R e v i e w

Controversy of Free Radical Hypothesis: Reactive Oxygen Species – Cause or Consequence of Tissue Injury?

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Abstract. For a decade or two, the hypothesis of causality of various disorders by reactive oxygen species (ROS), due to their potentially harmful effect towards cellular constituents, is one of the most frequently cited in biomedical sciences. In fact, the ROS-mediated alterations of biomacromolecules are considered to be essential events in the etiopathogenesis of those diseases where involvement of ROS has been indicated. ROS easily react *in vitro* with most biological molecules, causing their degradation and destruction. This may implicitly suggest that, when excessively produced *in vivo*, ROS are deleterious to integral components of the cell and cause their dysfunctions. Some experimental data indicate that ROS-mediated lipid peroxidation, protein oxidation and oxidative alterations to nucleic acids are crucial events of unfavorable actions of ROS. Yet the most convincing evidence, i.e. unambiguous inhibition of tissue injury by pretreatment with antioxidants, has not been provided. On the contrary, there are quite a few papers reporting failure in applying antioxidants to heal those pathologies where the causal role of ROS was supposed. Other papers reported serious complications arising from antioxidant therapy, which is quite in contradiction to its expected effect. On the other hand, an increasing number of recent findings have provided evidence of a key role of ROS in both intracellular signaling and intercellular communication, processes involved in maintaining homeostasis. Hence, some investigators consider excessive production of ROS to be rather a "smoke after the fire" than "a deleterious fire" itself, suggesting the occurrence of overproduced ROS as being the consequence of some primary damage. The present paper aims at summarizing some pros and cons of various opinions with an attempt to help better understand the involvement of ROS in tissue injury.

Key words: Reactive oxygen species — Antioxidants — Oxidative stress — Tissue injury — Ischemia-reperfusion — Hypoxia-reoxygenation

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Introduction

Some ten years ago, an increasing body of experimental evidence on the involvement of free oxygen radicals and reactive oxygen species (ROS) in various pathophysiological states stimulated assumptions on the role of ROS in the etiopathogenesis of different diseases. The essence of this view is based on findings that ROS easily react with most biological macromolecules causing their degradation and destruction (Horáková et al. 1990; Dargel 1992; Kvaltínová et al. 1993; de Groot 1994; Medocci et al. 1999). Consequently, the term of Free Radical Diseases was introduced for those disorders, where ROS were considered to play a causal role in tissue injury resulting in organ dysfunction. This has been suggested e.g. for adult respiratory distress syndrome, atherosclerosis, inflammation, rheumatoid arthritis and other autoimmune diseases, degenerative disorders associated with aging, diabetes complications, stress-induced injuries, processes of mutagenesis and cancerogenesis, postischemic and posthypoxic damages, organ transplantation complications, etc. (Harman 1986; Halliwell et al. 1992; Juránek et al. 1992; Phillis 1994; Nosálová et al. 1995; Dreher and Junod 1996; Beckman and Ames 1998; Bauerová and Bezek 1999; Baynes and Thorpe 1999).

More recently, investigators started to raise questions whether uncontrolled ROS production could be a common mechanism underlying so many various pathological processes and whether oxidative alterations to cellular components by ROS might be a uniform cause of tissue injury accompanying different diseases. Others have suggested that ROS produced in excess might be the consequence rather than the cause of damage and could mediate further course or/and serve as indicators of end-stage tissue injury (Baynes and Thorpe 1999; Brookes et al. 2004; Takuma et al. 2004). An increasing number of studies have appeared providing evidence of an important role of ROS in both intracellular signaling and intercellular communication, processes involved in maintaining homeostasis (for review, see Hensley et al. 2000; Thannickal and Fanburg 2000; Dröge 2002; Feinendegen 2002; Becker 2004). The present paper attempts to summarize some pros and cons of the free radical hypothesis with a purpose of better understanding the role of ROS in the etiology and pathogenesis of tissue injury.

