Editorial

Cogitation about free radicals and oxidative stress – an old concept with many new limitations

A. Ziegelhöffer

Institute for Heart Research, Center of Excellence in Cardiovascular Sciences, Slovak Academy of Sciences, Dúbravská cesta 9, P.O.Box 104, 840 05 Bratislava 45, Slovakia. E-mail: usrdzigy@savba.sk

Key words: Oxidative stress — Free radicals — Hydroxyl radical — Radical diseases — Antioxidants

Creation of free oxygen radicals (FR) and reactive oxygen species (ROS) in biological material as well as the mechanisms and consequences of their action, are employing the mind of many investigators already for more than three decades. During this time it became increasingly important and to some degree even fashionable to explore the actions of ROS, associated with some damage, as well as the possibilities and outcome of defense against their attack. Orientation on involvement of FR and ROS in pathophysiological processes gave birth to the classical concept of oxidative stress (OS) as early as at the beginning of the 80-ies in the previous century (Sies 1985). On the other hand, serious interest in the possible roles of FR, ROS and NO (not excluding peroxynitrite radicals) in non-destructive processes, such as in crosstalk between intracellular organelles and cell signaling (Suzuki et al. 1997; Hensley et al. 2000; Thannickal and Fanburg 2000; Becker 2004) as well as in compensation and adaptation mechanisms, like those in ischemic preconditioning of the heart (Ravingerová et al. 2004; Andelová et al. 2005) etc., started much later, around the year 2000 only.

The amount of studies offered to investigation of OS grew to thousands. They concerned different organs, tissues and cells, in nearly each physiological and pathological situation. However, only a small minority among the studies dealing with OS was really based on direct, quantitative estimation of relationships between the amounts of FR and ROS in action and their effects. Similarly, relatively little effort was offered to elucidation of exact space and distance relationships between the specific loci where the extremely reactive FR were expected to be created and the often very distant sites (structures) where their action was detected.

Great amount of studies on OS was based on the assumption that whether upon an external or an internal impulse, FR are created and attacking at once as an interdependent group forming a unified whole. Consequently they are overrunning the spacial unclearly (?) organized defense systems and doing damage to every available target. In this type of studies usually less attention was paid to specificities in the reactivity of single FR species. On the other hand, many studies on OS were focused on investigation of the role of some selected FR species or to follow-up of the reaction sequences of some ROS only. Hence, unfortunately, some studies on OS were leaving open more questions then they solved. In addition, more than one they yielded in broad minded generalization of partial conclusions and consequently in involuntary inter-exchange of causes with the consequences (Juránek and Bezek 2005), thus giving rise to some serious doubts about the current paradigm of involvement of the FR, ROS and oxidants in the presumptive mechanism of the OS (Baynes and Thorpe 1999; Ziegelhöffer et al. 1999; Ravingerová et al. 2000; Brookes et al. 2004; Juránek and Bezek 2005).

Probably because of challenging techniques requested to quantitative FR determination in tissues, the efforts were focused mainly to less sophisticated estimations of oxidized, predominantly more distant products of the radicals-triggered reaction sequences, indicated as markers of the OS (Kehrer and Starnes 1989; Juránek et al. 1992; de Zwaert et al. 1999; Nakashima et al. 2003). In these studies, ROS and FR were usually considered as pathogenic factors and, consequently, their evaluation lead to the notion that many diseases are either tightly associated with FR and ROS-induced perturbations in metabolism and function or that FR and ROS are at least involved in some phase of their etiopathogenesis. These diseases were then often termed as "free radical diseases" (Harman 1986; Halliwell et al. 1992; Halliwell 1994). Starting from this point of view, administration of diverse antioxidants was proposed, in order to prevent the damage believed to be caused by FR and ROS (Sies 1991, 1993; Halliwell 1995). Nevertheless, the antioxidants as therapeutic tools fulfilled the expectations only partially. The direct efficacy of global, systemic anti-oxidation treatment rarely exceeded 30–40% of what was anticipated

(Patterson et al. 2000; Ward and Croft 2006). Moreover, the treatment with diverse antioxidants was in numerous cases associated with undesirable side effects. It prevented, mostly completely, the genuine processes of endogenous protection leading to adaptation of tissues and cells to the given noxas (Ziegelhöffer et al. 1999; Ravingerová et al. 2000, 2001; Ferko et al. 2006).

