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Oxidative Stress and Methods of Its Determination in Experimental Brain Pathology

Maksimovich N.Ye., Bon L.I*., Holik S.V

Grodno State Medical University, Grodno, Republic of Belarus

***Corresponding Author**: - Bon L.I., Candidate of Biological Science, assistent professer of Chair of pathological physiology of the name of D.A. Maslakov, Education Establishment "Grodno State Medical University".

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Abstract

Oxidative reactions and the substances formed as a result of their course are important in the vital activity of the cells of the whole organism and the brain, in particular. It has been established that oxygen radicals function as a messenger, responsible for neuronal activity, regulate cerebral blood flow, apoptosis and other processes necessary for the functioning of the brain. It has been shown that nerve impulse conduction is also associated with the formation of free-radical forms of phospholipids.

The purpose of this article is to summarize and systematize literature data on the mechanisms of oxidative stress and describe the method for studying it in modeling experimental cerebral pathology.

Oxidative stress, which is the result of an imbalance between prooxidants and antioxidants towards the former due to excessive production of free radicals and/or a decrease in the activity of antioxidant defense, underlies the pathogenesis of many diseases and its study in modeling experimental pathology serves as a fundamental basis for clinical research.

Key words: Oxidative stress, brain pathology, experiment

Introduction

Oxidative reactions and the substances formed as a result of their course are important in the vital activity of the cells of the whole organism and the brain, in particular. It has been established that oxygen radicals function as a messenger, responsible for neuronal activity, regulate cerebral blood flow, apoptosis and other processes necessary for the functioning of the brain. It has been shown that nerve impulse conduction is also associated with the formation of free-radical forms of phospholipids.

To date, a general idea has been formed about the prooxidant-antioxidant system of the body. An imbalance between the prooxidant and antioxidant links of this system leads to hyperproduction of oxygen radicals and damage to cells and tissues. The general mechanisms underlying damage to body tissues with the participation of oxidative reactions are well studied and have a universal character. However, in the brain, as well as in other organs and systems, they have specific features, which is important to take into account when modeling the experimental pathology of the central nervous system.

The purpose of this article is to summarize and systematize literature data on the mechanisms of oxidative stress and describe the method for studying it in modeling experimental cerebral pathology.

Characterization of reactive oxygen species

Reactive oxygen species (ROS) or free radicals are particles (usually unstable) containing one or more unpaired electrons. An unpaired electron occupies an atomic or molecular orbital. Oxygen radicals have paramagnetic properties, since the presence of unpaired electrons causes them to interact with a magnetic field. The presence of an unpaired electron is the reason for the high reactivity of ROS.

The term "ROS" is broader than "free radicals of oxygen", since, in addition to the latter, it also includes the following molecules: H2O2, singlet oxygen (•O2), ozone (O3) and hypochlorite (HOCl).

ROS are physiological metabolites formed during normal metabolism. Oxygen-containing radicals are chemically highly toxic and highly reactive. Taking electrons from organic molecules, they turn them into peroxide compounds and start chain reactions.

Oxygen radicals formed in the body can be divided into natural and initiated.

In turn, natural oxygen radicals are divided into primary, secondary and tertiary.

The main source of oxygen radicals is molecular oxygen. Primary oxygen radicals include: O2• (superoxide anion radical or superoxide), HO2• (hydroperoxide radical), NO• (nitric oxide or nitroxide), semiquinones formed in the reactions of electron carriers such as coenzyme Q (Q•) and flavoproteins.

The formation of primary radicals is carried out with the participation of enzymatic systems (NADPH oxidase, NO synthase, cyclooxygenase, lipoxygenase, monooxygenase, xanthine oxidase, etc.). They perform important functions for the body: they are involved in tissue respiration, antimicrobial protection, and regulation of vascular tone.

From the primary radical - O2•, as well as as a result of other reactions in the body, active molecular compounds are formed: HO• (hydroxyl radical), RO• (alkoxy radical), RO2• (peroxide or peroxide radical), H2O2, HOCl, lipid hydroperoxides - secondary oxygen radicals. Normally, they are involved in the regulation of the permeability of cell membranes, they are messengers, and in excess they have a destructive effect on cell structures.

