OXIDATIVE AND NITROSATIVE STRESS AS WELL AS THE TRYPTOPHAN CATABOLITES PATHWAY IN DEPRESSIVE DISORDERS

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SUMMARY

The aim of this paper is to elucidate the role of oxidative and nitrosative stress as well as the tryptophan catabolites pathway in the development of depression and the mechanism of action of antidepressant drugs, based on the available literature. According to the World Health Organization (WHO), an estimated 350 million people worldwide suffer from depression. The pathogenesis of depressive disorders has not been fully explained yet and several causes of this disease have been suggested. There is evidence for the involvement of several interconnected biochemical pathways, including oxidative and nitrosative stress as well as the tryptophan catabolites pathway. Studies to date indicate that patients with depression have lower levels of enzymatic and non-enzymatic elements of an antioxidant response and, at the same time, they display an increased amount of oxidative stress markers, when compared to healthy individuals. The development of depression is also associated with excessive activity of nitric oxide synthase. Furthermore, decreased levels of tryptophan and increased levels of its harmful catabolites, i.e. kynurenine and quinolinic acid, may lead to progression of the disease. Changes in these biochemical pathways can be used as risk factors for the development of depression and, in the future, they could be utilized as diagnostic biomarkers. Moreover, regulation of biochemical processes may contribute to the development of a new, effective and personalized antidepressant therapy.

Key words: depression - oxidative and nitrosative stress - tryptophan catabolites pathway

List of shortcuts:
CoQ10 – coenzyme Q10;   E-EPA – ethyl ester of eicosapentaenoic;   GPx – glutathione peroxidase;   GSH – glutathione;   IDO – indoleamine 2,3-dioxygenase;   MDA – malondialdehyde;   MDM – human monocyte-derived macrophage;   NO – nitric oxide (II);   NAC – N-acetylcysteine;   PBMC – peripheral blood mononuclear cell;   PUFAs – polyunsaturated fatty acids;   RNS – reactive nitrogen species;   ROS – reactive oxygen species;   SOD – superoxide dismutase;   SSRIs – serotonin-specific reuptake inhibitors;   TCAs – tricyclic antidepressants;   TRD – treatment-resistant depression;   WHO – World Health Organization;   XO – xanthine oxidase;   ZD – depressive disorders;   Zn – zinc;   8-oxoG – 8-oxoguanine;   8-iso-PGF2 – 8-iso-prostaglandin F2

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Introduction

In the 1990s, a psychiatric examination made it possible to determine that mental disorders were much more common than any other somatic chronic disease. At the moment, according to data from the World Health Organization (WHO), depression (depressive disorder – DD) is fourth on the list of the most serious health problems. It is estimated that 350 million people around the world suffer from depression, which is approximately 5% of the global population, whereas in developed countries this percentage might be as high as 10% (The WHO World Mental Health Survey Consortium Prevalence, Severity, and Unmet Need for Treatment of Mental Disorders in the World Health Organization). This problem affects every eleventh person in Poland. Depression can occur in both sexes, but women are nearly twice as likely to suffer from DD than men (Kessler 2003). Suicide attempts are a serious threat during depression episodes. There are around one million suicide deaths from DD annually (about 3000 deaths a day) (Marcus et al. 2012). In addition to the coronary heart disease, depression will have been the second leading cause of world economic decline and social disability by 2020 (Poniatowska-Leszczyńska & Małysszczak 2013). The total cost of treating depression in the United States is about $ 83.1 billion per year. For comparison, the costs of heart failure treatment total about $ 39.2 billion, and in the case of AIDS – about $ 50 billion per year (Dutta et al. 2015, Greenberg et al. 2015, Voigt et al. 2015).

