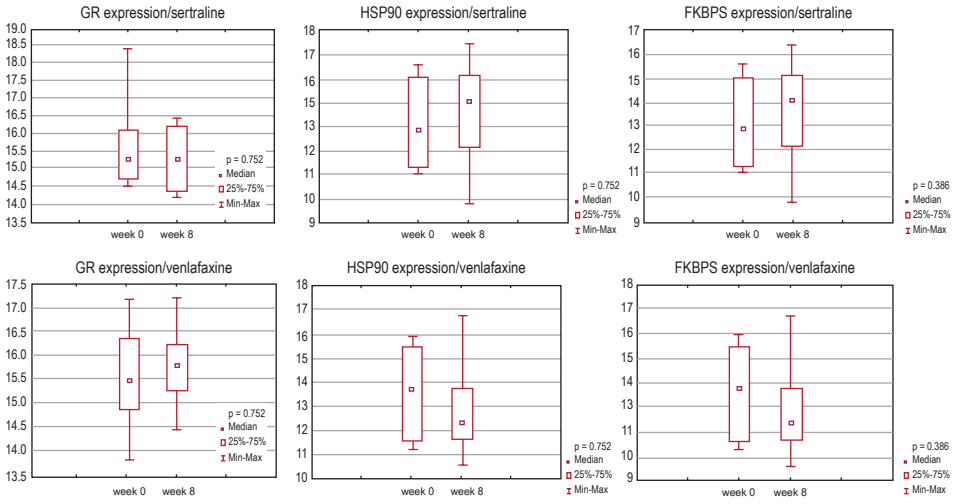

Figure 3. **The comparative analysis of the expression of 3 genes (*GR, HSP90* and *FKBP5*) stratified by antidepressant drug (sertraline/venlafaxine)**

Correlation with severity of depression

The correlation analysis of expression of the studied genes with the severity of depressive symptoms on the HDRS at the beginning of the study and after 8 weeks of antidepressant treatment did not show significant relationships ($p > 0.05$ for all genes), regardless of the drug.

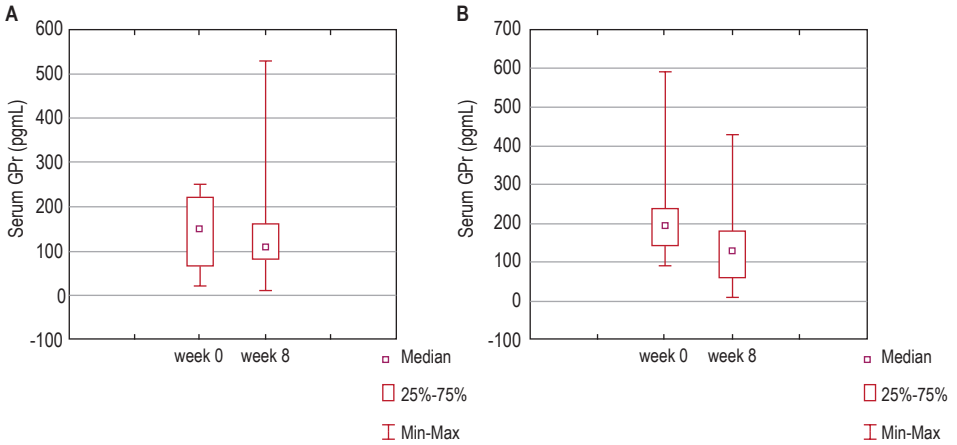

Figure 4. **Serum concentration of GRα isoform at the beginning of the study and after 8 weeks of treatment with a) sertraline (p = 0.498) and b) venlafaxine (p = 0.262)**

Concentration of GR isoforms

ELISA analysis showed that GRα serum concentration decreased after 8 weeks of pharmacotherapy, regardless of the drug, however, this decrease was not statistically significant (Figure 4). Detection of GRβ was possible only in one patient, in other patients GRβ serum concentration was below the detection limit, thus we excluded that isoform from further analysis.

Discussion

In our study we observed a decrease in cortisol level in our patients after 8 weeks of pharmacological therapy. It was consistent with clinical observation: the patients showed the reduction of depressive symptoms on the Hamilton scale and improvement in clinical symptoms.

Previous studies showed dysregulation of the HPA axis in 50–70% of patients with depression [7]. These disturbances mainly included: increased level of cortisol [5] and the reduced sensitivity of GR receptor [18]. The studies of Newport et al. confirm the increased response of the HPA axis to psychological stress in patients with depression [19]. Additionally, patients with childhood trauma showed higher post-DST cortisol concentrations, compared to patients without trauma [20]. In our study, DST showed the aberrant HPA axis function only in 10% of patients, which may be the cause of lack of visible effect of the therapy on the expression of the studied genes.

Both antidepressant drugs used in this study belong to the selective serotonin reuptake inhibitors, however, they have different mechanisms of action. Venlafaxine is a bicyclic molecule that blocks reuptake of serotonin, noradrenaline and, to a lesser extent, dopamine [21]. Sertraline acts directly on serotonin and dopamine transporter [22]. The analysis of expression of three genes involved in the HPA axis regulation: *GR*, *HSP90* and *FKBP5* in MDD patients before treatment and after 8 weeks of antidepressant treatment showed no significant differences. However, when we stratified patients by antidepressant drug, we observed a tendency towards a decrease of mRNA expression of the studied genes after sertraline, while after venlafaxine we observed a tendency towards an increase of expression after therapy. To date, no papers analyzing the relationship between the used drug and its effect on the HPA axis regulation or the expression of genes related to the stress axis have been published.

Previous study by Lukic et al. showed significantly increased amount of GR molecules in the cytoplasm of depressive patients as compared to the healthy control group and, at the same time, increased level of the gene *FKBP5*. *FKBP5* binds to the GR complex, which results in decreased affinity of GR receptor to ligand and impaired translocation to the nucleus. Increased activity of *FKBP5* contributes to a decreased amount of GR in the cytoplasm [23].

The analysis of GRα isoform concentration showed a decrease of the concentration after pharmacotherapy. Previous report by Matsubara et al. showed reduced GRα isoform in patients with major depressive disorder not only during severe depressive episode but also in remission [24].

Conclusions

Summarizing, the obtained results have not shown significant differences in expression of the studied genes during antidepressant therapy. The possible factors influencing that result could be relatively small sample size and also the fact that only 10% of patients showed disturbances in the HPA axis function (aberrant results of DST test) at the beginning of the study.

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