Branching chain reactions occurring in the presence of Fe2+ or Cu2+ lead to an increase in the amount of lipid radicals.

In this case, hydrogen peroxide decomposes with the formation of a hydroxyl radical - one of the most active radicals (Fenton reaction):

$H_2O_2+Fe^{2+} \rightarrow OH^{\bullet}+OH^{-}+Fe^{3+}$

$H_2O_2+Cu^{+} \rightarrow OH^{\bullet}+OH^{-}+Cu^{2+}$

Another source of hydroxyl radicals can be photolysis and radiolysis of water upon irradiation:

$H_2O_2+O_2^{-} \rightarrow OH^{\bullet}+OH^{-}+O_2$ (Haber-Weiss reaction)

The hydroxyl radical is extremely reactive and destroys almost any molecule it encounters. Acting on SH-groups, histidine and other amino acid residues of

proteins, OH• causes denaturation of the latter and inactivates enzymes. In nucleic acids, OH• destroys carbohydrate bridges between nucleotides and thus breaks DNA and RNA chains, resulting in mutations and cell death. Penetrating into the lipid layer of cell membranes, the hydroxyl radical initiates lipid chain oxidation reactions, which leads to membrane damage, disruption of their functions and cell death.

Radicals can be formed under the action of ultraviolet, ionizing radiation, illumination with intense visible light, for example, laser light. Initiated radicals are formed when xenobiotics enter the body during their metabolism and have a toxic effect.

ROS functions are normal

Interacting with cysteine residues, sulfhydryl groups in proteins, ROS change the activity of transcription factors, tyrosine protein kinases, Na/K ATPase.

ROS affect the rate of binding of cytoplasmic calcium to calmodulin, the activity of calcium ATP-ase, and regulate the phases of the cell cycle.

ROS-initiated free radical lipid peroxidation (SPOL) regulates the composition of the lipid bilayer of the plasma membrane (its fluidity changes), as a result, the activity of membrane-bound enzyme proteins, its permeability to various ions, and the transport of substances into the cell change.

Depending on the intensity of ROS generation, they can affect the mechanisms of intracellular signal transduction. Free oxygen radicals act as second messengers in signaling cascades triggered by angiotensin II, endothelin, etc. Thus, NO•, which is formed by the endothelium of blood vessels with the participation of the heme-containing enzyme NO-synthase, plays a key role in the regulation of vascular tone and blood pressure; its deficiency leads to hypertension, and its excess leads to hypotension.

Oxygen radicals formed in the cytosol of the cell in response to stimulation by growth factors are involved in the regulation of the proliferative process. The cell death inducer apolipoprotein A1 (APO-1) has pro-oxidant properties.

In addition to the complete four-electron reduction of the O2 molecule to water in the respiratory chain of mitochondria in aerobic cells, incomplete one-three-electron reduction of oxygen occurs with the successive formation of various ROS, which include the superoxide anion, hydrogen peroxide H2O2, and the most active radical, hydroxyl (HO•).

Electron donors can be Fe2+, Cu2+ or semiquinones.

In addition to the formation of ROS in the process of electron transport in the respiratory chain, their production occurs during the synthesis of prostaglandins and leukotrienes from arachidonic acid, the metabolism of catecholamines, etc.

Other less important sources of basal ROS formation in cells are some oxidases (aldehyde oxidase, dihydroorotate dehydrogenase, tryptophan dioxygenase, NO synthase).

Reactive oxygen species can be formed not only intracellularly, but also extracellularly with the participation of leukocytes. In the process of contact of a phagocyte with a microbial cell, the so-called "metabolic explosion" or "respiratory explosion" occurs, characterized by the activation of NADPH- and NADH-oxidase, myeloperoxidase and amino acid oxidases, which leads to the formation of ROS (O2•, H2O2, OH2•, HOCl) with powerful bactericidal action.

Oxidative stress and its causes

A prolonged and significant increase in ROS production leads to the activation of LPO. ROS and LPO products, formed in large quantities, have a toxic effect on the cell, which can even result in its death. This condition is referred to as "oxidative stress" or "oxidative stress".