Mediators of tissue injury and the healing process

Injury of tissue and its healing represents a sequence of various events, depending on the injurious cause itself, e.g. infection, inflammation, ischemia, and on other factors, such as the intensity of the damaging agent, type of tissue, condition of the whole organism, etc. (Chettibi and Ferguson 1999). To assess the severity of tissue injury, various biomarkers are being followed. In light of the understanding that the plasma membrane is impermeable under physiological conditions for most intracellular compounds, like proteins, no release of such macromolecules to extracellular space should occur. Yet under damaging action, disruption of membrane integrity and thus massive leakage of enzymes located intracellularly, e.g.

routinely followed lactate dehydrogenase release, may occur. Thus usually applied recordings concerned e.g. inhibition of oxidative phosphorylation and of energy-dependent processes, increased concentration of cytosolic calcium, and often loss of intracellular enzymes (Kehrer and Starnes 1989; Nosálová et al. 1991; de Zwart et al. 1999). Whether the enzyme leakage is associated with the reversible phase of tissue injury (Schmidt and Schmidt 1987) or with its irreversible phase (Janero et al. 1991) has not been conclusively established.

Since the healing process starts from the moment of injury, there is a certain pattern of tissue reactions, which are common to any damage. This concerns an effort to eliminate injurious action and to restore tissue integrity and its function by remodeling impaired structures. The responses are mediated by a variety of messengers released during the injurious/healing process. Thus, induction and release of stress proteins, acute phase proteins, chemokines and cytokines, resulting in activation of the immune system, occur in the early stages (Keyse and Tyrell 1989; Seitz et al. 1996; Gabay and Kushner 1999; Yenari et al. 1999). Activated phagocytes, producing cytotoxic agents, not only prevent the spread of the infection but also remove host cellular particles which were damaged. Activation of phagocytes depends largely on their high consumption of oxygen, called oxidative burst, during which ROS are produced (Klebanoff 1999; Leto 1999). In addition, to protect against invaded microbes, phagocytic cells possess also oxygen-independent mechanisms, including degranulation and secretion degradative enzymes, such as proteases, phospholipases, hydrolases, nucleases, etc. (Elsbach et al. 1999). Other mediators of tissue injury are, e.g. compounds derived from arachidonic acid, potent modulators of the inflammatory reaction (Griffiths 1999; Penrose et al. 1999). Interestingly, ROS have been reported to be released as by-products of both cyclooxygenase and lipoxygenase catalysis, which also depends on the partial pressure of oxygen (Yamamoto 1991; Landino et al. 1996; Sahnoun et al. 1998; Juránek et al. 1999; Juránek and Rekalov 2000; Juránek et al. 2002). Hence, along with others, ROS are likely to serve as mediators of injurious yet also of healing process.

Cytosolic calcium plays important role in tissue injury. Increasing calcium concentration in cytosol activates calcium-dependent regulatory proteins and degradative enzymes, such as proteases, phospholipases, nucleases, etc., which alter functions of the affected macromolecules (Kehrer and Starnes 1989; Nicotera et al. 1992; Phillis 1994; Kirschner 1999). Irreversible alterations to essential cellular constituents may ultimately result in cell death, necrosis or apoptosis, contributing to tissue injury. Intracellular overload by free ionized calcium has been suggested to be critical for transition of reversible cellular damage into irreversible one (Kristensen 1994). Indeed, intracellular calcium overload was observed to occur right before cell death (Lee and Allen 1991; Takuma et al. 2004). Calcium antagonists have been found effective in cytoprotection against both necrosis and apoptosis (Jacinto and Jandhyala 1992; Négre-Salvayre and Salvayre 1992) as well as in defending myocardium from postischemic arrhythmias (Lu et al. 1999). There are also other findings supporting the view that intracellular calcium plays a crucial role in the process of cell death (Goldhaber and Weiss 1992; Kristensen 1994; Rekalov et al.

