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Review article

S. KWIECIEN[1], K. JASNOS[1], M. MAGIEROWSKI[1], Z. SLIWOWSKI[1], R. PAJDO[1], B. BRZOZOWSKI[2], T. MACH[2], D. WOJCIK[1], T. BRZOZOWSKI[1]

LIPID PEROXIDATION, REACTIVE OXYGEN SPECIES AND ANTIOXIDATIVE FACTORS IN THE PATHOGENESIS OF GASTRIC MUCOSAL LESIONS AND MECHANISM OF PROTECTION AGAINST OXIDATIVE STRESS - INDUCED GASTRIC INJURY

[1]Department of Physiology Jagiellonian University Medical College, Cracow, Poland;
[2]Gastroenterology and Hepatology Clinic, Jagiellonian University Medical College, Cracow, Poland;

The gastric mucosa plays an important role in the physiological function of the stomach. This mucosa acts as gastric barrier, which protects deeper located cells against the detrimental action of the gastric secretory components, such as acid and pepsin. Integrity of the gastric mucosa depends upon a variety of factors, such as maintenance of microcirculation, mucus-alkaline secretion and activity of the antioxidizing factors. The pathogenesis of gastric mucosal damage includes reactive oxygen species (ROS), because of their high chemical reactivity, due to the presence of uncoupled electron within their molecules. Therefore they cause tissue damage, mainly due to enhanced lipid peroxidation. Lipid peroxides are metabolized to malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). The local increase of MDA and 4-HNE concentration indicates ROS-dependent tissue damage. Superoxide dismutase (SOD) is the main enzyme, which neutralizes ROS into less noxious hydrogen peroxide. A decrease of SOD activity is an indicator of impairment of the protective mechanisms and significantly contributes to cell damage. Hydrogen peroxide is further metabolized to water in the presence of reduced glutathione (GSH). GSH can also work synergetically with SOD to neutralize ROS. The reactions between GSH and ROS yields glutathione free radical ($GS^{\bullet}$), which further reacts with GSH leading to free radical of glutathione disulphide ($GSSG^{\bullet}$). This free radical of GSSG can then donate an electron to the oxygen molecule, producing $O_2^{\bullet -}$. Subsequently, $O_2^{\bullet -}$ is eliminated by SOD. A decrease of the GSH level has detrimental consequences for antioxidative defense cellular properties. Gastric mucosa, exposed to stress conditions, exhibits an enhancement of lipid peroxidation (increase of MDA and 4-HNE), as well as a decrease of SOD activity and GSH concentration. This chain reaction of ROS formation triggered by stress, appears to be an essential mechanism for understanding the pathogenesis of stress - induced functional disturbances in the gastric mucosa leading to ulcerogenesis.

Key words: *gastric mucosa, reactive oxygen species, lipid peroxidation, malondialdehyde, superoxide dismutase, glutathione, peroxynitrite*

INTRODUCTION

The gastric mucosa plays an essential role in maintaining the physiological functions of the stomach. This mucosa acts as gastric barrier, which protects deeper tissue against the damaging actions of the gastric juice components and ingested mucosal irritants (1, 2). In the classic approach, a gastric mucosal barrier is composed of cells from the gastric epithelium with intracellular tight junctions as well as adjacent layer of mucus. Gastric blood flow plays a crucial role in the maintenance of gastric integrity (3, 4). The undisturbed gastric blood flow is regulated by many physiological factors and mechanisms, including nitric oxide (NO), afferent capsaicin-sensitive C fibers and products of cyclooxygenase (COX) activity (5, 6).

NO is produced and released from the vascular endothelium, epithelial cells and sensory nerve endings (5, 7) via the activity of NO synthase (NOS). A substrate for this enzyme is aminoacid L-arginine and NO-synthase (NOS) puts molecules of oxygen (O_2) into molecule of L-arginine, capable of producing NO (7). NO diffuses from the endothelium to smooth muscles, located in the vascular wall, where NO reacts with guanylyl cyclase, leading to cellular enhancement of cyclic guanosine monophosphate (cGMP), acting as a second messenger. The increment of cGMP activity, in smooth endothelial muscle, causes relaxation of vascular wall, accompanied by an increase of blood flow through this vessel (8). This vasodilatatory effect is mimicked by exogenous administration of nitrates, namely NO donors, such as 3-morpholinosydnonimine (SIN-1), S-nitroso-N-acethyl-D,L-penicylamine (SNAP), gliceryl trinitrate (GTN) or NO-releasing aspirin (NO-ASA) (9). Other vasodilators, for example, pentoxifylline (PTX) may act on smooth myocytes, causing their relaxation and this effect seems to be NO independent (10).

The vasodilatatory effect of NO contributes to the maintenance of gastric mucosal barrier integrity and the inhibition of NO

production by a nonspecific N-nitro-L-arginine (L-NNA) was shown to markedly impair important functions of a gastric mucosa including gastric secretion and gastric motility (11, 12). The inhibition of NOS by the administration of exogenous synthetic inhibitors such as L-NNA, L-NAME or by the endogenous NOS inhibitor, ADMA have been shown to exacerbate the acute gastric mucosal lesions and delayed the healing of chronic gastric ulcers (10, 12, 14, 15). The adverse effect of blockade of NOS on gastric integrity by L-NNA or the aggravatory effect of ADMA on gastric mucosal lesions can be reversed by administration of L-arginine, a substrate for this enzyme, administered in the presence of these inhibitors (10, 14, 15) (*Fig. 1*).

The maintenance of gastric mucosa integrity and the gastroprotection depend upon the activity of afferent capsaicin-sensitive C fibers (16-18). Sensory nerves are involved in the regulation of blood microcirculation in the gastric mucosa, which is densely innervated by capsaicin-sensitive afferent neurons, containing vasodilator peptides, such as calcitonin gene-related peptide (CGRP) (8, 16, 17). The C fibers are sensitive to capsaicin administration, because the low doses of capsaicin stimulate of sensory nerves accompanied by the release of CGRP, whereas high doses of capsaicin lead to functional ablation of these fibers (10). Therefore, the ablation of sensory nerves by high doses of capsaicin provides the opportunity to determine their role in the regulation of gastric integrity (13).