“Oxidative stress” is the result of an imbalance between prooxidants and antioxidants towards the former due to excessive production of ROS and / or a decrease in the activity of antioxidant protection (AOP).

The role of excess oxygen free radicals in the mechanisms of ischemic and reperfusion organ damage, aging, carcinogenesis, the development of some autoimmune processes, atherosclerosis, cell death due to apoptosis, and a number of other pathological conditions has been revealed.

Molecular targets for ROS in the cell are membrane

polyunsaturated fatty acids, DNA carbohydrate bridges, and proteins.

There are two main mechanisms of the aggressiveness of free radicals (FR) and lipid peroxidation, causing damage to the cell membrane:

1. Excess formation of SR and activation of LPO. In this situation, excessive formation of SR and LPO activity exceeds the ability of the cell's antioxidant system to inactivate them. This is observed when:

- exposure of the cell to ultraviolet rays and ionizing radiation;
- prolonged stressful influences (formation of SR during the metabolism of a significant amount of catecholamines);
- hypervitaminosis D, K, A, excessive metabolism of prostaglandins;
- hypoxia;
- "Ischemia-reperfusion" syndrome;
- hyperoxia (with hyperbaric oxygenation);
- malignant neoplasms.

2. *Weakening of the antioxidant system.* In this case, the formation of SRs corresponds to the norm, but their inactivation is insufficient due to the deficiency of AOD factors.

This mechanism can take place when:

- hereditary disorders of synthesis and activity of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, etc.);
- senescence
- deficiency in food of iron, manganese, selenium and zinc;
- hypovitaminosis E, C, A, PP, B2;
- disturbances in the Krebs cycle leading to insufficient formation of NADPH and NADH to restore a number of antioxidants;
- exposure of cell membranes to substances with detergent action (bile acids, etc.).

Free radical lipid peroxidation

Free radical lipid peroxidation of cell membranes can be caused by both non-enzymatic and enzymatic processes. Non-enzymatic initiation of SPOL may be due to the action:

- physical factors (ionizing and ultraviolet radiation);
- chemicals (atmospheric pollutants - nitrogen oxides and ozone, various xenobiotics - herbicides, phytoncides, medicinal substances), which, when transformed in the human body, can be sources of ROS.

Enzymatic initiation of LPOL is primarily due to the activation of such enzymes as NADPH oxidase, xanthine oxidase, NO synthase, and lipoxygenase.

LPO is a chain reaction with the formation of lipid radicals and their peroxides, which initiate its further distribution.

The development of oxidative stress includes: oxygen initiation of free radical lipid peroxidation, formation of lipid free radicals and their peroxides.As a result of the attack of ROS, including the hydroxyl radical, of an unsaturated fatty acid (LH), a lipid alkyl radical (L.) is formed:

$LH + R^{\cdot} \rightarrow RH + L^{\cdot}$

Subsequently, the lipid alkyl radical interacts with molecular oxygen to form the lipid peroxyl radical (LOO.):

$L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$

As the oxidation chain continues, the lipid peroxyl radical attacks another unsaturated fatty acid (LH) to form lipid hydroperoxide (LOOH) and a new lipid alkyl radical (L.):

$LH + LOO^{\bullet} \rightarrow L^{\bullet} + LOOH$

The last two reactions alternate with each other.

Due to the interaction of lipid hydroperoxide with ferrous iron, the oxidation chain is branched with the formation of an alkoxy lipid radical (LO.), which, in reaction with a new polyunsaturated fatty acid, forms a hydroxy derivative of the lipid (LOH) and a lipid alkyl radical interacting with molecular oxygen:

$LOOH + Fe^{2+} \rightarrow LO^{\cdot} + OH^{-} + Fe^{3+}$

$LH + LO^{\cdot} \rightarrow L^{\cdot} + LOH$

The breakage of lipid chain oxidation can occur spontaneously, as well as under the action of tocopherol (vitamin E) and nitric oxide.