The essence of the problem stems from the lack of knowledge about the aetiological, neurobiological and pharmacological mechanisms of pathogenesis at cellular and molecular levels. Moreover, a diagnosis of the disease is only observational, so depressive syndromes still cannot be clearly codified and understood (Willner et al. 2013). It seems that a complex network of interconnected genetic, biological (variable levels of neurotransmitters, for example dopamine and serotonin), environmental, and psychosocial factors predisposes to the development of DD (Table 1) (Kendler et al. 1993,
Table 1. Risk factors for the development of depressive disorders

<table>
<thead>
<tr>
<th>Factors</th>
<th>Characteristic features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic factors</td>
<td>Genes encoding receptors, transporters, neurotransmitters, enzymes involved in signalling pathways</td>
</tr>
<tr>
<td>Monoaminergic factors</td>
<td>Deficiency of norepinephrine, serotonin, signalling disorders, low expression of serotonin receptor</td>
</tr>
<tr>
<td>Hormonal regulation</td>
<td>Disorders of the pituitary, adrenal, thyroid and gonadal functions (excessive production of cortisol)</td>
</tr>
<tr>
<td>disorders</td>
<td></td>
</tr>
<tr>
<td>Biological rhythm</td>
<td>Sleep disorders, insomnia, excessive sleepiness during the day</td>
</tr>
<tr>
<td>sleep-wake</td>
<td></td>
</tr>
<tr>
<td>Neuropeptides</td>
<td>Level change of Beta-endorphin, corticotropin releasing hormone, neuropeptide Y</td>
</tr>
<tr>
<td>Immune factors</td>
<td>Depression as a complication of inflammation</td>
</tr>
<tr>
<td>Structural changes in</td>
<td>Neuronal necrosis in the hippocampal area, frontal lobes, subcortical nuclei</td>
</tr>
<tr>
<td>the central nervous</td>
<td></td>
</tr>
<tr>
<td>system</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Somatic diseases – cardiovascular diseases, cancer</td>
</tr>
<tr>
<td></td>
<td>Diseases of the central nervous system – Alzheimer’s disease, epilepsy</td>
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<tr>
<td></td>
<td>Medications – antihypertensive drugs, steroids</td>
</tr>
</tbody>
</table>

Sullivan et al. 2000, Dröge 2002). Although the pathogenesis of the disease is not fully understood, there are reports about the role of related biochemical pathways – oxidative and nitrosative stress, and the pathway of tryptophan catabolites – in the development of depressive disorders.

Nitrosative and oxidative stress

Nitrosative and oxidative stress are inevitably associated with the occurrence of free radicals. In 1956, an American gerontology specialist, Denham Harman, described free radicals as compounds involved in the processes leading to cell damage, mutagenesis, tumour development and biological aging. Studies of reactive oxygen species (ROS) and nitrogen (RNS) exhibit the dual nature of the compounds. On the one hand, these reactive compounds can damage biomolecules, which may lead to tissue dysfunction. On the other hand, they constitute an essential element in signal transduction pathways that activate a stress response. The concentration of radicals decides on their nature – at low concentrations, they are modulators; at high levels, they have a toxic effect (Dröge 2002, Lusczewski et al. 2007).