The next component, undoubtedly essential for gastric mucosal barrier physiology, is prostaglandin cyclooxygenase (COX), which converts arachidonic acid, a substrate for COX-1 and COX-2 to prostaglandins, especially prostaglandin E_2 (PGE_2) (6, 8, 9). Prostaglandins (PGs) prevent damage of deeper structures due to an increase of mucus secretion, intensification of bicarbonate anions production (HCO_3^-), which neutralise acidic gastric content and the stimulation of mucosal phospholipids (6, 19, 20). PGs were shown to evoke increment of gastric blood flow (4, 21, 22) (*Fig. 2*), thus enhancing oxygen and nutrients delivery to the gastric mucosa. Two isoforms of COX: constitutive isoform, called COX-1, as well as inducible isoform, called COX-2 were proposed (3, 6, 8). Classic approach to their functions revealed that PGs derived from COX-1 exert gastroprotective effects, while high levels of PGs, generated *via* COX-2 are associated with inflammation causing an increase of vessels permeability, pain and fever (8, 23-25). The administration of non-selective COX inhibitors, for instance aspirin, which exerts a potent anti-inflammatory effect, resulting from COX-2 inhibition, can also cause side effects such as bleeding and haemorrhagic lesions of the gastrointestinal mucosa, predominately due to COX-1 inhibition (10, 13).

These adverse effects of aspirin's action and other NSAIDs ingested by patients with inflammatory disorders such as

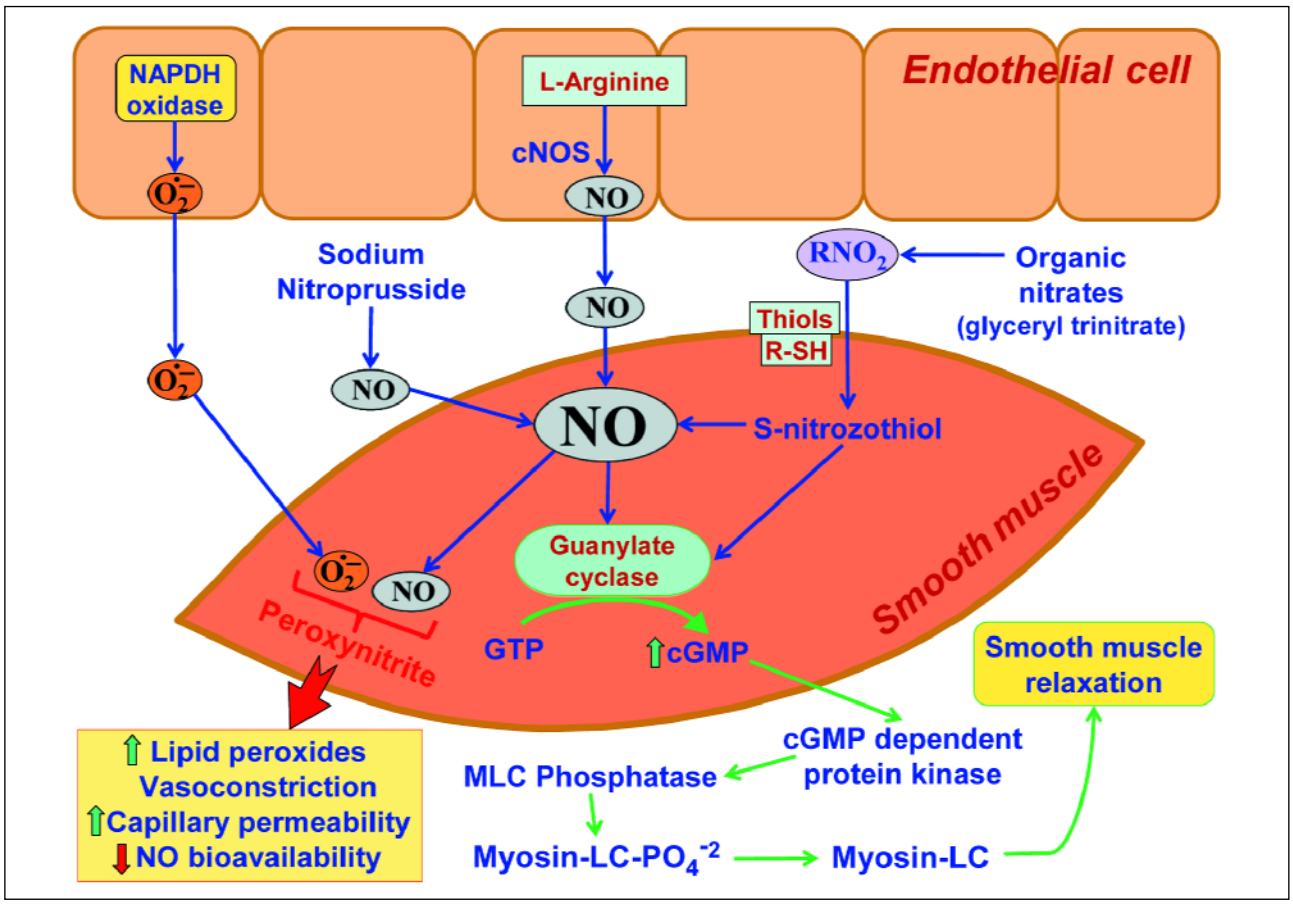

Fig. 1. Transformation pathways of a nitric oxide in the gastric tissue.
After stimulation of endothelial cells, constitutive NO synthase (cNOS) is activated, which transforms L-arginine (L-Arg) to nitric oxide (NO). NO diffuses to smooth muscle cells of gastric blood vessels. Inside smooth muscle, NO activates guanylate cyclase, transforming guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). This cGMP, acting *via* myosin light chains (MLC) phosphatase leads to relaxation of smooth muscle cell and subsequent increase of vessel diameter and larger blood flow. NO may be also deliver by exogenous NO donors, such as sodium nitrate, nitroprusside or other organic nitrates and then it acts the same way, as endogenous NO. Thiols (R-SH), for example, glutathione (GSH) cooperate with NO.

rheumatoid arthritis brought about the question, if new derivative of aspirin, chemically linked to NO moiety, and called NO-releasing NSAIDs (NO-aspirin, NO-naproxen) can counteract the mucosal damage and micro-bleeding associated with this novel NSAID therapy compared with classic NSAIDs therapy. Both novel NO-releasing NSAIDs were shown to possess COX-1-inhibitory and NO donating properties, thus diminishing side effects including both gastroduodenal bleedings and hemorrhagic lesions of gastrointestinal mucosa (24, 26, 27).