Spontaneous break:

$LOO^{\cdot} + LOO^{\cdot} \rightarrow$ non-radical products

Termination of chain oxidation under the action of α-tocopherol (TocOH):

$LOO^{\cdot} + TocOH \rightarrow LOOH + TocO^{\cdot}$

Some of the tocopheroxyl radicals (TocO.) are inactivated when interacting with each other to form covalently bound dimeric tocopherol molecules:

$TocO^{\cdot} + TocO^{\cdot} \rightarrow TocO\text{-}TocO$

Under the action of β-carotene, tocopheroxyl radicals can be converted into α-tocopherol.

Under certain conditions, the tocopheroxyl radical can initiate LPOL:

$$LH + TocO^{\cdot} \rightarrow L^{\cdot} + TocOH$$

Breaking of the chain oxidation of lipids is also possible when the lipid peroxyl radical interacts with nitric oxide, resulting in the formation of organic nitrate:

$LOO^{\cdot} + NO \rightarrow LONO_2$

The main damaging effects of ROS within the plasmalemma and other membranes are:

- *dysfunction of membranes due to the oxidation of thiol groups* (SH) of sulfhydryl proteins with the formation of a thiyl radical (Pt-S.), derivatives of sulfinic (Pt-SO2H) and sulfonic (P-SO3H) acids:

$Pt\text{-}SH + L^{\cdot} \rightarrow LH + Pt\text{-}S^{\cdot}$

$Pt\text{-}S^{\cdot} + O_2 \rightarrow Pt\text{-}SO_2H$

$PtSO_2H + O_2 \rightarrow PtSO_3H,$

and also due to the formation of disulfide bonds within one protein molecule and between proteins located nearby.

At the same time, the end sections of transmembrane glycoproteins, including cellular receptors, are split off or change their localization, which leads to a violation of the receptor function, a change in the functioning of ion pumps, and an increase in the permeability of the plasma membrane for cations and anions and other substances. Due to the “breakdown” of the Na-K pump, Na + accumulates in the cell, and K + leaves it, which leads to a smoothing of the sodium-potassium gradient and a decrease in the resting potential. Due to a change in the work of the Ca2+ ATP phase, an increase in the concentration of calcium in the cell occurs.

- *membrane lipid peroxidation.* This toxic effect is the most dangerous for the cell, since not only the lipid bilayer is damaged, but also the protein and carbohydrate components of the plasmalemma that are closely related to it. The most pathogenic effect of ROS is for membrane unsaturated fatty acids. In this case, the stratification of the lipid layer of the plasmalemma and the rupture of its outer edge occur due to the formation of hydroperoxides (LOOH) with hydrophilic properties.

The consequences of free radical oxidation of cell membrane lipids are:

an increase in the permeability of cell membranes for ions, in particular, the mitochondrial membrane for hydrogen and calcium ions, which leads to inhibition of the formation of ATP, the release of calcium from mitochondria into the cytosol and its increase in the cytosol;

- change in the mechanical properties of membranes with an increase in their rigidity;
- violation of lateral diffusion of lipids within one monolayer, which contributes to a change in the rate of formation of functionally active protein complexes in the membrane;
- dysfunction of ion channels;
- a decrease in the stability of the lipid bilayer, leading to an electrical breakdown of the membrane by its own membrane potential and the loss of the barrier properties of the cell membrane.

There is the formation of aggregated proteins, intracellular inclusions, fragments of protein molecules toxic to the cell, impaired reception, transmission of humoral effects, transmembrane transport of ions and molecules, excitability, generation and conduction of nerve impulses, metabolism and intercellular interaction.

Cellular Antioxidant Defense Mechanisms

The constant formation of prooxidants in the body is balanced by their inactivation by the antioxidant system. Cells have a number of properties that allow them to effectively resist the damaging effects of ROS and APs. To provide protection against oxidative stress, cells have

a well-developed antioxidant system that contains low- and high-molecular compounds that can inhibit the initiation of ROS formation, “intercept” free radicals (ROS scavengers) or neutralize the formation of lipid free radicals and lipid peroxides.

Antioxidant protection (AOD) is represented by enzymatic and non-enzymatic mechanisms.