1997; Kristián and Siesjö 1999; Takuma et al. 2004). It has been speculated that cytosolic calcium overload may be "one final common pathway" triggering various types of tissue injuries, even though if evoked by different agents (Goldhaber and Weiss 1992; Nicotera et al. 1992; Phillis 1994; Takuma et al. 2004). Importantly, attraction of phagocytic cells and their activation as well as production of ROS have been demonstrated to depend on calcium, too (Zimmerman et al. 1989; Dahlgren et al. 1992; Thiel and Bardenheuer 1992).

Formation and elimination of reactive oxygen species

Oxygen is not only an essential element for aerobic organisms in yielding their energy effectively but, due to its high oxidative potential towards most organic compounds, it is also a cause of serious hazard to the life of these organisms. That is why various forms of molecular oxygen (in this paper referred to as ROS) have been implicated to be deleterious for essential constituents of the cell.

ROS are generated under basal conditions as by-products of cellular metabolism, primarily in the mitochondria. Briefly, nearly 100 % of utilized oxygen enters mitochondria where it is reduced to water while passing through the electron transporting chain. However, about 1 % of the transported electrons may leak the respiration chain. This allows creation of partly reduced oxygen intermediates, e.g. superoxide anion radical, hydrogen peroxide and hydroxyl radical (for review, see e.g. Naqui et al. 1986). The amount of electrons leaking mitochondria and thus the amount of ROS formed has been reported to be proportional to partial pressure of oxygen in the tissue (Boveris and Chance 1973; Tribble and Jones 1990; Boveris et al. 2000). In addition to the mitochondrial electron transport chain, there are other ROS producing mechanisms, e.g. system of cytochrome P-450, oxidative enzymes, such as endothelial xanthine oxidase, NAD(P)H oxidases and myeloperoxidases of phagocytic cells, and arachidonate oxygenases (Goldhaber and Weiss 1992). Autooxidative reactions of endogenous substances such as catecholamines, or exogenous substrate, such as xenobiotics, as well as the oxidation of reduced metabolites accumulated e.g. in the process of anaerobic metabolism, have been reported to produce ROS, too (Kehrer 1993; Phillis 1994). It was also demonstrated that interaction of arachidonic acid with the mitochondrial electron transport chain promoted ROS generation (Cocco et al. 1999).

To be protected from potentially harmful effects of ROS, aerobic organisms evolved several specialized mechanisms. To detoxify ROS, they use system of antioxidants, including specific antioxidative enzymes, e.g. superoxide dismutase, catalase, glutathione peroxidase, and non-enzymatic antioxidants, e.g. glutathione, tocopherols, ascorbic acid, etc. (Yu 1994; Gaté et al. 1999). In fact, the activities of antioxidative enzymes were found to be compensatory enhanced, reflecting elevated metabolic rate and thus the increased ROS production (Selman et al. 2000). To remodel already impaired cellular constituents, a system of repair mechanisms has been developed. This system consists of mostly degradative yet also other enzymes

such as proteases, peptidases, phospholipases, acyl transferases, endonucleases, exonucleases, polymerases, ligases, etc., to cleave and replace irreversibly damaged macromolecules (Elliott et al. 2000). Importantly, the systems are integrated, they work in concert and their actions may be closely interconnected (Sies 1993; Berry and Kohen 1999; Gaté et al. 1999). So-called oxidative stress represents an imbalance between ROS production and ROS elimination in favor of prooxidant processes (Sies 1985).

Although, under physiological conditions, the distribution of antioxidative and repair mechanisms in living organisms do not allow ROS to harm essential components of the cell, there are many diseases where oxidative stress is considered to take place and ROS to mediate tissue injury (de Zwart et al. 1999; Gaté et al. 1999; Medocci et al. 1999). However, the real role of ROS in etiopathogenetic mechanisms has not been convincingly elucidated as yet. In fact, more and more papers are reporting that ROS and their products, e.g. lipid and protein oxidation metabolites, play important signaling functions and are involved in maintaining cell homeostasis, defending the tissue against further insults by promoting preconditioning, regulating gene transcription, removing abnormally proliferating cells by inducing their apoptosis, protecting against cancer, etc. (e.g., Kirschner 1999; González-Flecha and Demple 2000; Ravati et al. 2000; Thannickal and Fanburg 2000; Carmody and Cotter 2001; Martin and Barrett 2002; Nakashima et. al. 2003; Becker 2004). This paper focuses on an illustrative example – postischemic injury occurring upon reintroduction of oxygen into ischemic tissue.