REACTIVE OXYGEN SPECIES

As mentioned above, the reactive oxygen species (ROS) contribute to the pathogenesis of gastric damage and many agents were shown before to afford protection of the gastric mucosa *via* inhibition of the oxygen metabolic pathways (13, 28-30). The ROS are atoms or molecules, which exhibit higher chemical activity than molecular oxygen in the basic state (31). The most important ROS's include free radicals, such as superoxide radical anion ($O_2^{\bullet -}$), hydroperoxyl radical ($HO_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet}$) (32, 33). They exhibit high reactivity due to unpaired electron in the outermost shell. Non-free radicals, such as hydrogen peroxide (H_2O_2), ozone (O_3) and singlet oxygen ($^1O_2$) also belong to a class of ROS, because of their high oxidative reactivity. Interestingly, the ROS could be generated intracellulary and extracellulary (34-37). Intracellular mechanism of ROS production is predominantly based on local ischemic episodes within tissues followed by reperfusion (18, 21). Cellular ischemia results in diminished synthesis and release of adenosine triphosphate (ATP) in mitochondria. In this conditions ATP is breaking down to adenosine monophosphate (AMP) and then adenine and hypoxanthine. At the same time the mitochondria releases calcium ions (Ca^{2+}) from their internal space into cytoplasm. The increment of the cytoplasmatic pool of Ca^{2+} activates intracellular protease, which converts xanthine dehydrogenase (XDH) into xanthine oxidase (XO) (33). The XDH uses nicotinamide adenine dinucleotide (NAD^+) as the electron acceptor for the oxidation of hypoxanthine and xanthine into uric acid. This process is not accompanied by the generation of ROS, rather, XO uses molecular oxygen, which is delivered during reperfusion as an electron acceptor to produce superoxide radical anion. Another mode of ROS generation is related to the activation of the mitochondrial respiratory chain (38). Part of the total number of oxygen molecules, involved in the function of mitochondrial respiratory chain, is reduced in single - electron reaction, because electrons "leak" from the electron

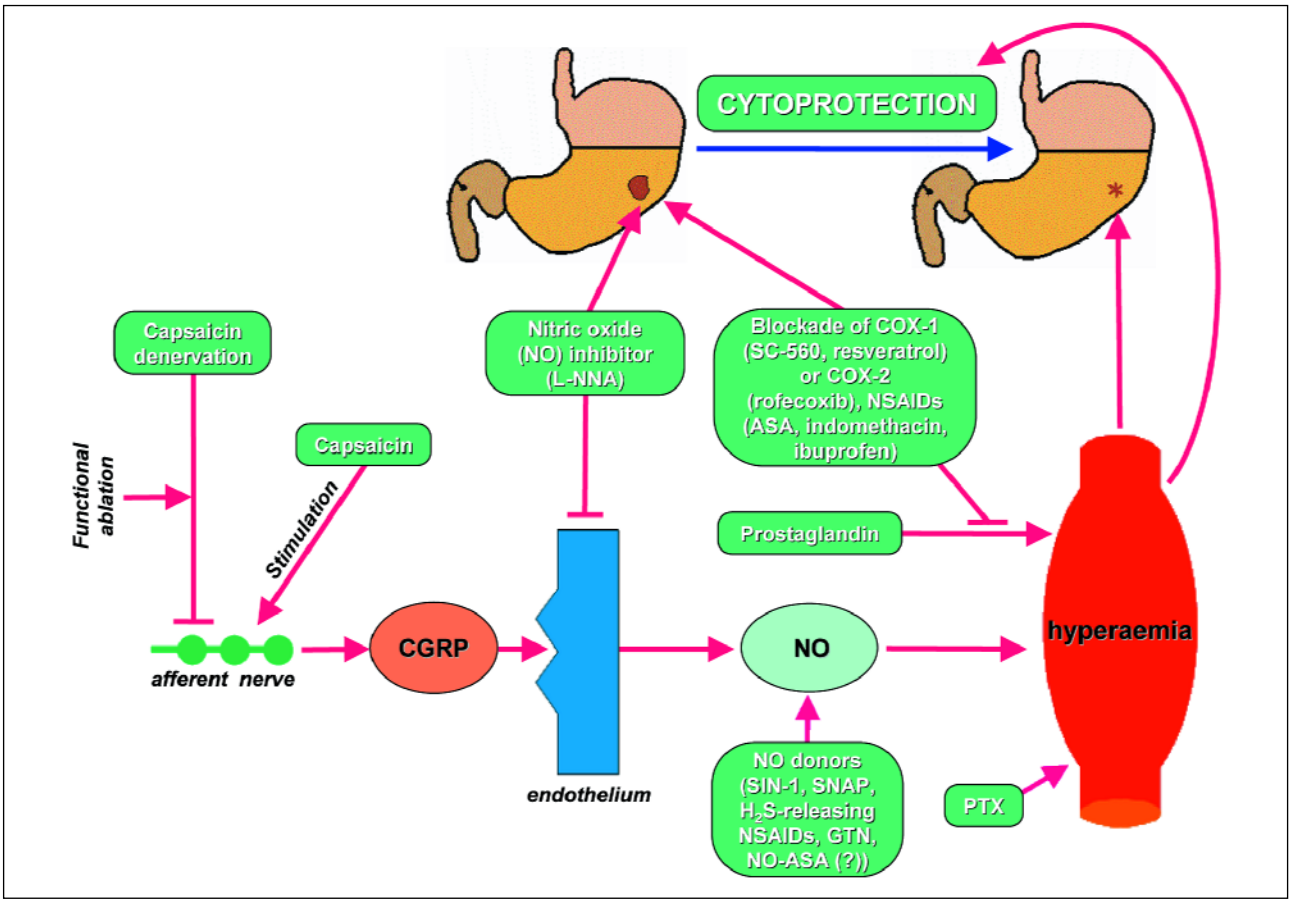

Fig. 2. Factors regulating gastric mucosal barrier function.
Calcitonin gene related peptide (CGRP) is released at the ending of an afferent nerve. Then, CGRP stimulates an endothelium to release nitric oxide (NO). NO influences a blood vessel, resulting in hyperemia. Hyperemia plays an important role in a cytoprotection. This pathway may be activated or blocked at different levels. Low dose of capsaicin stimulates an afferent nerve. High dose of capsaicin produces capsaicin denervation and blocks an afferent nerve. N-nitro-L-arginine (L-NNA) inhibits nitric oxide synthase (NOS) in an endothelium. NO donors, such as SIN-1 (3-morpholinosyndnoimine), SNAP (S-nitroso-N-acetyl-DL-penicillamine), nitroglycerin (GTN), NO-aspirin (NO-ASA) deliver NO exogenously. Pentoxifylline (PTX) and prostaglandins, similarly to NO, cause hyperaemia. COX (cyclooxygenase), producing prostaglandins, may be blocked by SC-560, rofecoxib, resveratrol or aspirin (ASA).

mitochondrial transport chain and this leads to the formation of superoxide radical anion (30) (*Fig. 3*).

