Research report

The expression of genes encoding for COX-2, MPO, iNOS, and sPLA2-IIA in patients with recurrent depressive disorder

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1. Introduction

Depressive disorder is the heterogenic disease. Activation of inflammatory, oxidative, and nitrosative stress (IO&NS) pathways plays an important role in the pathophysiology of depression (Maes et al., 2011a). There is evidence showing that the production of free radicals and their derivatives is increased in depression (Galecki et al., 2009; Kodydková et al., 2009; Kotan et al., 2011). Increased levels of proinflammatory mediators are now well established in depression (Maes, 2011). There are also results showing that key inflammatory enzymes, including cyclooxygenase-2 (COX-2), myeloperoxidase (MPO), inducible nitric oxide synthase (iNOS) are involved in the disease in question (Guo et al., 2009; Vaccarino et al., 2008; Wang et al., 2008). The aforementioned enzymes not only contribute to inflammatory processes, but also participate in the production of free radicals and damage to fatty acids, DNA and proteins (Aktan, 2004; Dubois et al., 1998; Hansson et al., 2006; Kuehl and Egan, 1980; Valko et al., 2007; Winterbourn, 2002).

COX-2 enzyme is one of the two isoforms of COX producing prostaglandins from arachidonic acid. The level of COX-2 is raised in chronic inflammatory diseases and may be induced by cytokines (Dubois et al., 1998). There is also evidence that COX-2 may participate in neurodegenerative processes (Mirjany et al., 2002) but it also plays a role in
brain physiology (Minghetti, 2004). The expression of COX-2 is observed not only in inflammatory cells, but also in the central nervous system (CNS) (Yasojima et al., 1999). The importance of COX-2 in depression is indicated by the therapeutic effectiveness of COX-2 inhibitors in the treatment of depression and in animal models (Müller et al., 2006; Myint et al., 2007). Moreover, in animal model of depression increased expression of mRNA for COX-2 was observed in hippocampal region (Cassano et al., 2006).

MPO is a heme enzyme expressed in brain cells (Jolivalt et al., 1996) and in immune cells and that plays an important role in the regulation of inflammatory processes and causes oxidative damage (Hansson et al., 2006). Activation of MPO leads to the production of hypochlorous acid and other toxic oxidants (Winterbourn, 2002) that function as antimicrobial agents (Heinecke, 1999; Podrez et al., 2000). There are studies showing that low levels of MPO reduces the risk of cardiovascular diseases (CVD) (Zhang et al., 2001), an inflammatory disorder (Carr and Frei, 2000) that co-exists with depression (Maes et al., 2011b; McCaffery et al., 2006). MPO deficiency also attenuates cytokine production (Haegens et al., 2009). Recently Vaccarino et al. (2008) found that higher MPO is one of the biomarkers of inflammation in depression. Additionally, increased expression in brain region is related to neurodegeneration (Green et al., 2004), a process that also accompanies depressive disorder (Maes et al., 2011a).

The next enzyme involved in inflammation and oxidative stress is iNOS producing nitric oxide (NO). The molecule is important in many physiological and pathological processes including inflammation and oxidative stress (Hirst and Robson, 2011). iNOS constitutes an inducible form, which expression is present in numerous inflammatory cells in the periphery and in brain. iNOS expression is also observed in neurons following biological stimuli including proinflammatory cytokines, lipopolysacharides, and stress as well (Aktan, 2004; Heneka and Feinstein, 2001; Kleinert et al., 2004). All those factors play a role in the onset of depression (Kubera et al., 2011; Maes, 2011). Evidence for the involvement of iNOS includes: increased level of iNOS-derived NO in patients suffering from depression (Suzuki et al., 2001), inhibition of the iNOS that lowers the depressive-like behaviors (Wang et al., 2008), reduced NO activity followed by antidepressant treatment (Wegener et al., 2003), both performed on animal model.

Secretothy phospholipases A2 (sPLA2s) are important enzymes detected in blood of patients with inflammatory disorders. These enzymes catalyze the reaction of fatty acids production from phospholipids (Triggiani et al., 2006). sPLA2s produce other inflammatory mediators and reactive oxygen species (Adibhatla and Hatcher, 2008; Triggiani et al., 2006). The expression of sPLA2 is low in resting inflammatory peripheral cells, but their release is mediated by pro-inflammatory factors (Balboa and Balsinde, 2006). The mainly investigated type of sPLA2 that is involved in inflammation is type IIA (sPLA2-IIA) enzyme. In the brain, mRNA for sPLA2-IIA is observed (Sun et al., 2004) and activation of sPLA2 type IIA contributes to neuronal death (Schaefier et al., 2010). Moreover, sPLA2-IIA appears to be a risk factor of CVD (Koenig et al., 2009), because sPLA2-IIA makes oxidized LDL more atherogenic (Divchev and Schieffer, 2008).

