Trends Article:

OH-Radical-Type Reactive Oxygen Species: A Short Review on the Mechanisms of OH-Radical- and Peroxynitrite Toxicity

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Introduction

Forming of reactive oxygen species (ROS) is biologically necessary for all aerobic cells (Halliwell and Gutteridge, 1989; Elstner, 1990). For the evaluation of oxygen toxicity it is of great importance, however, in which cellular compartment and to which extent activation occurs.

Two important insights have to be kept in mind:

⇒ There is ROS-formation concerning signal transfer, regulation of intermediary metabolism as well as influence on induction and efficiency of immune answers. These processes are under metabolic control and deviations from steady-state equilibria are rare under physiological conditions.

⇒ There are ROS on the other side which escape metabolic control and concommitantly also intrinsic scavenger systems thus inevitably leading to disease symptoms and destructions.

The first category comprises superoxide, hydrogen peroxide, organic peroxides (leukotriene- and prostaglandin precursors) and also NO (formerly EDRF: **e**ndothelium-**d**erived **r**elaxation factor) or protein-bound tyrosine radicals representing obligatory, intermediary products during enzyme catalysis, for example in ribonucleotide reductase. The second category essentially comprises diffusion-controlled reactions based on site-specific escape of strong oxidants of the OH·-type from their "solvent cages" (Pryor and Squadrito, 1995; Czapski, 1984; Elstner *et al.,* 1987; Youngman and Elstner, 1981). These species concern subcellular areas of only a few nanometers since site-specific production and destruction of neighbouring molecules occur in the same local environment. For at least three reasons these reactions are no longer under control of the otherwise responsible regulators and antioxidative defence chains:

⇒ the extremely rapid reaction of these species with most biomolecules;

⇒ their local limits due to the extremely reduced life time and thus diffusion distances;

⇒ the most important scavenger enzymes such as superoxide dismutase (SOD), catalase or glutathione peroxidase are ineffective in most cases.

Through oxidations caused by the second category oxidants primary molecular damage may occur mostly concerning proteins or membrane lipids. Damage can not be avoided but may be repaired by the cooperatively working enzymic "team work" comprising proteases and lipases, which excise damaged amino- or fatty acids. In a second step *de novo* synthesized molecules are reinstalled and repair is finished.

Aerobic cells and thus the corresponding organs are unable to establish or develope specific mechanisms to avoid or counteract these oxidatively damaging reactions due to basic chemical reasons: if molecules or atoms react with other molecules or atoms in a diffusion-controlled manner, i.e. with

Abbreviations: AM, alveolar macrophage; EDTA, ethylene diamine tetraacetate; EPO, eosinophile peroxidase; GC, guanylate cyclase; HSP, heat shock protein; LDL, low density lipoprotein; L-NAME, N^{ω}-nitro-L-arginine methyl ester; MPO, myeloperoxidase; NMMA, N^{G}-monomethyl-L-arginine; NOS, NO-synthase; ROS, reactive oxygen species; SIN-1, 3-morpholinosydnonimine; SNAP, S-nitroso-N-acetyl-penicillamine; X, xanthine; XOD, xanthine oxidase.

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reaction constants faster than $k = 10^8$ $M^{-1}sec^{-1}$ (and this holds for OH-radical-type oxidants) enzyme catalysis is not efficient. Free diffusion of the reaction partners is limiting, and not thermodynamic or kinetic parameters at the active sites. In order to control these types of reactions only small "Kamikaze"-type molecules have a chance to interact with the final oxidant directly at the point of its formation i.e. the "solvent chage" which it escapes. During these process the small molecules act as one-electron donors. These reaction is followed by either destruction of the donor molecule or by its repair via secondary donors. Primary donors are mostly phenols such as tocopherol, ubiquinol, probucol, butylated hydroxitoluene or butylated hydroxianisole, flavonoids, cumarins, purins, tryptophan, to mention just a few. During action these molecules are converted into more or less stable phenoxy radicals. Secondary donors suitable for "repair" of the one-electron deficient primary donors are ascorbic acid or glutathione. These antioxidants in turn are regenerated at the expense of NAD(P)H by their corresponding specific reductases.

The antioxidative system obeys a certain "pecking order" and takes care about the primary damage thus helping to avoid visible or measurable secondary damage commonly addressed as disease symptoms (Davies, 1993).