Oxidative and nitrosative stress in the development of depression

An imbalance between the production and neutralization of ROS is characteristic of patients with DD. A potential cause of DD seems to be reduced activity of antioxidants, mainly reduced levels of zinc (Zn), coenzyme Q10 (CoQ10), vitamins A, C and E, glutathione in the plasma, which intensify oxidation processes, including lipid peroxidation, and damage of proteins and DNA (Pandya et al. 2013). However, the results of studies over antioxidants are inconsistent. In some of them, patients did not differ from healthy individuals in terms of plasma levels of vitamins A, C and E (Kotan et al. 2011). On the other hand, Maes et al. (2000) found that levels of vitamin E in plasma of DD patients were lower as compared to healthy volunteers (Maes et al. 2000). This is confirmed by a study of serum alphatocopherol levels (Owen et al. 2005). Another study suggests that the severity of the disease is associated with increased levels of vitamin C in plasma (Kobrosly & Wijngaarden 2010). The differences in results may be due to their properties – vitamins A and E are fat soluble, and their distribution is determined by the amount of low density lipoproteins and triglycerides (Maes et al. 2000). In addition, some inaccuracies may arise from size differences of the studied groups, environmental impacts and the severity of the disease. Furthermore, patients with depression are characterized by decreased activity of glutathione peroxidase (GPx) and increased activity of xanthine oxidase (Ox), an enzyme responsible for the production of hydrogen peroxide and superoxide anion, and superoxide dismutase (SOD) (Maes et al. 2011a, Wei et al. 2009). In the case of SOD and GPx, there are also some discrepancies between studies. Some of them confirm a link between depression and increased activity of SOD (Wei et al. 2009, Sarandol et al. 2007). Some researchers confirmed that the occurrence of depression was associated with excessive activity of Gpx (Bilici et al. 2001); while others did not detect any difference between the study and control groups (Kotan et al. 2011, Kodydkova et al. 2009).

From the perspective of depression, zinc (Zn) is an important microelement. Zn is involved in the regulation of learning and mental functions. Researchers noticed a reduction in the number of progenitor cells and immature nerve cells in the hippocampus of rats treated with a diet low in Zn (Suh et al. 2009). Further studies involving these animals showed that a deficiency of Zn led to the occurrence of DD symptoms (Ho & Wang 2010). Cellular biomolecule damage is one of the consequences of oxidative and nitrosative stress. Some of the products of these processes can serve as specific markers, which may be used in early and accurate diagnostics in the future. One example of such markers includes 8-oxoguanine (8-oxoG), an indicator of elevated oxidative DNA damage, the increased levels of which were found in urine, cerebrospinal fluid, serum, and peripheral blood mononuclear cells (PBMCs) of depressed
patients (Poniatowska-Leszczyńska & Małyszek 2013, Galecki et al. 2009). Subsequent research conducted using PBMC derived from patients with DD confirmed that depression was accompanied by an increased amount of oxidatively modified nucleobases (Czarny et al. 2015).

Another potential marker of depression can include an increased concentration of malondialdehyde (MDA), a product of polyunsaturated fatty acids (PUFAs) peroxidation caused by elevated levels of ROS and RNS (Herken et al. 2007). In addition, patients with subsequent recurrent depressive episodes show a higher level of MDA than patients with the first episode of this disease. Studies showed that patients with DD were characterized by reduced levels of polyunsaturated fatty acids (PUFAs) in the cell membrane of erythrocytes. Degradation of membrane lipids can be caused by the intensification of peroxidation processes (Herken et al. 2007, Stefanescu & Ciobica 2012). Another product of fatty acid oxidation is 8-iso-prostaglandin F2 (8-iso-PGF2), which is generated during oxidation of arachidonic acid. 8-iso-PGF 2 can be a potential biomarker in the diagnosis of DD because people with depressive disorders have elevated levels of this compound (Dimopoulos et al. 2008).

Patients with depression are also characterized by abnormalities in the RNS production pathway as manifested by elevated levels of nitric oxide (NO). NO can have a highly toxic nature. This is due to the reaction of NO with superoxide, which leads to the formation of peroxynitrite – a compound that is highly reactive with aromatic amino acids, especially with tyrosine and phenylalanine, resulting in nitrated forms of these amino acids (Maes et al. 2011b). Furthermore, patients with depression are characterized by elevated levels of IgM antibodies against nitrated protein substrates (mainly nitrated form of tyrosine) that arise due to the high reactivity of peroxynitrite (Dimopoulos et al. 2008).

In addition, patients after suicidal attempts had higher levels of NO in serum as compared to patients without such attempts. An analysis using neurons originating from the suprachiasmatic nucleus of patients who committed suicide confirmed an increased expression of the nitric oxide synthase. Moreover, studies showed that patients with recurrent depression had significantly elevated levels of NO in serum (Dimopoulos et al. 2008). However, Talarowska et al. did not detect any significant differences in the expression of inducible nitric oxide synthase between patients with a single depression episode and a group suffering from the recurrent form of the disease (Talarowska et al. 2012).