Let us try to analyze at least some among the weak points that make the concept of OS vulnerable and provide reasons for limited success of the anti-OS treatment. The original concept of the OS is based on the observation that ischemia/reperfusion (I/R) as well as numerous chronic, degenerative, autoimmune and inflammatory diseases are accompanied with enhanced formation of FR, particularly the most biologically damaging hydroxyl radical (HR). Use of the adjective oxidative probably may be ascribed to the fact that at least the end products and/or markers of the FR-induced damage represent oxidized derivatives of lipids, proteins and nucleic acids (Maupoil and Rochette 1988) and that they themselves may represent a source of toxicity.

Further support for use of the term oxidative was provided by the experience that the FR- and particularly the HR-induced damage became apparent in reperfusion phase of the I/R process (Ravingerová et al. 1999) and that some antioxidants are capable to alleviate I/R-induced damage (Halliwell et al. 1992; Halliwell 1994).

Limitation 1 – terminological

A characteristic feature of HR is their capability to react with double bonds of diverse aliphatic and aromatic compounds in a way that is difficult to understand as oxidation, at least in respect to laws of electrochemistry. Moreover, it is clearly demonstrated that HR are created and attacking exclusively in the oxygen lacking, hypoxic-ischemic phase of the I/R process, whereas oxygen only enters the stage in subsequent steps of the reaction sequences running during reperfusion and yielding in formation of markers of the OS (Maupoil and Rochette 1988; Kehrer and Starnes 1989; Eaton et al. 1999). Although, admitting that in some different cases oxidation might be the primary reaction, it is paramount that the FR will always act at the beginning. Hence, from the aspect of priority of action it would be less deceiving and more exact to use the term radical-induced stress, instead of the indication OS. Nevertheless, it can not be excluded either that in many cases the oxidized intermediary or end-products of the FR-triggered reactions represent the truly active elements that induce the damage. However, this may concern a large group of compounds with not always satisfactorily defined degree of toxicity. Therefore, at the present state of arts, the available data neither confirm in each case the exclusivity of FR and oxidation process nor the toxicity of oxidized metabolites as true initiators of all events understood under the term OS. Hence, the latter term seemed to loose its exact meaning. Many investigators became aware of this fact. They either stopped to use the term OS completely or tried to bypass it by speaking about redox metabolism or redox signaling (Maulik and Das 2002). Other, more conservative authors still admit the use of the term, but in a non-promiscuous way and in the aim to avoid any misunderstanding they attempt to explain thoroughly what do they mean when speaking about the OS (Vasdev et al. 2006).

Limitation 2 – substantial

Most studies dealing with biological effects of FR still quote to the old definition of Sies (1991) referring to OS as a "disbalance between formation and removal of reactive metabolites of oxygen and nitrogen in favour of their formation which may yield in potential damage". In context of this definition, the indication reactive metabolites of oxygen and nitrogen also included the FR. Later on Halliwell (1995) supported the above definition by indicating OS as a "disbalance between FR formation and the anti-oxidation defence that leads to damage of tissues and organism." However, unfortunately, more than a decade after their formulation both this definitions became very vague since they were based on the assumption that in normal conditions, the formation of FR and of all reactive metabolites of oxygen and nitrogen is in equilibrium with their removal. Nevertheless, this would work only if all potential sites of FR and ROS formation in tissues and cells would be also equipped with systems with adequate capacity for their elimination. However, since yet, it is missing any evidence for that. To eliminate the anticipated discrepancies in this respect, Sies armored his definition as follows: "About OS we may only speak in cases when ROS formation is increased significantly". However, the development since 1991 revealed that a local increase in FR in a small cell compartment may have already high significance for signaling towards activation of diverse intracellular systems (Hensley et al. 2000; Nakashima et al. 2003; Brookes et al. 2004) including those of intrinsic protective mechanisms (Ziegelhöffer et al. 2002; Ferko et al. 2006). These important signals neither require an intensity to be visible in the bulk concentration of radicals, nor to be reflected in increase in the damage to the cell indicated by accumulation of reaction products of FR. Hence, the old definitions of OS starts to become slowly outdated also from this aspect.

Limitation 3 – stress or cumulative action of FR and ROS?