In the extracellular model of ROS generation, $O_2^{\bullet-}$ is released from the outer surface of the cellular membrane to the extracellular fluid. Classic example of this process is superoxide ($O_2^{\bullet-}$) production by neutrophils. A neutrophil possesses in its cellular membrane a specific enzyme NADPH oxidase, which is composed by two subunits: flavoprotein and cytochrome b558 (30, 39). This enzyme catalyzes a double - electrons reduction of oxygen molecule (O_2), finally leading to generation of $O_2^{\bullet-}$. The source of electrons, in this process, is reduced nicotinamide adenine dinucleotide (NADPH) (36, 38). NADPH oxidase manifests higher affinity to NADPH, than NADH, so NADPH is only substrate for this enzyme in a cell. Neutrophil derived $O_2^{\bullet-}$ diffuses to adjacent tissues. Due to stability of $O_2^{\bullet-}$ in physiological pH, it can reach distant organelles from its place of generation (28, 36). Kasazaki *et al.* (40) have documented the association between both extracellular and intracellular sources of ROS. The radical $O_2^{\bullet-}$ produced by XO (intracellular mechanism) facilitates tissue infiltration by neutrophils and this effect leads to an augmentation of $O_2^{\bullet-}$ generation from extracellular sources.

Further transformations of $O_2^{\bullet-}$ take place in tissues, because the two $O_2^{\bullet-}$ radical react to each other leading to the formation of hydrogen peroxide (H_2O_2) (41, 42). This reaction can occur spontaneously or it could be catalyzed by an enzyme - superoxide dismutase (SOD). H_2O_2 reacts with $O_2^{\bullet-}$, resulting in the generation of $OH^{\bullet}$ according to the Haber-Weiss reaction. This process is accelerated by the presence of iron (Fe^{2+}) ions (the Fenton reaction) (41, 46). The formation of ROS may serve as a prerequisite for damage to the surrounding tissues. Using electron paramagnetic resonance with spinal trapping, Kasazaki *et al.* (40) and Yasukawa *et al.* (42) have revealed that $OH^{\bullet}$ seems to play a major role in the formation of gastric mucosal injury.

LIPID PEROXIDATION

Irrespective of ROS type, the first stage of ROS-mediated cellular damage is peroxidation of cellular membrane components, especially membrane lipids in the process, called lipid peroxidation (47, 48). This process particularly involves the ROS-mediated oxidative degradation of components of cellular membrane phospholipids, such as polyunsaturated fatty acids (PUFA). In first step of lipid peroxidation, ROS detaches hydrogen the atom from a chain of PUFA, followed by the reduction of ROS to water and the transformation of fatty acid to free radical. This radical of fatty acid attaches to oxygen molecule loading to the generation of peroxyl radical. Free peroxyl radical