Postischemic injury – role of ROS and intracellular calcium

Involvement of ROS in tissue injury occurring due to organ ischemia and its subsequent reperfusion has been repeatedly reported, e.g. for the brain, heart, gastrointestinal tract, etc. (for review, see e.g. Goldhaber and Weiss 1992; Kehrer 1993; Phillis 1994). The mechanism of ROS generation by the xanthine oxidase-mediated process has been already described in detail (Inauen et al. 1989; Phillis 1994). Briefly, oxidative phosphorylation is inhibited due to lack of oxygen in ischemia. On the other hand, to maintain functioning of the tissue, its demands in energy are usually increased. Subsequently, enhanced catabolism of adenosine triphosphate results in accumulation of adenosine monophosphate, which is metabolized to adenosine and further to inosine and hypoxanthine. The latter is in normoxia metabolized by the enzyme xanthine dehydrogenase. Under hypoxic conditions, however, xanthine dehydrogenase converts to its oxidase form. The conversion of the enzyme is mediated by the calcium-dependent protease calpain (Iwamoto et al. 1999). Then, upon reoxygenation, while consuming molecular oxygen, xanthine oxidase produces superoxide anion radical. On the other hand, intracellular calcium concentration is known to increase due to hypoxia. Thus, calcium release from intracellular stores was reported to occur in neurons soon after the onset of anoxia (Katchman and Hershkowitz 1993). Implicitly, in case of the increased cytosolic calcium concentration, conversion into xanthine oxidase may occur with following production of ROS

as a consequence (Phillis 1994). In addition to ROS produced by xanthine oxidase, ROS have been indicated to generate during direct interaction of electrons, in accumulated partly reduced anaerobic intermediates, with oxygen molecules (Nohl and Jordan 1986; Baker and Kalyanaraman 1989). Besides, neutrophils have been reported to be attracted and activated by superoxide produced by the endothelial xanthine oxidase during reperfusion (Goldhaber and Weiss 1992; Sahnoun et al. 1998). Indeed, it was demonstrated that administration of antioxidants inhibited involvement of neutrophils in the postischemic tissue, and neutrophil depletion reduced reperfusion injury (Grisham et al. 1998; Klebanoff 1999). Superoxide, produced e.g. by xanthine oxidase, was also evidenced to release iron from ferritin (Ryan and Aust 1992), thus providing iron to Haber–Weiss reaction yielding hydroxyl radical. Hence, *via* involving other pathways, xanthine oxidase activated by calcium-dependent mechanism can mediate far more massive generation of other ROS upon the reoxygenation.

Pharmacologic inhibition of ROS production was reported to reduce the extent of damage caused by ischemia and reperfusion, yet tissue protection was often found to be only partial (Ravingerová et al. 1991; Goldhaber and Weiss 1992; Bezek and Juránek 2000). On the other hand, both inhibitors of calcium entry and chelators of intracellular calcium were shown to protect various tissues from ROS action and to inhibit postischemic injury (Gabauer et al. 1991; Jacinto and Jandhyala 1992; Dyatlov et al. 1998). The protective effect of calcium blockers was earlier attributed to their ability to inhibit lipid peroxidation and to prevent ROS formation (Mak and Weglicki 1990; Henry 1991; Nègre-Salvayre and Salvayre 1992; Mišík et al. 1993). However, it has been suggested that, in order of importance, decreased availability of oxygen, calcium overload, and ROS overproduction, were responsible for reperfusion injury (Ravingerová et al. 1991; Takuma et al. 2004). Accordingly, one may expect at least a few mechanisms of calcium-dependent ROS formation to be involved in postischemic tissue: e.g. the calcium-dependent and calpain-mediated conversion of xanthine dehydrogenase to xanthine oxidase, calcium-mediated upregulation of NADPH oxidase, and calcium-dependent activation of phospholipases A_2 (Phillis 1994; Takuma et al. 2004).