Hans Selye (1936) indicated stress as a 3-stage sequence of changes which occur upon exposure to various noxious agents which he termed "the general adaptation syndrome". The first stage Selye called "the alarm reaction". It was accompanied, beside others, with loss of cortical lipoid and

medullary chromaffin substances from the adrenals. The second stage was indicated as "the stage of resistance" and it could only be reached if the stressed object survived. In this stage, the adrenal remain enlarged but regain their lipoid, while the medullary cells show vacuolization. If the stressing impulse was sufficiently severe, it followed "the stage of exhaustion".

Since the discovery of Selye, numerous new stressors including FR and ROS were studied from the aspect of the mechanism of their action. These studies confirmed that stress is a 3-stage single, basically non-cumulative reaction which may have different intensity but always have limited duration. Stresses may follow repeatedly, but one stress reaction, although if coupled with production of radicals, can't be prolonged indefinitely. However, in long lasting diseases, FR are produced more or less continually and may show cumulative action. Therefore, it would be fair to distinguish: in I/R and in similar situations admit the participation of a process resembling radicals-induced stress. Opposite case represents the cumulative effect of FR produced permanently in noxas such as diabetes, hypertension etc., where a stress reaction following directly from the sickness may be only expected at the beginning of the disease. In such cases would be more correct to replace the term OS by using expressions like: damage by oxidants and radicals, overload with oxidants and radicals, cumulative action of radicals, etc.

Summary

Since its creation, the concept of OS became very popular. Manifestations of the OS were investigated, verified, proved and disproved in thousands of studies.

However, the enormous amount of knowledge about OS that accumulated in the last decades had dual influence: it extended the original concept of OS considerably, often even in an undesirable way, but it also pointed to its vulnerability. The present treatise is a cogitation about some main limitations that can make the original concept of OS outdated. No matter whether outdated or only less exact, it would be better to think it over three times prior using the term OS.

Acknowledgments. Supported by grants VEGA 2/5110/26, 2/7126/27, APVV 51-027404 and the NATO project CBP.NUKR. CLG 981884.

References

Andelová E., Barteková M., Pancza D., Styk J., Ravingerová T. (2005): The role of NO in ischemia/reperfusion injury in isolated rat heart. Gen. Physiol. Biophys. **24**, 411–426

Baynes J. V., Thorpe S.R. (1999): Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes **48**, 1–9

Brookes P. S., Yoon Y., Robotham J. L., Anders M. V., Sheu S. S. (2004): Calcium, ATP and ROS: a mitochondrial love-hate triangle. Am. J. Physiol., Cell Physiol. **287**, C817–833

Becker L. B. (2004): New concepts in reactive oxygen species and cardiovascular reperfusion physiology. Cardiovasc. Res. **61**, 461–470

de Zwart L. L., Meerman J. H. N., Commandeur J. N. M., Vermeulen P. E. (1999): Biomarkers of free radical damage applications in experimental animals and in humans. Free Radic. Biol. Med. **26**, 202–226

Eaton P., Li J. M., Hearse D. J., Shattock M. J. (1999): Formation of 4-hydroxy-2 nonenal modified proteins in ischemic rat heart. Am. J. Physiol., Heart Circ. Physiol. **276**, H935–943

Ferko M., Gvozdjaková A., Kucharská J., Mujkošová J., Waczulíková I., Styk J., Ravingerová T., Ziegelhöffer-Mihalovičová B., Ziegelhöffer A. (2006): Functional remodeling of heart mitochondria in acute diabetes: interrelationships between damage, endogenous protection and adaptation. Gen. Physiol. Biophys. **25**, 397–413

Halliwell B. (1994): Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet **344**, 721–724

Halliwell B. (1995): Antioxidant characterization. Methodology and mechanism. Biochem. Pharmacol. **49**, 1341–1348

Halliwell B., Gutteridge J. M. C., Cross C. E. (1992): Free radicals, antioxidants and human disease: where we are now? J. Lab. Clin. Med. **119**, 598–620

Harman D. (1986): Free radical theory of aging. In: Free Radicals, Aging and Degenerative Diseases (Eds. J. E. Johnson Jr., R. Walford, D. Harman and J. Miguel), pp. 3–49, Liss, New York

Hensley K., Robison K. A., Gabbita S. P., Salsman S., Floyd R. A. (2000): Reactive oxygen species, cell signaling and cell injury. Free Radic. Biol. Med. **28**, 1456–1462