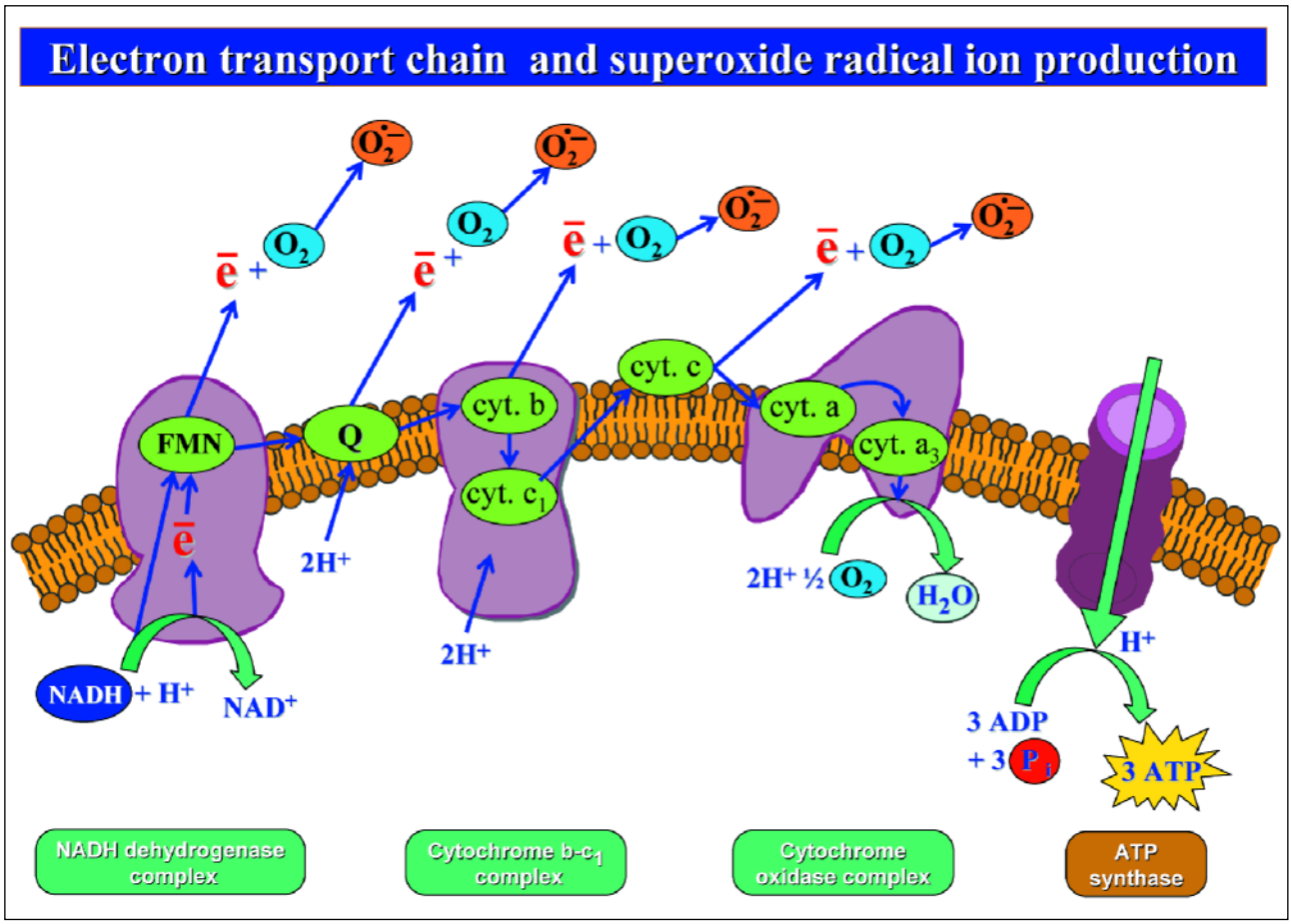

Fig. 3. Electron transport chain in a mitochondrion.
Reactive oxygen species generation is related to the activation of mitochondrial respiratory chain. Correct pathway of mitochondrial respiratory chain involves NADH (reduced nicotinamide adenine dinucleotide) dehydrogenase complex, cytochrome (cyt.) b - c_1 complex and cytochrome oxidase complex. Electron transport along these complexes results in complete reduction of oxygen molecule (O_2), in presence of hydrogen ions (H^+), to water molecule (H_2O). Part of total number of oxygen molecules, involved in the function of mitochondrial respiratory chain is reduced in single electron reaction, because electrons "leak" from the electron mitochondrial transport chain and this leads to formation of superoxide radical anion.

of fatty acid has the ability to detach hydrogen atoms from other PUFAs to generate lipid peroxides. Compared with lipids, lipid peroxides are less stable and may break down to free radicals. This process is accelerated by the presence of iron and copper ions (Fe^{2+}, Cu^{2+}) (46, 49, 50). High reactivity of peroxyl radicals with lipids molecules, as well as chemical instability of lipid peroxides, contribute to positive feedback in lipid peroxidation, thus quickly involving of majority of lipids at cellular membrane (*Fig. 4*). Lipid peroxides are metabolized, *via* β-oxidation pathway to malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) (51-53). Other constituents of cellular membrane, as aminoacids or proteins, are also involved in the process of lipid peroxidation, however, in contrast to peroxidation of lipids, the speed of this reaction is slowed down (33, 47, 48).

The products of lipid peroxidation, MDA and 4-HNE, are advisable to be used as indicators of ROS-dependent tissue damage in various organs including stomach and intestine (33, 47, 54). Lipid peroxidation products modify properties of cellular membranes, by inserting polar groups into phospholipid molecules, located inside the lipid bilayer; this way the lipid internal part of the membrane becomes hydrophobic and more permeable (47). Lipid peroxidation also causes alterations in the membrane potential toward a depolarization. Moreover, the peroxidation inhibits activity of protein transporters, leading to a derangement of the active transport through the membrane (32, 51). The products of lipid peroxidation uncouples the respiratory chain phosphorylation within the mitochondria, resulting from an increase in permeability of internal mitochondrial membrane for protons. This mechanism creates the equilibrium of proton concentrations between both sides of the internal mitochondrial membrane (39, 52). Aldehyde products of lipid peroxidation, such as MDA, react with amine groups of membrane protein to yield Schiff bases and therefore cellular membrane becomes more stiff. Moreover, reaction between MDA and cellular membrane protein changes its antigenic characteristic. Thiols also undergo oxidation causing inactivation of the active enzymatic centers (33, 47, 48).

The next product of lipid peroxidation, namely 4-HNE, was also shown to participate in the disorder of cellular functioning during oxidative stress (46, 52, 53). This toxic product of lipid peroxidation is probably involved in the pathogenesis of many diseases including e.g. atherosclerosis, Alzheimer disease and peptic ulcer disease (52, 54). Moreover, 4-HNE modifies cellular