Sources and Reactivities of OH·-Type Oxidants

The balance between effective cellular regulation and velocity of the damage-evoking reaction cascade finally determines whether repair mechanisms are governing the scenario or a "radical-chaos" takes over. Not desirable reactions therefore should be ameliorated or abolished by the administration of more or less specific antioxidants predominately or exclusively reacting with strongly electropositive agents.

Which reactions have to be envisaged as main producers of strongly electropositive reacting substances such as the OH-radical? The most important ones are summarized in Table I.

For the first two reactions (A, B) transition-metal catalysis is required, for reactions C, D and E apparently not. These observation has tremendous consequences on estimation and evaluation of the localisation of OH·-formation.

The most prominent ways of generation of OH-radical and its equivalents shall be shortly discussed here.

Generation and reactivity of the OH-radical

Xanthine oxidase (XO, E. C. 1.2.3.1.) is located in several cell types such as endothelial cells and is mostly present as xanthine dehydrogenase (oxidizing hypoxanthine, xanthine (X) or certain aldehydes via NAD^+-reduction). During certain stress reactions the dehydrogenase is converted (for example protease-catalyzed; c.f. Hassoun *et al.*, 1995) into xanthine oxidase, which reacts with the same electron donors, but reducing oxygen instead of NAD^+ thus producing superoxide and hydrogen peroxide.

Impurities of, or willingly added, iron ions create strong oxidants ("Fenton"-oxidants) which can very sensitively be identified via several appropriate methods (Elstner, 1990). In the presence of reductants (E_1 (xanthine in the XO-reaction)) with sufficient affinity for oxygen and an appropriately negative redox potential (E_o of the redox pair $O_2/O_2^{\cdot-}$ = -330 mV), superoxide may be formed from atmospheric oxygen:

$$E_1\text{-} + O_2 \rightleftarrows E_1^{\cdot} + O_2^{\cdot-} \text{ (superoxide formation)} \quad (1)$$

Superoxide dismutates at neutral pH in aqueous media with a rate constant k=2 x 10^5 ($M^{-1}sec^{-1}$), yielding hydrogen peroxide:

Table I. OH-radical forming reactions.

Reaction	Overview; Reference
A. Xanthine oxidase catalyzed reactions; Fenton-type reactions;	Elstner (1993)
B. NAD(P)H-oxidase catalyzed reactions;	Elstner (1993)
C. MPO/EPO-catalyzed reactions;	Pagano *et al.* (1995)
D. Reaction of hypochlorite with superoxide	Pryor and Squadrito (1995)
E. Decay of peroxynitrite	Candeias *et al.* (1993)

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2 \text{ (dismutation)} \quad (2)$$

Hydrogen peroxide in turn can be monovalently reduced by several electron donors such as reduced transition-metal ions (Fe^{2+};Cu^+) yielding the extremely reactive hydroxyl radical, $OH^{\cdot}$:

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^{\cdot} \quad (3)$$

Reaction (3) is addressed as "Fenton"-reaction.

Fe^{3+} in turn can be reduced to Fe^{2+} by superoxide ion:

$$Fe^{3+} + O_2^{\cdot-} \rightarrow Fe^{2+} + O_2 \quad (4)$$

The sum of reactions (3) and (4)

$$H_2O_2 + O_2^{\cdot-} \xrightarrow{Fe} OH^{\cdot} + OH^- + O_2 \quad (5)$$

is known as "Haber-Weiss"-cycle and is not existant as such in the absence of catalytic amounts of iron. EDTA stimulates $OH^{\cdot}$-formation by facilitating iron redox cycling whereas desferroxamine (desferal) blocks the cycle.

$OH^{\cdot}$ has a very positive redox potential (close to +2V) and a life time of approximately 1 μsec thus reacting closely to the site of its generation producing "site specific" oxidative damage. The oxygen species $OH^{\cdot}$ is the major target of the antioxidative power of phenolics: due to its kinetic properties, $OH^{\cdot}$ is a reactive oxygen species not under the control of specific enzymes and/or detoxification chains. Since superoxide is able to reductively release iron ions from transport molecules such as ferritin or transferrin or from hemoglobin, Fenton- or Haber-Weiss-chemistry is of utmost relevance in a wealth of disease processes such as inflammation.

Besides the X/XO-reaction activated leukocytes are another important source of superoxide. The NAD(P)H-oxidases of activated phagocytes primarily produce superoxide and hydrogen peroxide. The formation of $OH^{\cdot}$ is a secondary effect dependent on the catalysis mainly of iron derived from storage- or transport-proteins or from hemoglobin as already mentioned.