Non-Enzymatic Mechanisms of the Antioxidant System

Important elements of AOP are low-molecular non-enzymatic antioxidants - "scavengers" of ROS and AFA. Non-enzymatic antioxidants are water-soluble (ascorbic acid, glutathione, thioredoxin, bilirubin, urates - act in the aqueous phase (cytoplasm, mitochondria, nucleus)) and fat-soluble (tocopherols, carotenoids, ubiquinone - act in the lipid phase). Antioxidants realize their action both intra- and extracellularly.

Ascorbate (AN) is a "scavenger" for hydroxyl radicals, alkoxy radicals (RO•), peroxyl radicals (RO2•), hypochloric acid (HOCl), N2O3, peroxynitrite (ONOO) and others**.** Takes part in the reduction of tocopheroloxyl radical (TocO.) to α-tocopherol with the appearance of an ascorbate radical (semidehydroascorbate):

$TocO^{\bullet} + AH \rightarrow TocOH + A^{\bullet}$

The ascorbate radical (A.), in turn, is reduced to ascorbate (AN) with the help of reduced glutathione and the enzyme glutaredoxin:

$A^{\cdot} + 2GSH \rightarrow AH^{-} + CSSG + H^{+}$, as well as

NADH-dependent semidihydroascorbine reductase:

$A^{\cdot} + НАДН \rightarrow AH^{-} + НАД^{+}$ and

NADPH-dependent thioredoxin-reductase:

$A^{\cdot} + НАДФН \rightarrow AH^{-} + НАДФ^{+}$

Reduced glutathione

Tripeptide glutathione (GSH) - γ-L-glutamyl-L-cysteine-glycine is the most common sulfhydryl compound in cells, which has its own antioxidant activity, directly inactivates the superoxide radical, hydroxyl radical and peroxynitrite, acts as a cofactor of antioxidant enzymes containing thiol group, hydrogen donor, metabolite and substrate of enzymes of the glutathione system. Glutathione is constantly synthesized in the liver and released into the blood, enters all cells of the body, except for red blood cells. It takes part in the synthesis of proteins and nucleic acids, protects against ROS by restoring disulfide bonds, affects the activity of enzymes and other proteins, supports membrane functions, takes part in the metabolism of eicosanoids, is a reserve of cysteine, takes part in the metabolism of xenobiotics, increases cell resistance to pathogenic influences, affects proliferation. The exceptional importance of the role of reduced glutathione in protecting cells from damage. So, glutathione is involved in the reduction reaction of the ascorbate radical (A.) to ascorbate (AH-), catalyzed by the enzyme glutaredoxin.

Glutathione is used in the cell to reduce the sulfene groups in the protein molecule (Pt-SOH), which are formed during the oxidation of the SH groups of cysteine residues.

In this case, the interaction of the reduced glutathione with the sulfene group first leads to the appearance of a mixed disulfide formed by the protein and glutathione and then to the reduction of the thiol group of the protein

$Pt\text{-}SOH + GSH \rightarrow PtS\text{-}SG + H_2O$

$PtS\text{-}SG + GSH \rightarrow Pt\text{-}SH + GSSG$

The importance of the reduction reaction of thiol groups of proteins with glutathione is explained by the fact that the formation of sulfene ionic groups in the molecules of a number of proteins that regulate the phenotypic properties of cells changes the properties of these proteins. As a result, the function of cells as a whole can change.

Ergothioneine (natural betaine) inactivates hypochlorous acid (hypochlorite), hydroxyl radical and peroxynitrite;

α-lipoic acid is a trap for hydrogen peroxide and hypochlorite, and dihydrolipoic acid is for superoxide radical.

Vitamin E (α-tocopherol), carotenoids (lycopene, β-carotene, oxycarotenoids and xanthophylls) and ubiquinone Q act in the lipid (hydrophobic) phase.

Vitamin E (α-tocopherol) blocks the free radical oxidation of cell membrane lipids by interacting with the

lipid peroxyl radical.