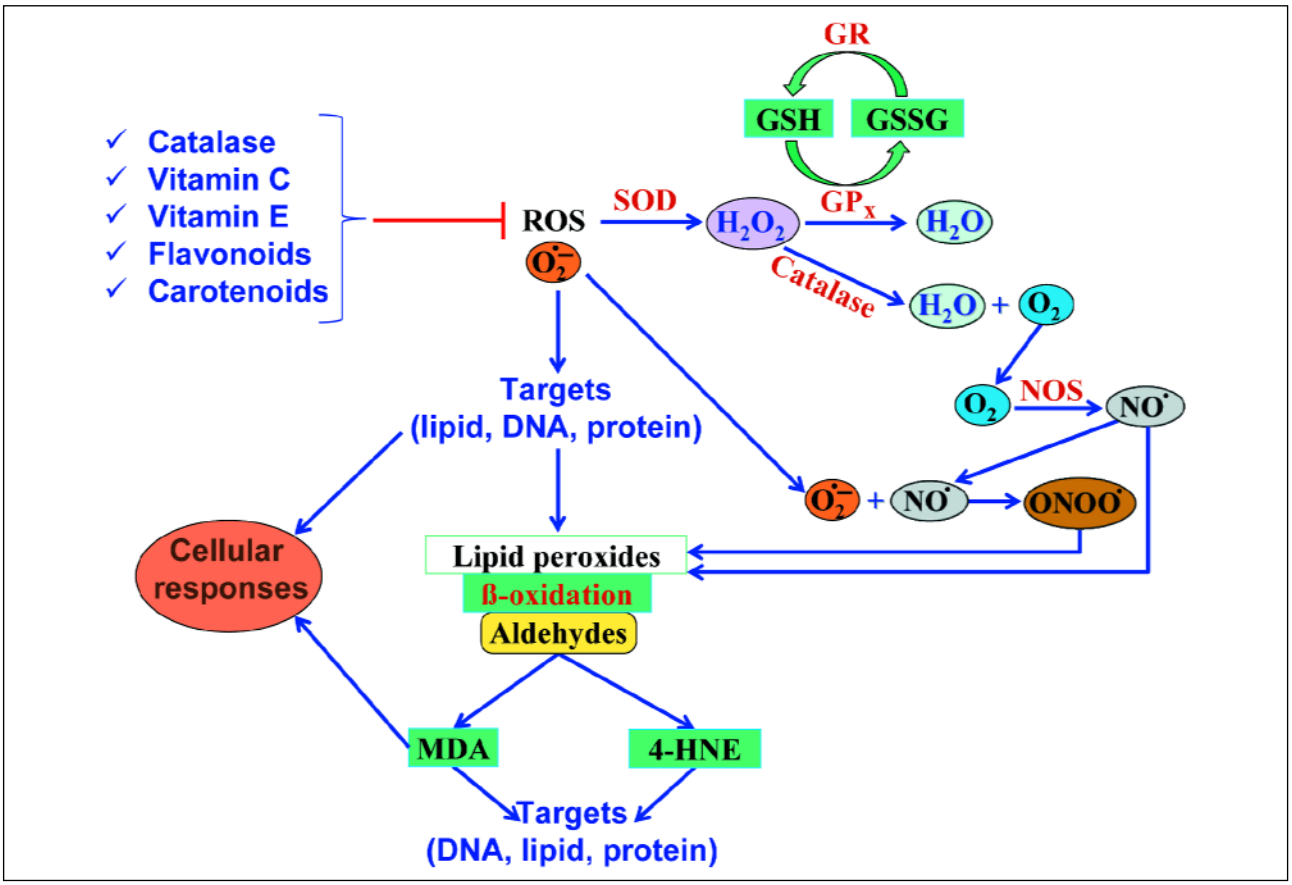

Fig. 4. Transformation of a superoxide radical anion in an organism (lipid peroxidation or neutralization of the radical). Superoxide radical anion ($O_2^{\bullet-}$) reacts with cellular lipids, leading to the formation of lipid peroxides, that are metabolized to malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). Other cellular compounds, such as DNA, proteins also may be modified by $O_2^{\bullet-}$, however peroxidation of lipids is more intensive, because it has property of chain reaction. Nitric oxide synthase (NOS) produces nitric oxide (NO), which reacts with $O_2^{\bullet-}$ to produce peroxynitrite ($ONOO^{\bullet}$). $ONOO^{\bullet}$ also participates in lipid peroxides formation. Superoxide dismutase (SOD) catalyzes the dismutation of superoxide radical anion ($O_2^{\bullet-}$) into less noxious hydrogen peroxide (H_2O_2), that is further degraded by glutathione peroxidase (GPx) and cooperating glutathione reductase (GR). The reduction of H_2O_2 into water by GPx is accompanied by the conversion of glutathione from reduced form (GSH) into oxidized form (GSSG). GR restores pool of GSH by reduction of GSSG, by means of reduced nicotinamide adenine dinucleotide phosphate (NADPH). Another enzyme - catalase - also participates in H_2O_2 neutralization, releasing oxygen molecule (O_2). Antioxidants, such as vitamins C and E, flavonoids and carotenoids counteract reactive oxygen species (ROS) generation.

growth and exhibits signaling properties (53). 4-HNE easily diffuses within the tissues making distant lesions and, similarly to MDA may react with thiol and amine groups of cellular proteins, resulting in cell metabolic disturbances. Other important properties of 4-HNE are stimulation of neutrophil chemotaxis, activation of phospholipase C (PLC) and the activation of adenylyl kinase. Stimulation of neutrophil chemotaxis facilitates ROS - dependent tissue destruction, resulting from intracellular generation of ROS (52, 53). In conclusion, lipid peroxidation products (MDA and 4-HNE) are responsible for a plethora of cellular pathophysiological events, therefore measurement of their concentrations, which reflects the damage induced by oxidative stress can be considered to be a useful tool in the experimental and clinical settings (39, 48, 53, 55).

SUPEROXIDE DISMUTASE

Superoxide radical anions ($O_2^{\bullet -}$) are transformed to hydrogen peroxide (H_2O_2) during spontaneous or a catalyzed reaction, named a dismutation (43-45). SOD is an enzyme, which catalyzes the dismutation of $O_2^{\bullet -}$ into less noxious H_2O_2 (49). Three types of SOD can be distinguished: cytoplasmatic, mitochondrial or extracellular ones, all containing metal ions in their catalytic active site. Both the cytoplasmatic and extracellular SOD contains copper and zinc ions; while the mitochondrial SOD is equipped with manganous ion. Another isoform of SOD may contain iron ion in the catalytic active site, but this SOD is predominantly present in prokaryotes, for example, in bacteria (50, 51). The cytoplasmic form of SOD consists of two identical, chemical stable subunits. Chemical stability of this SOD is maintained by hydrogen bonds, disulfide linkages inside subunit, as well as the presence of zinc ion in the catalytic active site (45), while copper ion plays a crucial role in the chemical activity of this enzyme. The activity of this Cu/Zn-SOD enzyme involves the oxidation of cuprous ion (Cu^+) and reduction of cupric ion (Cu^{2+}) by $O_2^{\bullet -}$, in the presence of hydrogen ions (H^+), leading to H_2O_2 generation. To augment effectiveness of $O_2^{\bullet -}$ neutralization, SOD possesses a complex of amino acids with electric charges on the enzyme molecule surface, necessary for the creation of an electrical gradient. This gradient drags $O_2^{\bullet -}$ directly to the active center of the enzyme (49, 50, 57).

SOD plays a crucial role in scavenger cascade responsible for ROS neutralization (55). H_2O_2 formed due to SOD activity is further broken down by antioxidazing enzymes: a catalase or a glutathione peroxidase (13, 53, 58). The catalase accelerates H_2O_2 breakdown to water and oxygen. The second biochemical pathway of H_2O_2 metabolism depends on the activity of glutathione peroxidase, which cooperates with the action of glutathione reductase. Glutathione peroxidase - induced breakdown of H_2O_2 to water and is accompanied by transformation of reduced glutathione (GSH) to its oxidized form (GSSG) (*Fig. 5*). Glutathione peroxidase has a major affinity to H_2O_2, suggesting the potent antioxidizing activity of this enzyme in the maintenance of physiological conditions and cell homeostasis (56, 58-60).

REDUCED FORM OF GLUTATHIONE

Reduced form of GSH is believed to act as a main intracellular antioxidative buffer with multifaceted action against tissue oxidative stressors. Chemically, glutathione is a peptide composed of 3 amino acids, namely: glutaminic acid, cysteine and glycine (γ-glutamylcysteinylglycine), and all containing thiol (SH) groups originating from cysteine. The availability of the glutathione SH group to oxidative action of ROS leads to the formation of glutathione free radical ($GS^{\bullet}$) or, as mentioned above, glutathione disulphide (GSSG), also known as oxidized glutathione. Both forms undergo further biochemical transformations, leading to regeneration of a reduced form of GSH with a SH group, possessing the activity of digestive peptidases (33). Isopeptic bond in the GSH molecule, composed of γ-carboxylic residue of glutaminic acid with an amine group of cysteine protects GSH from intracellular degradation (58).