Another principle of the production of strong oxidants is the oxidation of halides (X^-) by hydrogen peroxide producing hypohalides (OX^-; c.f. Folkes *et al.*, 1995). The hypohalides in turn may be converted into $OH^{\cdot}$ in the presence of superoxide or Fe^{2+}. Neutrophil myeloperoxidase (MPO) catalyzes the formation of hypochloric acid at the expense of hydrogen peroxide and chloride ion, while the eosinophile peroxidase (EPO; c.f. Mc Cormick *et al.*, 1994) catalyzes an analogous reaction with bromide; both types of enzymes can utilize the pseudohalide, rhodanide (SCN^-), as substrate.

The sum of the above mentioned reactions may be formulated as follows (Cadeinas *et al.*, 1993; 1994):

$$H_2O_2 + X^- \xrightarrow{MPO/EPO} HOX + OH^- \quad (6)$$

$$HOX + O_2^{\cdot-} \rightarrow OH^{\cdot} + O_2 + X^- \quad (7)$$

$$HOX + Fe^{2+} \rightarrow OH^{\cdot} + X^- + Fe^{3+}. \quad (8)$$

Reactions (7) and (8) represent new reaction mechanisms known for only a few years.

Generation and reactivity of peroxynitrite (ONOOH): an OH-radical-type oxidant

Peroxynitrite, $ONOO^-$, is formed from nitrogen monoxide and superoxide according to:

$$NO^{\cdot} + O_2^{\cdot-} \rightarrow ONOO^-. \quad (9)$$

The reaction (9) is extremely fast and proceeds with a rate konstant of $k = 6.7 \times 10^9$ ($M^{-1}sec^{-1}$) indicating that ONOOH-formation is only diffusion-limited (Refs. 11–16 in Rubbo *et al.*, (1994)). ONOOH is mainly produced under pathophysiological conditions: rapid superoxide formation by XOD or activated neutrophiles together with NO-synthase-activation.

The role of ONOOH on shock, inflammation and ischemia-reperfusion has recently been reviewed by Szabo (1996a). ONOOH is an extremely destructive ROS: several indications lead to the assumption that disease induction is mediated by these reactive species. Firstly, stimulated chemiluminescence in pre-stimulated neutrophils (as indicator of a "further"-activating reaction) is enhanced by ONOOH (Bednar *et al.*, 1996). Parkinson's disease, evident as a lack of biologically active aromatic amines such as DOPA, is potentiated by ONOOH indicating its role in neurodegenerative processes (Ischiropoulos *et al.*, 1995). ONOOH also causes changes in protein organization, protein nitration and lipid peroxidation in erythrocytes or erythrocyte membranes (Soszynski and Bartosz, 1996). Furthermore ONOOH may

play an important role in sperm dysfunction (Mitropoulos *et al.*, 1996). DNA damage in human pancreatic islands after ONOOH exposure has been described as well (Delaney *et al.*, 1996).

ONOOH may contribute to the pathogenesis of various diseases by modifying ROS-generating capacity in the body through the induction of the conversion of xanthine dehydrogenase to XOD (Sakuma *et al.*, 1997).

Model reactions with inhibitors could demonstrate that only in the presence of both radicals, NO and O_2.-, and through their interaction, damage of endothelial cells occured (Bratt and Gyllenhammer, 1995). Very similar results were obtained by Lamarque and Whittle (1995) who showed that in the simultaneous presence of both, NO-donors such as sodium nitroprusside and superoxide generators such as XOD, gastric mucosal cells became damaged. Volk *et al.* (1995) concluded that the strong inhibition of a model reaction by catalase indicates also an interaction between NO and H_2O_2 which results in the damage of liver-endothelial cells. In addition, exposure of pulmonary arteria-catalase towards NO resulted in its inactivation as indicated by a change in H_2O_2-mediated vessel relaxation (Mohazzab *et al.*, 1996). A controversial but in some details similar observation was made by Mohsen *et al.*, (1995), who observed a damage of cardiomyocytes by H_2O_2 which was prevented in the presence of NO or catalase.