The role of **ubiquinone Q** (from the French word ubiquitaire - to be everywhere, "ubiquitous quinone") as an antioxidant is to reduce the tocopheroxyl radical (TocO.) to α-tocopherol. In this case, ubiquinone Q itself is converted into a ubisemiquinone radical, which is then reduced to ubiquinone Q either in the membrane electron transport chain or with the participation of vitamin C.

The antioxidant properties of carotenoids have not been studied enough. They are singlet oxygen traps and have the ability to reduce the tocopheroxyl radical (TocO) to α-tocopherol.

In addition to low molecular weight non-enzymatic antioxidants, scavengers ACF and APA, there are **enzymatic defense mechanisms**. Enzymatic antioxidants include membrane-bound and cytosolic enzymes (superoxide dismutase, catalase, glutathione-dependent peroxidases and transferases), which provide the reduction of ubiquinone Q (DT-diaphora), ascorbate radical, oxidized glutathione (glutathione reductase). AOP enzymes contain in the active center metal ions with variable valence, which, depending on the conditions, act as both an oxidizing agent and a reducing agent.

Superoxide dismutase (SOD) catalyzes the dismutation reaction of superoxide anion radicals:

$O_2^- + O_2^- = O_2 + H_2O_2$

During the reaction, hydrogen peroxide is formed, which is able to inactivate SOD, so SOD always “works” in tandem with **catalase**, which quickly and efficiently breaks down hydrogen peroxide into neutral compounds:

$2H_2O_2 = 2H_2O + O_2$

The property of SOD to reduce the formation of peroxynitrite is due to its ability to reduce the concentration of the superoxide radical in the cell, which also limits its availability for the Fenton reaction. The superoxide radical stimulates the release of iron from ferritin and from the “iron-sulfur centers” of cellular proteins. There are other mechanisms that provide the protective effect of SOD as an antioxidant enzyme.

Peroxidase in most cells is localized in peroxisomes, and is found in significant amounts in mitochondria.

System "glutathione - enzymes of the antioxidant system"

The glutathione enzyme system includes three glutathione-dependent enzymes: glutathione peroxidase (GPO), glutathione reductase (GR), and glutathione transferase (GT).

Glutathione peroxidases are selenium-containing enzymes. Currently, *four types* of glutathione peroxidases have been identified that differ in their structure and localization within cells.

Glutathione peroxidase catalyzes reactions in which the enzyme reduces hydrogen peroxide to water, as well as organic hydroperoxides (ROOH) to hydroxy derivatives, resulting in the conversion to the oxidized disulfide form GS-SG:

$H_2O_2 + 2GSH = GS\text{-}SG + H_2O$

$ROOH + 2GSH = GS\text{-}SG + ROH + H_2O$

Glutathione peroxidase neutralizes peroxynitrite:

$ONOO^- + 2GSH \rightarrow NO_2^- + GSSG + 2H_2O$

Glutathione reductase is a flavoprotein with a flavin adenine dinucleotide prosthetic group and consists of two identical subunits. Catalyzes the reduction of glutathione from the oxidized form of GS-SG, and all other enzymes of the glutathione system use it:

$2НАДФН + GS\text{-}SG \rightarrow 2НАДФ + 2\ GSH$

The reduction of oxidized glutathione is carried out with the participation of NADP + reduction systems on the pentose phosphate pathway of glucose-6-phosphate oxidation (“Embden-Meyerhoff shunt”):

$НАДФ^+ + Г\text{-}6\text{-}Ф \rightarrow НАДФН + H^+ + 6 – phosphogluconate$

Glutathione transferase catalyzes the reaction:

$RX + GSH = HX + GS\text{-}SG$

Some forms of glutathione transferases, like glutathione peroxidases, are capable of reducing lipid hydroperoxides to hydroxy acids (LOH).

Under normal conditions, more than 90% of glutathione is in the reduced form in the cell. Pronounced oxidative stress of the cell can reduce the content of reduced glutathione with an increase in the concentration of oxidized glutathione. Oxidized glutathione stimulates the

formation of disulfide bonds between adjacent thiol groups in the protein molecule and changes their properties:

$GSSG + Pt\text{-}(SH)_2 \leftrightarrow Pt(S\text{-}S) + 2GSH$,

and also interacts with SH-groups of proteins and forms "mixed disulfides", which changes the properties of proteins, causing dysfunction of cells.