Recently, biological studies observed that polymorphism of the genes encoding COX-2, MPO and iNOS are related to the risk of recurrent depressive disorder (rDD) (Galecki et al., 2010a, 2010b, 2011).

The aim of the present study was to investigate mRNA expression of PTGS2, MPO, NOS2A, PLA2G2A coding aforementioned four enzymes in patients suffering from rDD versus normal controls.

The reason to investigate the gene expression in the whole blood resulted from the fact that all these enzymes are not only expressed in brain but mainly in the periphery. There is also strong evidence that peripheral molecules might affect brain cells. Moreover, whole blood is more accessible. Peripheral blood cells share more than 80% of the transcriptome with nine tissues including brain (Mehta et al., 2010).

2. Materials and methods

2.1. Subjects

A group of 181 patients, treated for rDD (102 females; 56.4%), were enrolled into the study. The mean age in that group was 42.6 ± 8.2 years (mean ± SD). The diagnosis was established according to ICD-10 criteria (F33.0–F33.8) (World Health Organization, 1992). In all qualified cases, medical history was obtained, using the standardized Composite International Diagnostic Interview (CIDI) (World Health Organization, 1992). Additionally, the number of depressive episodes, duration of the disease and the age at onset were assessed in each patient. The control group consisted of 149 healthy subjects (83 females; 55.7%) with family history negative for psychiatric disorders. The mean age in that group was 38.7 ± 6.7 years (mean ± SD). The control subjects included community volunteers, enrolled to the study following the criteria of the psychiatric CIDI interview (World Health Organization, 1992). Both patients and controls with other psychiatric diagnoses, concerning axes I and II disorders and inflammatory diseases were excluded from the study. All the patients and control subjects were native, unrelated inhabitants of the central Poland. Written informed consent was obtained from all the participants of the study. The study protocol had earlier been approved by the Local Bioethics Committee No. RNN/626/09/KB.

2.2. Quantitative real-time PCR

Total RNA (1 μg) was extracted from the blood cells using Trizol reagent (Life Technologies Inc), and was processed directly to cDNA synthesis using the TaqMan Reverse Transcription Reagents kit (Applied Biosystem) according to the manufacturer’s protocol. The human PLA2G2, NOS2A, PTGS2, MPO and GAPDH expressions were quantified by real-time quantitative PCR using ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s protocol. Briefly 2.5, 2.0; 1.5, 1.0; 0.5 and 0.25 μl of synthesized cDNA were amplified in triplicate for both GADPH and each of the target genes, to create a standard curve. Like-wise 2 μl of cDNA was amplified in triplicate in all isolated samples for each primer/probe combination and GAPDH. Each sample was supplemented with both respective
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0.3 μM forward and reverse primers, fluorescent probe, and made up to 50 μl using qPCR™Mastermix for SYBR Green 1 (Eurogentec Seraing Belgium). All following PCR primers were designed using software Primer Express (Applied Biosystems) forward 5’CTCTACGGGTCCACGTATC3’, reverse 5’CAACAGTCATGAGTGACACG3’, forward 5’CAGTCTGGCCTTTGCTCAT3’, reverse 5’GGTTGGCCGTCGGACTTT3’, forward 5’GTCAAGAACGATGTTTGCATC3’, reverse 5’GGTGCTGCCTTTGCTCAT3’, forward 5’CCAGGAAGCCCGGAAGAT3’, reverse 5’CCGAAGACATTTCCACTTCT3’, forward 5’TGCCTGCTACACCTTCT3’ specific for PLAZG2, NOS2A, PTGS2, MPO and GAPDH cDNA respectively. GAPDH was used as an active and endogenous reference to correct for differences in the amount of total RNA added to reaction and to compensate for different levels of inhibition during reverse transcription of RNA and during PCR. Each target probe was amplified in separate 96-well plate. All samples were incubated at 50 °C for 2 min. and at 95 °C for 10 min. and then cycled at 95 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min. for 40 cycles. SYBR Green 1 fluorescence emission data were captured and mRNA levels were quantified using the critical threshold (CT) value. Analysis was performed with ABI Prism 7000 (SDS Software). Controls without RT and with no template cDNA were performed with each assay. To compensate for variations in input RNA amounts, and efficiency of reverse transcription, GAPDH mRNA was quantified and results were normalized to these values. Relative gene expression levels were obtained using the ΔΔCt standard 2−ΔΔCt calculations and expressed as a fold change of a control sample (Winchester et al., 1999). Amplification specific transcripts were further confirmed by obtaining melting curve profiles.