Juránek I., Bauer V., Horáková L. (1992): Influence of acute stress on lipid peroxidation *in vivo*. Pharmazie **47**, 716–717

Juránek I., Bezek Š. (2005): Controversy of free radical hypothesis: reactive oxygen species – cause or consequence of tissue injury? Gen. Physiol. Biophys. **24**, 263–278

Kehrer J. P., Starnes J. W. (1989): Models and markers used to study cardiac reperfusion injury. Pharmacol. Ther. **44**, 123–145

Maulik N., Das D. K. (2002): Redox signaling in vascular angiogenesis. Free Radic. Biol. Med. **33**, 1047–1060

Maupoil V., Rochette l. (1988): Evaluation of free radical and lipid peroxide formation during global ischemia and reperfusion in isolated perfused rat heart. Cardiovasc. Drugs Ther. **2**, 615–621

Nakashima I., Liu W., Akhand A. A., Takeda K., Kawamoto I., Kato M., Suzuki H. (2003): 4-hydroxynonenal triggers multistep signal transduction cascades for suppression of cellular functions. Mol. Aspects Med. **24**, 231–238

Patterson C., Madamanchi N. R., Runge M. S. (2000): The oxidative paradox. Another piece in the puzzle, Circ. Res. **87**, 1074–1076

Ravingerová T., Slezák J., Tribulová N., Džurba A., Uhrík B., Ziegelhöffer A. (1999): Free oxygen radicals contribute to high incidence of reperfusion-induced arrhythmias in isolated rat heart. Life Sci. **65**, 1927–1930

Ravingerová T., Pancza D., Volkovová K., Džurba A., Štetka R., Ziegelhöffer A., Styk J. (2000): Response to myocardial ischemia is modulated in diabetic rat heart: adaptation to disease? Exp. Clin. Cardiol. **5**, 122–128

Ravingerová T., Štetka R., Barančík M., Volkovová K., Pancza D., Ziegelhöffer A., Styk J. (2001): Response to ischemia and endogenous myocardial protection in the diabetic heart. In: Diabetes and Cardiovascular Disease. Ethiology, Treatment and Outcomes. pp. 285–293, Kluwer Academic/Plenum Publishers, New York

Ravingerová T., Neckář J., Šimončíková P., Barančík M., Pecháňová O., Ziegelhöffer A., Kolář F. (2004): Oxidant signals and cardioprotection: dual role in susceptibility to ischemia/reperfusion injury. J. Mol. Cell. Cardiol. **37**, 322

Selye H. (1936): A syndrome produced by diverse nocuous agents. Nature (London) **138**, 32

Sies H. (1985): Oxidative stress: introductory remarks. In: Oxidative Stress. pp. 1–8, Academic Press, London

Sies H. (1991): Oxidative Stress: Oxidants and Antioxidants. Academic Press, London

Sies H. (1993): Strategies of antioxidant defense. Eur. J. Biochem. **215**, 213–219

Suzuki Y. J., Forman H. J., Sevanian A. (1997): Oxidants as stimulators of signal transduction. Free Radic. Biol. Med. **22**, 269–285

Thannickal V. J., Fanburg R. L. (2000): Reactive oxygen species in cell signaling. Am. J. Physiol., Lung Cell Mol. Physiol. **279**, L1005–1028

Vasdev S., Gill V. D., Singal P. K. (2006): Modulation of oxidative stress-induced changes in hypertension and atherosclerosis by antioxidants. Exp. Clin. Cardiol. **11**, 206–216

Ward N. C., Croft K. D. (2006): Hypertension and oxidative stress. Clin. Exp. Pharmacol. Physiol. **33**, 872–876

Ziegelhöffer A., Styk J., Ravingerová T., Šeboková J., Volkovová K., Čársky J., Waczulíková I. (1999): Prevention of processes coupled with free radical formation prevents also the development of calcium resistance in the diabetic heart. Life Sci. **65**, 1999–2001

Ziegelhöffer A., Ravingerová T., Waczulíková I., Čársky J., Neckář J., Ziegelhöffer-Mihalovičová B., Styk J. (2002): Energy transfer in acute diabetic rat hearts. Adaptation to increased energy demands due to augmented calcium transients. Ann. N. Y. Acad. Sci. **967**, 463–468