The HOONO-Precursor NO

NO is formed from arginine catalyzed by the enzyme NO-synthase (NOS; E. C. 1.14.13.39) according to the generalized formula:

$$\begin{array}{l} \mathrm{NH_2} \\ \mid \\ \mathrm{RC{=}NH} + 1.5\ \mathrm{NADPH/H^+} + 2\ \mathrm{O_2} \rightarrow \\ \mathrm{NH_2} \\ \mid \\ \mathrm{RC{=}O} + \mathrm{NO^{\cdot}} + 1.5\ \mathrm{NADP^+} + 2\ \mathrm{H_2O} \end{array} \qquad (10)$$

It has been suggested that the first product of the reaction might not be NO but NO^-, which is subsequently converted to NO by SOD (Schmidt *et al.*, 1996). NO-synthesis seems to proceed in a two-step reaction: the first reaction converts arginine into N^G-hydroxyl-L-arginine and is catalyzed by NOS, which seems to be activated by Cu^{2+}-ions (Ohnishi *et al.*, 1997). The second step is apparently independent of NOS and seems to be driven by superoxide generated by NADPH-oxidases or by XOD (Modolell *et al.*, 1997) in a reaction analogous to nitrite formation from hydroxylamine (Elstner and Heupel, 1976).

According to another very recent report, NOS not only produces NO but also superoxide at low arginine concentrations. These would explain why under certain conditions NO-synthesis yields cellular damage, but under other conditions not (Xia *et al.*, 1996). Likewise, proliferating bovine aortic endothelial cells produce both NO and superoxide upon stimulation by calcium ionophore (1 μM A-23187). In this case administration of 100U/ml SOD enhances guanylate cyclase (GC) activity indicating an interaction between superoxide and NO, where NO is rendered to be inactive in GC-activation (Arnal *et al.*, 1996).

The simple molecule NO (which should actually be written $NO^{\cdot}$ since it is a free radical like nitrogen dioxide, $NO_2^{\cdot}$) plays a very important role in the regulation of vascular tonus (c.f Elstner (1990, 1993)) activating GC thus producing the vasorelaxating cGMP. NO is present in arterial walls in a concentration of 100–400 nmol/l (Moore *et al.*, 1995). It therefore also plays a role in the pathophysiology of asthma where it acts as modulator of airway functions (Massaro and Drazen, 1996). Since NO is synthesized from arginine, administration of L-arginine during reperfusion significantly ameliorates pulmonary functions of blocked neonatal piglet heart-lung preparations (Shiraishi *et al.*, 1996).

NO also plays an important role in the central nervous system (Szabo, 1996b) where it regulates cerebral blood flow. As shown for the invertebrate mussel, *Mytilus edulis*, morphine stimulates NO-release from microglia (Liu *et al.*, 1996).

Leukocytes, activated for example by lipoxin A_4 (LXA_4), and also endothelial cells produce NO. Since alveolar macrophages (AM) of newborn rats exhibit amplified NO-production it has been suggested that NO triggers differentiation of AM thus strengthening the immunoresponses in early life (Sherman *et al.*, 1996). NO-production seems to be self-regulated since exogenous NO inhibits basal NO-release from vascular endothelia indicating negative feedback control (Ma *et al.*, 1996). NO

also seems to posses immunemodulatory functions via regulation of the production of proinflammatory interleukin-1 (IL-1) by murine macrophages. N^G-monomethyl-arginine (NMMA), a NOS-inhibitor, blocks IL-1-release from activated macrophages (Hill *et al.,* 1996). Likewise, prostaglandin F_{2a}-production in brain macrophages, the microglia, is activated by endogenous NO (Janabi *et al.,* 1996).

Endothelial protection by NO following reperfusion is due to upregulation of cell adhesion molecules (CAMs such as P-selectin) in its absence. This results in leukocyte adherance onto endothelia ("stickyness" of endothelium) and neutrophil infiltration yielding oxidative stress and necrotic reactions (Niu *et al.,* 1996; Lefer and Lefer, 1996). NO on the other hand seems to be important for the fungicidal ("anti-Candida")-activity of neutrophils since the NOS-inhibitor NMMA impairs killing of *C.albicans.* This effect is reversed by the NO-precursor, arginine (Fierro *et al.,* 1996).

In general NO seems to be „nature's naturally occuring leukocyte inhibitor" via two main mechanisms: first, NO decreases leukocyte adherence onto the endothelium by downregulation of the expression of both P-selectin and ICAM-1/VCAM-1; second, NO inhibits leukocyte action by inhibiting the cytoassembly of NADPH-oxidase (for review see Lefer, 1997).

In the early phase of diabetes NO may also act as a mediator of pancreatic ß-cell damage (Delaney and Eizirk, 1996).