All the above-presented findings may suggest that postischemic tissue injury could occur just as an inevitable consequence of increased cytosolic calcium concentration leading to upregulation of enzymatic breakdown of essential intracellular constituents. Intracellular calcium overload also seems to play a crucial role in the transition of reversible alterations into irreversible ones, resulting in cell death. In fact, the cytosolic calcium concentration increased due to hypoxia may be readily responsible for both the initiation of degradative processes and ROS overproduction occurring upon reoxygenation of hypoxic/ischemic tissue (Kristensen 1994; Phillis 1994). Thus, ROS would be excessively produced as a consequence of calcium overload. Therefore, we wish to suggest that though ROS are important mediators in postischemic injury, they are unlikely to be its primary cause. This assumption may be of considerable interest since it would also help to explain some of the

apparent discrepancies on the effects of antioxidants in tissue injury evoked by ischemia-reperfusion or hypoxia-reoxygenation.

Controversies on ROS involvement in postischemic injury

Classically ROS have been considered injurious. According to the free radical hypothesis, the tissue injury induced by ischemia and reperfusion occurs due to the uncontrolled production of deleterious ROS, which impair cellular constituents (Ďuračková et al. 1993; de Groot 1994; Phillis 1994; Kovacs et al. 1996; Gaté et al. 1999; de Zwart et al. 1999; Medocci et al. 1999). Easily oxidizable fatty acids of membrane phospholipids are a good target for peroxidative attack, leading to alterations of membrane permeability and fluidity. That in turn may result in dysfunction of proteins, such as ionic pumps, whose activity depend on the membrane lipid milieu. Additionally, direct oxidation of sulfhydryl groups in enzymes and proteins were found to affect their functions, including those maintaining membrane potential (Grover et al. 1992; Gaté et al. 1999; Ravingerová et al. 1999). The impaired ion distribution and increased cytosolic calcium concentration were thus considered an ultimate consequence of ROS action, produced e.g. during reperfusion of the ischemic tissue (Suzuki et al. 1997; Wang and Joseph 1999; Lounsbury et al. 2000). Whether ROS induce cytosolic calcium increase (Lounsbury et al. 2000; Lehotský et al. 2002) or whether the latter evokes enhancement of ROS generation (Kehrer 1993; Phillis 1994; Kristián and Siesjö 1999) has not yet been conclusively established. Anyway, calcium overload and ROS overproduction are closely interconnected in both positive and negative sense (Zoccarato et al. 2004). Some experimental findings obtained from *in vitro* and *in vivo* models appear to support the free radical hypothesis. Thus, a causal role of ROS was suggested in ischemia-reperfusion injury to the brain, myocardium, liver, kidney, intestine, etc. (Horáková et al. 1991; Goldhaber and Weiss 1992; Juránek et al. 1992; Bauer et al. 1995; McDonald et al. 1999; Bezek and Juránek 2000). Yet, much of the evidence presented is indirect and hardly valid, since the data were derived from observations where antioxidants improved just free-radical-derived parameters, such as intensity of lipid peroxidation or content of reduced thiol groups, presumably expected to reflect the extent of injury. On the other hand, there is a considerable number of negative reports concerning inhibition of tissue injury by antioxidants (Liu et al. 1989; Barinaga 1991; Armstead et al. 1992; Horáková et al. 1992).