GSH is a substrate for glutathione peroxidase (GPx) - an enzyme which reduces H_2O_2. Both, GPx and GSH may also inhibit lipid peroxidation directly or indirectly by mediation of lipid peroxides peroxidase (10, 33). The important function of GSH is protection of cellular proteins against oxidative injury. ROS oxidizes proteins both through formation of protein free radicals and oxidation of protein thiols. This latter action can be dangerous to enzymatic proteins, because of their inactivation. GSH reduces protein free radicals yielding glutathione free radical ($GS^{\bullet}$). In the case of oxidation of protein thiols, activity of GSH is mediated by specific enzymes called thiol transferases, which acts as catalyzed, and are involved in the reduction of protein thiols by GSH, then transformed to an oxidized form GSSG (31, 34). GSH can act as a substrate for glutathione transferases (61, 62). These enzymes conjugate GSH to xenobiotics, which enables their removal from an organism. Glutathione transferases contribute to elimination of lipid peroxidation products, namely GSH and 4-HNE. GSH+4-HNE complex may be removed from a cell by active membrane transport (61, 62). GSH can also cooperate with SOD to neutralize ROS. Reactions between GSH and ROS yield glutathione free radical ($GS^{\bullet}$), as described above. $GS^{\bullet}$ reacts with GSH to yield free radical of glutathione disulphide ($GSSG^{\bullet}$), which in turn donates an electron to the oxygen molecule, converting it to $O_2^{\bullet -}$, and is then eliminated by SOD (31, 33). This clearly suggest that GSH and SOD cooperate in cell protective action against oxidizing stress that may lead to the formation of gastric mucosal lesions in the gastric mucosa caused by cellular damage *via* reactive oxygen metabolites such as $O_2^{\bullet -}$ and lipid peroxidation products (63, 64).

EXPERIMENTAL MODELS OF GASTRIC MUCOSA INJURY

The intragastric application of ethanol (3, 5, 63), the exposure of rodents to water immersion and restraint stress (8, 10, 13, 26) as well as the ischemia followed by reperfusion (I/R) (21, 43, 47, 64) are widely accepted model of experimental injury to gastric mucosa. In the majority of these methods, the development of inflammation, often hemorrhagic type of inflammation, serves as a prerequisite for mucosal erosions and even ulcers. The mechanism of gastroprotection against gastric mucosal lesions induced by ethanol, stress or I/R can involve the alterations in gastric blood flow, mucus production and the role of prostaglandins (21, 63-65), nitric oxide (9, 63, 65), growth factors (66, 67), appetite controlling peptides such as nesfatin-1 (15), leptin (19) or ghrelin (68-70). The pathogenesis of mucosal damage include the effect of damaging agents on gastric acid secretion and neural regulation *via* brain-gut axis (15, 19) and the participation of microorganism infecting human stomach, such as *Helicobacter pylori* (37, 50 71) and *Candida albicans* (72, 73). The ROS and oxidative metabolism can contribute to a disturbances of the gastric mucosal barrier and the formation of gastric lesions and their role in pathogenesis of gastric mucosal injury has been described in a numerous studies (10, 20, 26, 33, 47, 65). The neutrophil induced gastric tissue infiltration have also been documented, as well as the increase of tissue MDA, 4-HNE levels and diminution of antioxidative mechanisms. The alteration in antioxidative status of gastric mucosa is accompanied by the decrease of SOD activity, as well as the depletion of the GSH pool, both implicated in the pathogenesis of I/R gastric lesions (21, 43, 47). In rats exposed to 3.5 hours of water cold stress

(WRS), numerous gastric mucosal bleeding erosions accompanied by the decrease of gastric blood flow (GBF) were observed (10, 13, 26, 47). Moreover, in a majority of these studies, an increase in MDA and 4-HNE considered as an indicator of lipid peroxidation with a decrease in gastric mucosal expression and activity of antioxidative enzymes SOD and GSH were also notified. The blockade of COX-1 and COX-2 activity by administration of SC-560 and rofecoxib, respectively, and the capsaicin denervation had magnified the number of gastric lesions and these effects were accompanied by a further reduction of GBF (*Table 1*), SOD activity, GSH concentration and enhancement in lipid peroxidation, as reflected by higher MDA and 4-HNE levels, when compared to animals exposed to WRS only (*Table 2*). On the other hand, NO donors, such as SIN-1, SNAP, glyceryl trinitrate and NO-releasing aspirin (26, 28) or antioxidazing compounds, such as resveratrol (65, 74) and pentoxifylline (75), afforded the protection of the gastric mucosa against WRS (*Table 3*), in part *via* activation of antioxidative parameters involving a decrease of MDA and 4-HNE, and an increase of SOD and GSH activities (*Table 4*). Although resveratrol inhibits COX-1 (65), it acts as a radical scavenger within mucosa injured by stress, thus affording some protection (*Table 3* and *Table 4*). On the contrary, aspirin possesses scavenger's properties (*Table 2*), but this NSAID aggravates stress-induced gastric lesions (*Table 1*), suggesting that its damaging action depends rather on the more potent inhibition of prostaglandin production than the scavenging activity against the formation of ROS.

Some of these compounds can exert beneficial influence on the gastric mucosa (glyceryl trinitrite , pentoxifylline) (75) or they act as metabolites of medications (e.g. SIN-1 is a metabolite of molsydomine) (10, 13). The adverse effects in the stomach such as the acute microbleedings and gastric hemorrhagic lesions evoked by stress are potentiated in the presence of NSAID such as aspirin or indomethacin. Thus, the more detailed determination of oxidative stress and the associated pathology in the GI tract may contribute in design of new, noninvasive method of prevention of gastrointestinal injury caused by various ulcerogenes.

The lipid peroxidation products, specifically the activity of (SOD) and the levels of GSH, play an important role as an

Table 1. Mean number of gastric lesions and gastric blood flow (GBF), as % of blood flow in the intact mucosa, in rats exposed to 3.5 hours of water immersion restraint stress (WRS) with placebo, aspirin (ASA) 40 mg/kg, SC-560 in dose 5 mg/kg, rofecoxib 10 mg/kg, given intragastrically (i.g.) or capsaicin denervation. Asterisk (*) indicates a significant change as compared to the value obtained in group: placebo + WRS.