Moreover, research showed that decreased activity of defence mechanisms against ROS and RNS, in both peripheral blood cells and nerve cells, may be accompanied by the development of DD (Maes et al. 2009). It suggests that the factors located in the periphery and involved in both oxidative and nitrosative stress may penetrate the blood-brain barrier. ROS and RNS can act neurotoxicity in the brain (Talarowska et al. 2015). A meta-analysis confirmed that elevated levels of ROS in depression might cause degradation of neurons in the hippocampus (Leonard & Maes 2012). In addition, tests of the blood-brain barrier permeability suggest that it is hyperpermeable in depression – protein elements (e.g. TRYCATs catabolites) can easily penetrate the brain (Buczko et al. 2005).

Tryptophan catabolites pathway

Tryptophan is an essential aromatic exogenous amino acid. About 30% of the total pool of tryptophan delivered into the body is used for protein synthesis. The remainder of tryptophan is degraded through the kynurenine pathway and undergoes non-protein transition to serotonin and melatonin. Tryptophan metabolism can be divided according to the place of its course. On the periphery, tryptophan undergoes three types of transformations: rupture of the indole ring leading to the generation of kynurenine, hydroxylation leading to the formation of serotonin, and decarboxylation leading to the formation of tryptamine. In the central nervous system, tryptophan is a substrate for the production of melatonin (the hormone responsible for maintaining normal circadian rhythm) and serotonin (the neurotransmitter responsible for emotions) (Keszthelyi et al. 2008).

Tryptophan catabolites pathway in the development of depression

The development of depression is linked with a reduced level of tryptophan and increased levels of its harmful metabolites (kynurenine and quinolinic acid) in plasma. DD patients show increased activity of key enzymes in the pathway of tryptophan catabolites – tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase (Buczko et al. 2005). Studies revealed that kynurenic acid might have antidepressant and neuroprotective properties. The levels of this compound in patients with depressive symptoms are reduced (Maes et al. 2011b). Elements of the TRYCATs pathway were also analysed as potential markers in the diagnosis of DD. Maes et al. (2012) confirmed that tryptophan metabolites (kynurenine, kynurenic acid, quinolinic acid) could be used as DD biomarkers in the future. Moreover, reduced levels of tryptophan in plasma may suggest the existence of mood disorders in patients (Maes et al. 2011b).

A deficit of serotonin is also believed to be one of the causes of depression (Maes et al. 2012). The essence of this problem has led to propose the foundations for the serotonin hypothesis of depression by Lapin and Oxenkrug. This hypothesis implies that a reduced level of serotonin in the brain is a risk factor for depression development. However, inaccuracies resulting from subsequent studies have led to a modification of this hypothesis. The currently applicable theory emphasizes the role of functional deficiency in the transmission signal by monoaminergic neurotransmitters and their
postsynaptic receptors in DD development. Molecular studies showed that monoamine deficiency is associated with impaired gene expression of receptors for these neurotransmitters and the enzymes involved in their metabolism (Lapin & Oxenkrug et al. 1969).

Melatonin, the neurohormone responsible for circadian rhythm regulation, is another derivative of tryptophan associated with DD. Eighty percent of depressed patients suffer from sleep disorders. Among them, 85% struggle with insomnia, while 15% have to deal with excessive daytime sleepiness. In addition, intensifying insomnia may contribute to the risk of recurrence and increase in the severity of a DD episode. Furthermore, it was demonstrated that insomnia was characteristic of the people who have attempted suicide. Untreated insomnia can also cause recurrence of full-blown depression (Heitzman 2009). Moreover, seasonal depressive episodes may indicate the involvement of circadian rhythms in the pathophysiology of the disease. An increased number of patients are hospitalized due to DD in spring and autumn (Gawlik & Nowak 2006).