2.3. Statistical analysis

Statistical analysis of the data was performed using Statistica 8.0. Results are expressed as the mean standard deviation (±SD). In order to select the type of measurement carried out an analysis of the variables tested, which showed that they do not have a normal distribution. The comparison of mRNA levels, and such clinical variables as the age at onset and the number of episodes (Mann–Whitney U-test; p > 0.05) of previous studies which showed that the levels or activities of all four enzymes are significantly increased in depressive disorders and/or processes characteristic for depression (Balboa and Balsinde, 2006; Guo et al., 2009; Vaccarino et al., 2008; Wang et al., 2008). Obtained data are in line with those obtained previously using animal depression model (Guo et al., 2009; Wang et al., 2008) and might explain the effectiveness of COX-2 inhibitors in depression treatment (Müller et al., 2006).

Findings of this study contribute to better understanding of the association between molecules related to IO&NS and investigated disease. Moreover, such result may partially explain the source of overproduction of free radicals and presence of oxidative stress in depression (Forlenza and Miller, 2006; Galecki et al., 2009). For example, there are data showing COX-2 involvement in increased low density lipoprotein LDL oxidation (Cheney et al., 2003). Study by Maki et al. (2009) observed a strong relationship between lipid peroxidation and MPO expression.

COX-2, MPO, iNOS, and sPLA2 play a role in other diseases that co-occur with depression and that are characterized by (neuro)inflammation and activation of O&NS pathways. Therefore, our data suggest that “a common etiology”, i.e., induction of IO&NS genes, may underpin both depression and other diseases characterized by activated IO&NS pathways. For example, an increased expression of gene coding COX-2 was found in the frontal cortex of Alzheimer’s disease (AD) (Pasinetti and Aisen, 1998), a neuroinflammatory disorder that shows a strong comorbidity with depression (Leonard, 2007). Significantly increased of mRNA level for COX-2 was demonstrated in asthma (Long et al., 2004). An elevated expression of MPO protein was found in AD (Maki et al., 2009), multiple sclerosis (Gray et al., 2008) and asthmatic patients (Ekmecki et al., 2004) all IO&NS disorders that often co-exist with depression (Chwastik et al., 2002; de Miguel Diez et al., 2011; Usman et al., 2010). Increased expression of NOS2A mRNA was found in asthmatic subjects (Redington et al., 2001) and in peripheral blood monocyte-derived macrophages in patients with inflammatory arthritis (Pham et al., 2003). High amounts of transcripts for sPLA2-IIA were also observed in patients with CVD (Dutour et al., 2010). It seems important as PLA2 inhibitors are becoming

| Table 1 Statistical analysis comparing the results of mRNA expression of NOS2A, PLAZG2, MPO, and PTGS2 in patients with RDD and normal controls. |
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| Groups | NOS2A (±SD) | PLAZG2 (±SD) | MPO (±SD) | PTGS2 (±SD) |
| RDD (181) | 0.1 (±0.02) | 0.12 (±0.02) | 0.3 (±0.004) | 0.22 (±0.03) |
| Control (149) | 0.07 (±0.02) | 0.07 (±0.02) | 0.28 (±0.04) | 0.2 (±0.03) |
| Z-value | Z = −9.3; | Z = −14.5; | Z = −4.9; | Z = −6.81; |
| p-value | p < 0.001 | p < 0.001 | p < 0.001 | p < 0.001 |

N = number of patients; RDD = recurrent depressive disorders patients; Control = control group subjects; NOS2A = inducible nitric oxide synthase gene, PLAZG2 = secretory phospholipase A2 type IIA gene; MPO = myeloperoxidase gene; PTGS2 = cyclooxygenase-2 gene; Z = value of U Mann–Whitney’s test; p = level of statistical significance; SD = standard deviation.
a promising therapeutic agent for the treatment of inflammatory disease (Magrioti and Kokotos, 2010). sPLA2-IIA is associated with atherosclerosis (Divchev and Schieffer, 2008). Increased expression of sPLA2-IIA and increased oxidized LDL antibodies in depression (Maes et al., 2010) may explain the co-incidence between atherosclerosis and depression (Paranthaman et al., 2010). Moreover, PLA2G2 mRNA is up-regulated in AD (Moses et al., 2006) and sPLA2-IIA has been recently shown to contribute to neuronal death (Schaeffer et al., 2010) which also has been observed in depressive disorder (Maes et al., 2011a).