Investigated group	Mean lesion number	Gastric blood flow (% control)
Placebo + WRS	24 ± 1,5	60 ± 2
ASA 40 mg/kg (i.g.) + WRS	29 ± 2 *	50 ± 3 *
SC-560 5 mg/kg (i.g.) + WRS	27 ± 1 *	52 ± 2 *
rofecoxib 10 mg/kg (i.g.) + WRS	27.5 ± 1 *	54 ± 1 *
capsaicin denervation + WRS	29 ± 1.5*	56 ± 4 *

Table 2. Concentration of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) in nanomoles per gram of tissue (nmol/g), superoxide dismutase (SOD) activity in units per gram of tissue (U/g) and concentration of reduced glutathione (GSH), expressed as micromoles per gram of tissue (μmol/g), in the gastric mucosa of rats exposed to 3.5 hours of WRS with placebo, aspirin (ASA) 40 mg/kg, SC-560 in dose 5 mg/kg, rofecoxib 10 mg/kg, given intragastrically (i.g.) or in rats with capsaicin denervation. Asterisk (*) indicates a significant change as compared to the value obtained in group: placebo + WRS.

Investigated group	MDA+4-HNE	SOD	GSH
Placebo + WRS	15.85 ± 1.27	245.20 ± 12.00	0.63 ± 0.02
ASA 40 mg/kg (i.g.) + WRS	11.00 ± 2.00*	287.20 ± 15.00*	0.62 ± 0.02
SC-560 5 mg/kg (i.g.) + WRS	15.70 ± 1.30	290.00 ± 35.00	0.55 ± 0.03*
rofecoxib 10 mg/kg (i.g.) + WRS	15.80 ± 0.60	280.00 ± 46.00	0.52 ± 0.03*
capsaicin denervation + WRS	17.40 ± 0.20*	217.20 ± 15.00*	0.50 ± 0.02*

Table 3. Mean number of gastric lesions and gastric blood flow (GBF), as % of flow in the intact mucosa, in rats exposed to 3.5 hours of water immersion restraint stress (WRS) with placebo, SIN-1 (3-morpholinosyndnoimine) 5 mg/kg, SNAP(S-nitroso-N-acetyl-DL-penicillamine) 5 mg/kg, nitroglycerin (NTG) 10 mg/kg given, NO-aspirin (NO-ASA) 40 mg/kg, resveratrol 10 mg/kg, given intragastrically (i.g.) and pentoxifylline (PTX) 10 mg/kg, given intraperitoneally (i.p.). Asterisk (*) indicates a significant change as compared to the value obtained in group: placebo + WRS.

Investigated group	Mean lesion number	Gastric blood flow (% control)
Placebo + WRS	24 ± 1.5	60 ± 2
SIN-1 5 mg/kg (i.g.) + WRS	7 ± 1*	90 ± 3 *
SNAP 5 mg/kg (i.g.) + WRS	10 ± 1*	89 ± 2 *
NTG 10 mg/kg (i.g.) + WRS	14 ± 1 *	86 ± 1 *
NO-ASA 40 mg/kg (i.g.) + WRS	14 ± 2*	85 ± 1.5 *
resveratrol 10 mg/kg (i.g.) + WRS	16 ± 1*	72 ± 2 *
PTX 10 mg/kg (i.p.) + WRS	9.5 ± 4*	88 ± 1 *

Table 4. Concentration of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) in nanomoles per gram of tissue (nmol/g), superoxide dismutase (SOD) activity in units per gram of tissue (U/g) and concentration of reduced glutathione (GSH), expressed as micromoles per gram of tissue (μmol/g), in the gastric mucosa of rats exposed to 3.5 hours of WRS with placebo, SIN-1 (3-morpholinosyndnoimine) 5 mg/kg, SNAP (S-nitroso-N-acetyl-DL-penicillamine) 5 mg/kg, nitroglycerin (NTG) 10 mg/kg given, NO-aspirin (NO-ASA) 40 mg/kg, resveratrol 10 mg/kg, given intragastrically (i.g.) and pentoxifylline (PTX) 10 mg/kg, given intraperitoneally (i.p.). Asterisk (*) indicates a significant change as compared to the value obtained in group: placebo + WRS.

Investigated group	MDA + 4-HNE	SOD	GSH
Placebo + WRS	15.85 ± 1.27	245.20 ± 12.00	0.63 ± 0.02
SIN-1 5 mg/kg (i.g.) + WRS	11.66 ± 0.80*	334.85 ± 44.10*	0.89 ± 0.05*
SNAP 5 mg/kg (i.g.) + WRS	12.82 ± 0.20*	459.65 ± 53.60*	0.93 ± 0.03*
NTG 10 mg/kg (i.g.) + WRS	9.52 ± 0.11*	600.30 ± 10.10*	0.91 ± 0.06*
NO-ASA 40 mg/kg (i.g.) + WRS	9.10 ± 1.05*	466.56 ± 29.40*	0.85 ± 0.09*
resveratrol 10 mg/kg (i.g.) + WRS	9.70 ± 0.30*	292.00 ± 34.00	0.74 ± 0.04*
PTX 10 mg/kg (i.p.) + WRS	6.00 ± 0.70*	530.00 ± 25.00*	0.74 ± 0.01*

indicator of tissue damage by ROS, known to contribute to the pathogenesis of gastrointestinal damage (31, 34, 76). The oxidative stress, amplified during stress (ulcerogenesis) was accompanied by an elevation of MDA + 4-HNE and diminution of SOD and GSH content. These ROS-mediated effects in gastric mucosa exposed to stress were reversed by gastroprotective substances releasing of NO, such as SIN-1, SNAP, nitroglycerin, NO-releasingASA, and the antioxidizing compound resveratrol resulting in reduction of stress-induced gastric damage and attenuation of MDA + 4-HNE, content and an increase in activity of antioxidizing enzymes SOD and GSH.

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Author's address: Assoc. Prof. Slawomir Kwiecien, Department of Physiology Jagiellonian University Medical College, 16 Grzegórzecka Street, 31-531 Cracow, Poland.
E-mail: skwiecien@cm-uj.krakow.pl