Disorders of biochemical pathways in the diagnosis and antidepressant treatment

The main aim of antidepressant treatment is to restore a patient’s well-being and high functional efficiency. Nevertheless, treatment of up to one third of patients with mood disorders is not effective. The problem of treatment-resistant depression (TRD) is a challenge for both doctors and researchers (Fava 2003). So far, studies have shown that DD is accompanied by reduced tryptophan levels in blood serum and a reduced ratio of tryptophan to CAA concentrations (competing amino acids, including valine, leucine, tyrosine, phenylalanine, isoleucine). Furthermore, studies demonstrated that patients who were resistant to antidepressant treatment using SSRIs (serotonin-specific reuptake inhibitors), TCAs (tricyclic antidepressants), and heterocyclic drugs had lower treatment efficiency than patients without such resistance (Maes et al. 1997a).

Dysfunctions of the biological clock are another feature of depression. Therefore, methods enabling the use of melatonin in the treatment of patients with TRD have been developed. These studies confirmed that melatonin could be used as an adjuvant therapy. The studies also demonstrated that treatment with the use of SSRIs and derivatives of melatonin (agomelatine) was more effective than a monotherapy with SSRIs (Benedetti et al. 1997a).

As was already pointed out, the levels of adenosine deaminase and xanthine oxidase were significantly higher, and the levels of superoxide dismutase were lower, in patients with DD as compared with healthy volunteers. SSRI treatment contributed to lowering the activity of xanthine oxidase and adenosine deaminase. Therefore, their activity can be used to monitor the efficacy of antidepressant treatment using SSRI (Stefanescu & Ciobica 2012).

The antioxidants which simultaneously modulate oxidative and nitrosative stress pathways are important in DD treatment. Ebselen (an organic compound containing selenium), which mimics the effect of GPx and simultaneously inhibits IDO, is one of such examples. Studies on mice showed that ebselen affected the behaviour of experimental animals (improving behavioural testing results), thus indicated the potential of organic selenium as a therapeutic agent for mood disorders (Posser et al. 2009). On the other hand, a research study on human monocyte-derived macrophages (MDM) confirmed that ebselen inhibited cellular activity of IDO at the post-translational level. Ebselen acts by binding cysteine residues of the enzyme, resulting in a conformational change of the protein and preventing the formation of bonds in the substrate binding pocket (Terentis et al. 2010).

As noted above, depression is associated with low levels of CoQ10. Additional studies showed that patients with TRD had significantly lower plasma levels of CoQ10 compared to patients without TRD. Therefore, this parameter can be used to distinguish TRD from the classic forms of depression (Stefanescu & Ciobica 2012). Another experiment suggests that patients with TRD depression are characterized by low Zn2+ concentrations in serum (Siwek et al. 2010). Patients with no response to SSRIs, TCAs and electroshock have significantly reduced levels of Zn2+ in blood plasma (Swardfager et al. 2013, Maes et al. 1997b). SSRI therapy supplemented with zinc may cause a reduction in the severity of the disease according to Beck’s Depression Inventory as compared to the placebo group (Nowak et al. 2003, Ranjbar et al. 2013). Sawada and Yokoi (2010) indicated the potential use of zinc supplementation in the prevention of depressive symptoms. However, research within this area is not clear (Sawada & Yokoi 2010). Nguyen et al. (2009) did not confirm the hypothesis about the medicinal properties of zinc (Nguyen et al. 2009).

Depression is also linked with low levels of vitamin A. Studies on rats demonstrated that treatment with SSRIs helped to optimize the level of vitamin A in the brain (Eren et al. 2007). After 24 weeks of treatment, patients with SSRI displayed normalized levels of vitamin A in plasma. Regulating the level of vitamin A in the serum of treated patients may prove to be useful in monitoring the effectiveness of SSRI treatment (Koton et al. 2011).