$GSSG + Pt\text{-}SH \rightarrow PtS\text{-}SG + GSH$

Deficiency of reduced glutathione deprives the cell of its antioxidant reserve, making it more susceptible to the damaging effects of ROS.

The study of the prooxidant-antioxidant state of the brain in the simulation of experimental cerebral pathology.

To determine the prooxidant-antioxidant state of the brain in its homogenates (20% dilution in PBS (pH-7.2)) determine the activity of lipid peroxidation processes (the content of products that react with thiobarbituric acid (TBKRS)), the concentration of reduced glutathione (GSH) , thiol groups (TSH) and glutathione peroxidase activity.

To determine the content of TBCRS, 2.4 ml of a 0.07 N sulfuric solution and 0.3 ml of a 10% solution of phosphotungstic acid are sequentially added to the test sample of 10% brain homogenate (0.3 ml). To the precipitate washed twice, dissolved in 3.0 ml of bidistilled water, add 1 ml of a 0.85% aqueous solution of TBA, dissolved in 25 ml of acetic acid with the addition of 5 ml of H2O. The color reaction takes place in hermetically sealed tubes at 96˚C for 60 minutes. After cooling them in water for 5 minutes, the optical density of the centrifuged supernatant is determined on a PV 1251C spectrophotometer (Solar, Belarus) at wavelengths of 532 nm and 580 nm. The concentration of TBCR is calculated by the formula: TBCR = (E532 - E580)/0.156 □ K, where E is the extinction at the corresponding wavelengths, V1 is the volume of the TBA solution; V2 is the volume of the test sample; K is the dilution factor of the brain sample (147.7). Calculation of the concentration of TBBS is carried out using the absorption coefficient for the resulting product ε532 = 1.56 × 105 M-1 × cm-1 and expressed in nanomoles per gram of protein (gram of tissue).

When measuring the concentration of GSH to 1 ml of 15% brain homogenate add 0.2 ml of 25% trichloroacetic acid, shake and centrifuge at 5000 rpm for five minutes. To the resulting supernatant (0.2 ml) was added 1.2 ml of 0.5 M phosphate buffer (pH 7.8) and 50 µl of Ellman's reagent. The GSH concentration is calculated taking into account the molar extinction coefficient (ε412 =13600 M-1 cm-1) by determining the optical density of the samples under study at λ= 412 nm on a PV 1251C spectrophotometer.

The determination of the TSH concentration is carried out as follows. Add 30 µl of 3% dodecyl sulfate sodium salt solution to 60 µl of brain homogenate, take 25 µl of the resulting mixture and combine with 1.2 ml of 0.5 M phosphate buffer (pH 7.8) and 50 µl of Ellman's reagent, after 10 minutes of incubation at room temperature determine the optical density on the PV 1251C spectrophotometer at λ=412 nm, taking into account the molar extinction coefficient. The molar extinction coefficient in determining the content of TSH is 13600 M-1 cm-1.

To measure the activity of glutathione peroxidase, 0.1 ml of 0.1 ml of brain homogenate and 20 mm tert-butyl hydroperoxide, incubated for 10 minutes at a temperature of 37˚C, the reaction is stopped with 0.02 ml of a solution of 25% trichloroacetic acid; to obtain a zero point, a similar procedure is carried out immediately after the introduction of tert-butyl hydroperoxide. The samples are centrifuged (5000 rpm, 5 min), 30 µl of the resulting supernatant and 30 µl of the Ellman reagent are added to 1 ml of phosphate buffer (pH 7.8), and the optical density is measured at λ = 412 nm and λ = 700 nm.

Conclusion

Thus, oxidative stress, which is the result of an imbalance between prooxidants and antioxidants towards the former due to excessive production of free radicals and/or a decrease in the activity of antioxidant defense, underlies the pathogenesis of many diseases and its study in modeling experimental pathology serves as a fundamental basis for clinical research.

Conflict of interests

Authors declare lack of the possible conflicts of interests.

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