Beside NO-formation via NOS-catalysis NO can be generated spontaneously from S-nitroso-N-acetyl-DL-penicillamine (SNAP), sodium nitroprusside and 3-morpholinosydnonimine (SIN-1), where SIN-1 produces simultaneously both NO as well as $O_2^{\cdot-}$ (Feelisch *et al.,* 1989; Bohn and Schönafinger, 1989).

Peroxynitrite as Precursor of the OH-Radical?

As mentioned above SIN-1 produces simultaneously both NO and $O_2^{\cdot-}$. SIN-1 was used as peroxynitrite generating substance by Inoue and Kawanishi (1995); they concluded that the damage of calf thymus DNA, measured as the formation of 8-hydroxy-deoxyguanosin (8-OHdG) by SIN-1, was due to the formation of an OH·-like oxidant. Effects of several inhibitors on damage of isolated bacteriophage DNA also seem to be due to a "single" reactive intermediate, but not to ONOOH itself (Epe *et al.,* 1996). This strong oxidant, which is produced in slightly acidic milieu ($pK_{a,app}$ for ONOOH = 6.8), corresponds with the "free" OH-radical formation via homolytic decay of peroxynitrite according to reaction (11) (Pryor and Squadrito, 1995):

$$ONOOH \rightarrow \{ONO^{\cdot} \cdot OH\} \rightarrow NO_2^{\cdot} + OH^{\cdot}. \qquad (11)$$

Even if the percentage of free OH· (relative to other generated radicals as calculated by electron paramagnetic resonance (EPR)-results) seems to be as small as 1 to 4% of total ONOOH (Pou *et al.,* 1995), the effect may be again due to "site-specificity" of the "cage", {ONO· ·OH}, and especially to the "escaping" OH· in the immediate proximity of the locus of formation.

But there are other peroxynitrite-dependent mechanisms which lead to an increase of vascular or neurological pathogenesis, independent of OH·:

⇒ the non-enzyme-catalysed formation of vasoconstrictory F_2-isoprostanes from arachidonic acid (AA; ca. 10% of all unsaturated fatty acids in LDL are AA!) via oxidation by ONOOH (Moore *et al.,* 1995). This process might simultaneously lead to oxidative modification of LDL and thus induce atherogenic processes;

⇒ the superoxide dismutase-catalyzed formation of highly reactive nitronium ions ($O{=}N^{+}{=}O$) from peroxynitrite according to:

$$ONOO^{-} \cdots SOD\text{-}Cu^{2+} \rightarrow SOD\text{-}Cu^{1+}O^{-} \cdots O{=}N^{+}{=}O^{\cdot}. \qquad (12)$$

which are able to nitrify for example tyrosine residues in proteins and thus work in competition to signal-chains where tyrosine phosphorylations have central regulatory functions (protein kinases!) (Beckman *et al.,* 1994):

$$SOD\text{-}Cu^{1+}O^{-} \cdots O{=}N^{+}{=}O + \text{tyrosine} \rightarrow SOD\text{-}Cu^{2+} + OH^{-} + NO_2\text{-tyrosine}. \qquad (13)$$

(formation of nitrotyrosine)

Carbon dioxide reacts rapidly with ONOOH (k = 2.9×10^4 $M^{-1}sec^{-1}$) forming $ONOOCO_2$-, which in turn enhances tyrosine nitration (Gow *et al.,* 1996).

Regulatory Phenomena during the Interaction between $NO^{\cdot}$ and $O_2^{\cdot -}$

If Haber-Weiss- (or Fenton-type-) reactions are predominating NO might act as an antioxidant by binding $O_2^{\cdot -}$ and thus avoiding both the formation of hydrogen peroxide via dismutation as well as reduction of Fe^{3+}.

Capillary leaks in rat lungs are induced by oxidative damage; breathing of NO protects from this damaging effect. This protection is not predominately due to vasodilatation but more likely to interaction with ROS produced by activated leukocytes. Thus NO under certain conditions may have antioxidative and/or antiinflammatory properties in addition to its "messenger"-role in vasodilatation (Guidot *et al.*, 1995).

Vice-versa, ROS have an inhibitory influence on NO-synthesis thus acting vasoconstrictory as shown in a rat-shock-model (hemorrhagia/reperfusion model; Myers *et al.*, 1995).