Studies suggest that diet is of importance in the prevention and treatment of depression. The results of epidemiological studies indicate that a diet rich in omega-3 fatty acids (mainly of fish origin) may protect against the development of depression (Tanskanen et al. 2001). Omega-3 acids impact the metabolism of neurotransmitters and modulate the effects of signal transduction pathways in cells (Horrobin 2001). Maes et al. (1999) detected a change in the composition of fatty acids in the serum of patients with DD. Depressed patients were characterized by low levels of not only...
PUFA but also omega-3 and omega-6 fatty acids (Maes et al. 1999). Furthermore, Nements et al. (2002) confirmed enhanced efficacy of antidepressant treatment supplemented by the ethyl ester of eicosapentaenoic acid (acids from the omega-3 group). The patients who were additionally given the ethyl ester of eicosapentaenoic acid (E-EPA) were characterized by a lower mean score on the Hamilton scale as compared to the patients in the placebo group (treated with antidepressants and placebo) (Nemets et al. 2002).

Another compound that can effectively modulate the treatment of depression is N-acetylcysteine (NAC). It is a precursor of glutathione (the main antioxidant) and is particularly important for maintaining the normal level of glutathione (GSH) in the brain. In addition, it was demonstrated that NAC modulates neurogenesis and prolongs survival of neurons (Dean et al. 2011). Studies showed that treatment with the addition of NAC reduced the severity of depressive symptoms in comparison to classical treatment. Patients undergoing an assisted therapy showed an improvement in the quality and satisfaction with life as well as feeling pleasure (Berk 2011). A summary of potential biomarkers is presented in Table 2.

### Table 2. Potential biomarkers in depressive disorders

<table>
<thead>
<tr>
<th>Potential biomarkers</th>
<th>Level of biomarkers in depressed patients</th>
</tr>
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<tbody>
<tr>
<td>Oxidative stress</td>
<td></td>
</tr>
<tr>
<td>malondialdehyde (plasma)</td>
<td>↑</td>
</tr>
<tr>
<td>8-oxoguanine (plasma)</td>
<td>↓</td>
</tr>
<tr>
<td>8-iso-prostaglandin F2 (urine)</td>
<td>↑</td>
</tr>
<tr>
<td>superoxide dismutase (plasma)</td>
<td>↓</td>
</tr>
<tr>
<td>glutathione peroxidise (plasma)</td>
<td>↓</td>
</tr>
<tr>
<td>glutathione reductase (plasma)</td>
<td></td>
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<tr>
<td>Nitrosative stress</td>
<td></td>
</tr>
<tr>
<td>nitrified protein forms</td>
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<tr>
<td>TRYCATs pathway</td>
<td></td>
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<tr>
<td>level of tryptophan (serum)</td>
<td>↓</td>
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<tr>
<td>tryptophan catabolites</td>
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</table>

### Conclusion

Depression is a serious mental disorder which affects an increasing number of people. In addition, DD is a disease that may vary depending on the patient. Therefore, a correct diagnosis as well as an appropriate and effective therapy are so difficult. The level of knowledge regarding the mechanisms of DD development is still insufficient. Previous studies confirmed the participation of certain processes in the development of depression symptoms and resistance to traditional pharmacotherapy. They include oxidative and nitrosative stress, and the tryptophan catabolites pathway. Being aware of the molecular mechanisms of depression development may lead to the development of effective biomarkers in the future. Regulation of specific processes may also enable the development of new, effective and personalized treatment options. Therefore, it is essential to continue research in order to confirm the links between the biochemical pathways – oxidative and nitrosative stress, and the tryptophan catabolites pathway – and depressive disorders.

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None to declare.

### Contribution of individual authors:

Paulina Wigner – conception and design of the publication, literature searches and analyses, acquisition of data, participate in drafting the article, execution of tables, approval of the final version.

Piotr Czarny – design of the publication, acquisition of data, participate in drafting the article, approval of the final version.

Piotr Galecki – design of the publication, participate in revising it critically for important intellectual content, approval of the final version.

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### References