Other findings, some from over a decade ago, clearly pointed out obvious discrepancies between the timing of tissue injury and ROS overproduction. Thus, it has been demonstrated that peak of hydroxyl radical did not coincide with cell damage (Khalid and Ashraf 1993). It was also found that hypoxia and reoxygenation damage in the liver occurred without any contribution of ROS (de Groot and Littauer 1989). Later Villa et al. (1992) reported no significant amount of ROS generated intracellularly in the liver during ischemia and reperfusion. In agreement, ischemic vasoconstriction of hepatovasculature has been sug-

gested to be a contributing factor to the reperfusion injury of the liver (Kukan et al. 1996). Further, based on measurements of xanthine oxidase activity, de Jong et al. (1991) concluded that free radical damage of the human heart, mediated by xanthine oxidase, is unlikely. Moreover, no protection from the postischemic injury to the kidney or skeletal muscle was found after administration of superoxide dismutase and catalase, indicating no involvement of superoxide anion radical or hydrogen peroxide (Borkan and Schwartz 1989; Long et al. 1989). It was also shown that despite the fact that ROS production depended on oxygen tension in the reperfusion phase, the cerebral injury evoked by ischemia failed to do so, suggesting that ROS were not the cause of brain postischemic damage (Agardh et al. 1991). Further, although lipid peroxidation in the gastric mucosa was inhibited by vitamin E or allopurinol, ischemia-reperfusion-induced lesions were not reduced at all (Armario et al. 1990; Tarnasky et al. 1990). It was also reported that absence of protection by superoxide dismutase indicated superoxide to be not a factor in cell killing in reperfused hearts (Downey et al. 1991). Later Kehrer (1993) concluded: "free radicals formed at reperfusion are innocuous".

Supporting evidence has come from further studies demonstrating that ROS are generated mostly in reperfusion. If the duration of the hypoxic phase is sufficient, xanthine dehydrogenase is converted to its oxidase form, and ROS may be generated upon reoxygenation (Battelli et al. 1992; Kukan et al. 1996; Juránek et al. 2002). There are only scarce findings on ROS production in the ischemic phase. In fact, ROS generation, as recorded by electron paramagnetic resonance, increased markedly from the moment of reoxygenation (de Santis and Pinelli 1994). Enhanced ROS formation was detected in the isolated rat heart within the first 20 s of its reperfusion following 10 min of global ischemia. This ROS overproduction did, however, not contribute either to the dysfunction of the myocardium or to the rate of lipid peroxidation in the tissue (Maupoil and Rochette 1988). Other investigators have also observed enhanced ROS production occurring upon reperfusion of a variety of postischemic tissues (Davies 1989; de Santis and Pinelli 1994). If ROS production does occur during hypoxia or ischemia, it may result from inadequate experimental conditions, i.e. as a methodological artifact. Indeed, even at a very low concentration of oxygen, originating for instance from the surrounding air atmosphere, its level was found to be sufficient to induce peroxidative processes in the hypoxic tissue (Salaris and Babbs 1989). To distinguish such artificial ROS production, differential comparison of the spectra of reduced metabolites occurring during hypoxia and those of peroxy radicals appearing upon reoxygenation has been suggested (Baker and Kalyanaraman 1989).

Some epidemiological studies indicated that ischemic heart disease and certain types of cancer were inversely related to the endogenous antioxidant status (Gey et al. 1991). Other studies reported that antioxidant supplementation might even be deleterious, e.g. in lung cancer or cardiovascular diseases (Riemersma et al. 1991; Omen et al. 1996). In the presence of α-tocopherol ischemia was found to cause a dramatic increase in cell death, accompanied by a large increase in in-

tracellular calcium concentration and lipid peroxidation products (Dyatlov et al. 1998).

Hence many findings indicate that ROS are unlikely a causal factor of postischemic injury. This represents a remarkable shift in perception of ROS playing a pivotal role in the etiology of tissue damage. That is why ROS overproduction should be apprehended rather as the consequence than the cause of tissue injury except for the injury caused by ionizing radiation forming hydroxyl radical in process of water radiolysis (Mitchell et al. 2000; Lehnert and Iyer 2002), proved also by the effectiveness of antioxidants as radiprotectors (Weiss and Landauer 2000).