This activity of ROS is presumably again mainly due to superoxide as demonstrated in another model with diabetic (streptozotocin-treated) rats, in contrast to control-rats: Superoxide dismutase-application diminuated the constrictory effect of N^G-nitro-L-arginine methyl ester (L-NAME, a NOS-inhibitor) on renal arteriole diameter, interpreted as a desactivation of $O_2^{\cdot -}$ as NO-"scavenger" (Ohishi and Carmines, 1995). Numerous natural and synthetic flavonoids (such as 6,7-dimethoxy-8-methyl-3',4',5-trihydroxyflavon; Girard *et al.*, 1995) react with products of XO such as superoxide and may thus be able to protect relaxating and antioxidative properties of NO indirectly.

An excellent review concerning the extremely complex interactions between NO and ROS and their effects on vascular endothelia, especially addressing regulatory processes, has been given by Marin and Rodriguez-Martinez (1995).

A tonic-protective effect of endogenously produced NO during ischemia-reperfusion on damage of rabbit myokard was also reported very recently: L-NAME increased the infarction seize after reperfusion of the myokard (Williams *et al.*, 1995). Likewise, pulmonary oxygen toxicity and death rate after exposure of rats towards 99% oxygen for 72 h (2 l/min) was significantly enhanced by L-NAME, indicating a protecting role of endogenous NO-synthesis (Capellier *et al.*, 1996). Similarly, ischemia-reperfusion-induced permeability increase as a measure for microvasculare damage was prevented by NO-donors (Moore *et al.*, 1996). It does not seem unjustified, therefore, to conclude that an inclusion of NO-donors (such as L-arginine or SNAP) in cardioplegic solutions might have positive effects on myokard contraction (Bing, 1995).

Similar positive effects of the NO-donors SNAP and Spermine-NO on macrophage-dependent oxidation of LDL are discussed as additional properties of NO, namely as radical chain-breaker during lipid peroxidation within LDL-molecules (Hogg *et al.*, 1995). Lipid peroxidation under production of novel nitrogen-containing lipids by ONOOH is also attenuated by NO probably by interacting with lipid radicals thus acting again as a "chain-breaker" (Rubbo *et al.*, 1994).

Endogenous and Exogenous Regulators of ONOOH-Functions

As already outlined above, {ONOOH} seems to mainly act as a pathogenic molecule.

The release of $OH^{\cdot}$ from the cage seems to be a rate-limiting step and seems to proceed at a low percentage (3–4% of total peroxynitrite) and extremely site specific. Nevertheless there are yet other reactions which the cage undergoes in carbonate containing media (see above), where the reaction with bicarbonate (HCO_3^-) under formation of $ONO_2CO_2^-$ occurs at physiological pH and physiological CO_2-concentrations of 25 mM and with a reaction constant of k=3 x $10^4 M^{-1}sec^{-1}$ (Lymar and Hurst, 1995). This reaction is probably responsible for the limited life time of {ONOOH} in physiological media. It may well be also responsible for the detoxification of ONOOH, but also for the formation of reactive and thus potentially toxic $HCO_3^{\cdot}$-radicals after homolytic splitting of ONO_2CO_2 or production of the nitrating nitronium cation NO_2^+ (see above) after heterolytic splitting.

Protection against ONOOH toxicity at inflammatory sites has been described for ascorbic acid (ASC), glutathione (GSH) and uric acid (UA): ASC and GSH protected both tyrosine nitration and α_1-antiprotease inactivation while UA only inhibited tyrosine nitration (Whiteman and Halliwell, 1996).

ONOOH cytotoxicity seems to involve **p**oly-(**A**DP)**r**ibosyl**t**ransferase (PART): this enzyme modifyes proteins, polymerases, nucleotides and cleaves NAD^+ into ADP-ribose and nicotinamide. ADP-ribose is covalently attached to various proteins and cells are rapidly depleted from NAD^+. As a consequence, glycolysis, mitochondrial electron transport and thus ATP-synthesis are slowed down followed by cell dysfunction and death. If, however, cultured J774 macrophages are subject to heat shock (60 mins. 43 °C, followed by recovery at 37 °C for several h) the heat shock protein HSP 70 is expressed and the cells are protected from ONOOH-induced suppression of mitochondrial respiration. Thus the authors (Szabo *et al.*, 1996) conclude that HSP 70 "may protect against peroxynitrite induced cytotoxicity".

Since the genes for the constitutive NOS (there are two NOS-isoenzymes: cNOS : isoenzyme in endothelial cells; iNOS: isoenzyme in activated immune cells and myocytes), have been localized (cNOS on chromosome 7 und iNOS on chromosome 17) and also cloned (Refs. in Bing (1995)), a genotechnical intervention into the pathological scenario of HOONO is no longer out of view.

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