Previously we have shown that functional single nucleotide polymorphisms in the genes encoding COX-2 and MPO confer susceptibility to develop rDD (Galecki et al., 2010a, 2010b). Therefore, one might conclude that the increased gene expression for COX-2 and MPO established in the present study might be genotype dependent. In our previous study we also examined polymorphism G/A in exon 22 of NOS2A gene coding iNOS. We found AA genotype decreasing the risk of depression, but G allele is a risk factor. Investigating exon 22G/A NOS2A polymorphism we wanted to find whether NOS2A gene and its variants are worth being examined in depressive disorder and can extend a group of susceptibility genetic variants to depression. The investigated variant is not functional, and there is rather no evidence for a relation between the single nucleotide polymorphism and expression. The variant might be in linkage disequilibrium with other variants that affect expression. The expression might also be related to another completely different variant and results showing G allele as a risk factor and increased expression might be circumstances. In addition, increased expression of the gene might not be genotype dependent. Considering the fact that we do not know if there is a relation between gene variants and expression, we should consider our results separately and take into account the fact the mRNA level is increased in rDD. Such results might possibly explain: increased level of NO in the periphery, effects of iNOS inhibitor in depression and generally effectiveness of non-steroid anti-inflammatory drugs (NSAID) in depression management. There are many factors inducing gene expression measured. For example, overexpression of mRNA for COX-2, iNOS and sPLA2-IIA was observed after stimulation with lipopolysaccharides (LPS) and/or proinflammatory cytokines (Anthonsen et al., 2000; Dubois et al., 1998; Kleinert et al., 2004; Long et al., 2004; Pham et al., 2003) — factors that are involved in the pathophysiology of depression (Maes, 2011). Gene expression for sPLA2 is induced by n-3 polyunsaturated fatty acid (PUFA) deprivation (Rao et al., 2007), another factor that is implied in the pathophysiology of the disease in question (Lin et al., 2010).

Although our results on increased gene expression are obtained in peripheral blood, these peripheral inflammatory aberrations might affect brain functions. Interestingly, there are studies reporting that altered mRNA expression in the
periphery might reflect the changes that take place in the brain (Belzeaux et al., 2010; Pandey et al., 2010). There are indeed many different pathways by which peripheral inflammation is translated into the brain and may in turn cause microglial activation and changes in brain areas that modulate depressive-like behaviors (Dantzer et al., 2008; Dilger and Johnson, 2008; Goehler et al., 2007). To sum up, even if the expression of the gene is not activated in the brain of depressed patients there is a strong possibility that brain pathogenic process may result from the peripheral disturbances.

4.1 Limitation

The presented study has limitations related to the fact that patient were being treated with antidepressant. This is relevant due to the fact that some antidepressants may affect mRNA expression (Raeder et al., 2006; Tordera et al., 2005). The present study is not able to answer whether antidepressant drugs may cause a change in mRNA expression. Nevertheless, to the our best knowledge, searching the literature there is no available data regarding the influence of the widely used antidepressants, including selective serotonin reuptake inhibitors (SSRI), on the type of gene expression studied here. In conclusion, we emphasize the importance of increased oxidative and nitrosative stress in recurrent depression and suggest further studies.

5. Conclusion

Our study suggests that COX-2, MPO, iNOS and sPLA2-IIA expression is increased during depression in the peripheral cells. The data indicate that increased expression of COX-2, MPO, iNOS, and sPLA2-IIA, which are key iO&NS enzymes, might be one of the mechanisms of underpinning the pathophysiology of rDD. Our results further extent the role of inflammation and O&NS in the etiology of depression. Our findings suggest that peripheral iO&NS gene expression may explain the molecular mechanism and be a molecular marker of the disease. Investigation of the gene expression can be followed by the examination of protein level and may indicate neuroinflammatory abnormalities in the brain. In some cases, dependent link between gene variants, gene expression, protein level and/or enzyme activity is observed. In such cases genes might act as a marker and may participate in the development of periphery-based diagnostic.

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Conflicts of interest

No conflicts of interest were declared.

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References


World Health Organization, 1992. The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Description and Diagnostic Guidelines.