Concluding remarks

Although the reaction of ROS with macromolecules may result in destruction and degradation of the given compounds, the mechanisms of ROS-mediated cytotoxicity *in vivo* are not well understood as yet. Free radical-mediated lipid peroxidation, protein oxidation and oxidative damage to nucleic acids are considered to be crucial events of the cytotoxic actions of ROS. Though, there are authors understanding excessive production of ROS as smoke after the fire, i.e. as an inevitable consequence of destruction of the affected macromolecules because of some primary damage to tissue (Halliwell 1994), implicating that ROS overproduction is actually a consequence of injury itself. Moreover, Sahnoun et al. (1998) suggested ROS to play a critical role in limiting tissue lesion and preventing infection. Feinendegen (2002) even concluded: "since apoptosis caused by elevated ROS concentrations eliminates a damaged cell, low ROS concentrations during antioxidant administration may prevent apoptosis in favor of cell survival and proliferation also in neoplastic cells and thus rather promote cancer than protect against it". It has been also demonstrated that free radical scavengers blocked ROS-mediated neuroprotection evoked by preconditioning (Ravatti et al. 2000). Thus, a major concern ought to be given to intentions of attenuating toxic effects of ROS by their scavenging since that should also affect some essential cellular functions. Therefore, with faith of pharmacological intervention, one needs to carefully consider any antioxidant supplementation.

Postischemic tissue injury may, on the other hand, develop as a result of intracellular calcium overload, occurring due to hypoxia (Brecht et al. 1992). Activation of calcium-dependent processes with following ROS generation may trigger degradation of the integral cellular components causing serious problems to the cell. If untreated, these may result in cell death and, in a massive scale, in tissue injury and organ dysfunction. Thus, enhanced concentration of cytosolic calcium i) activates degradative enzymes, and ii) converts xanthine dehydrogenase to xanthine oxidase by calcium-dependent enzyme calpain. The first process may lead to irreversible injury as a consequence of hypoxia itself (Kristensen 1994); calcium-mediated glutamate release with subsequent upregulation of N-methyl-D-aspartate receptors resulting in hypoxia-induced neuronal damage represents a typical example (Katchman and Hershkowitz 1993; Takuma et al. 2004). The second process is

essential for the production of xanthine oxidase-derived ROS (Phillis 1994), which may then serve as a signal for other processes, such as neutrophil activation (Grisham et al. 1998), usually resulting in even more ROS generation.

Hence, any time after tissue ischemia or hypoxia, leading to accumulation of reduced metabolites, increased ROS production may be expected upon oxygen reintoduction. This allows excessively accumulated electrons to react rapidly with the potent and universal electron acceptor, molecular oxygen, and thus enables ROS creating. Produced ROS, on the other hand, may have no significance in triggering the development of postischemic injury. Yet, once generated, they may aggravate the postischemic injury, either directly or indirectly, for example by neutrophil activation.

In conclusion, ROS were first implicated as deleterious, however, later findings acknowledged their importance in cell signaling. Although enhanced ROS concentration is a useful biomarker of ongoing macromolecule destruction, accompanying wide a range of injuries of different etiology, it is difficult to clearly differentiate injurious and healing events in the process of tissue damage. Thus, ROS have to be considered to act not only as mediators of injury but also of healing. One has to be cautious in interpreting the experimental data indicating an involvement of ROS in tissue damage as there is convincing evidence that ROS are unlikely to be a uniform cause of tissue injury, even though they are able to promote and exacerbate the pathogenic process. The intriguing possibility is that they may actually be important mediators of the healing process and therefore may reflect the effort of the organism to reestablish homeostasis.

Acknowledgements. This work was supported in part by grants from the Slovak VEGA (2/4127/04 and 2/5052/25).

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Final version accepted: June 15, 2005