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ROLE OF FREE RADICALS IN SEPTIC SHOCK

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The available data supporting the role of oxygen radicals (OR^0) as fundamental — though not unique — mediators in the pathogenesis of sepsis and septic shock are reviewed.

The main questions are 1) Are there any signs of OR^0 overgeneration during sepsis and septic shock? 2) Are there any indirect signs of OR^0 damage during sepsis and septic shock? 3) Does OR^0 inactivation affect the evolution and severity of sepsis and septic shock? 4) May the pathophysiology of sepsis and septick shock be viewed as a consequence of OR^0 overgeneration?

All these questions might receive affirmative answers; thus the relationships of OR^0 with other mediators of sepsis and septic shock are discussed.

It emerges that all the molecules which are being considered as mediators of sepsis and septic shock (cytokines, endothelins, platalet activating factor, complement) may act on granulocytes and other phagocytic cells to stimulate OR^0 production. There are also very close relationships with nitric oxide, another free radical.

The data in favour of an antioxidant therapy of sepsis and septic shock in humans are too few to be conclusive.

Key words: *oxygen free radicals, septic shock, multiple organ failure, antioxidants.*

INTRODUCTION

Oxygen radicals (OR^0) play a fundamental role in the complex pathogenesis of systemic inflammatory response syndrome (SIRS), sepsis, septic shock and multiple organ dysfunction syndrome (MODS). Their precise significance has not been completely clarified, probably due to the lack of methods for their direct detection and quantitation.

OR^0 (O_2^-, H_2O_2, OH^0, O_2^*) are the highly reactive intermediates of the mono- or divalent reduction of molecular oxygen. Owing to the presence of an uncoupled electron in the outer orbital they react with the very nearest molecules, thus initiating a lot of biochemical processes which lead to profound disturbances of cell biochemistry such as lipid peroxidation, protein

denaturation, polysaccharide depolymerization, genome oxidation, cellular and interstitial structure destabilization etc.

The generation of small amounts of OR^0 is a physiological process which is neutralized by the activity of scavenging substances (superoxide dismutases, peroxidases, vitamin E, vitamin C, methionine), so that no acute damage occurs; when the OR^0 generation massively increases, the balance with the defence systems fails and damage occurs.

The putative sources of OR^0 are: a) The mitochondrial respiratory chains during energy deprivation; b) The metabolic cascade of arachidonic acid overactivated by phospholipases; c) The enzyme xanthine-oxidase derived from xanthine-dehydrogenase in the presence of proteases; d) The granulocytes and other phagocytes activated by complement, bacteria, endotoxin, proteases, lysosomal enzymes, etc. All these events occur during SIRS, sepsis, septic shock and MODS; hence the involvement of OR^0 unbalance and overgeneration in their pathogenetic chains would seem highly probable.

Unfortunately, all the available data on the involvement of OR^0 in the aforementioned conditions are indirect and derived from assessment of byproducts of OR^0 reactions or from the consequences of OR^0 inactivation, without any direct measurements of OR^0 themselves, thus giving rise to some scepticism.

The aim of this paper is to present the main available data supporting the role of OR^0 in the pathogenesis of sepsis and septic shock as fundamental, though not unique, mediators.

Some questions need to be answered and discussed in order to arrive at conclusions and putative therapeutic applications.

ARE THERE ANY SIGNS OF OR^0 OVERGENERATION DURING SEPSIS AND SEPTIC SHOCK?

The direct signs of OR^0 overgeneration following experimental stimuli capable of producing sepsis and septic shock are few, but the data concerning damage produced by OR^0 are quite numerous.

Direct signs of OR^0 overgeneration

Neutrophils taken from endotoxemic rats produced significantly more O_2^- than controls, both spontaneously and after exogenous stimulation [1]. Jackson and coworkers [2], using murine macrophages incubated with endotoxin, recorded an electron spin responance signal of oxygen-centered radicals which disappeared after immission of superoxide dismutase into the system. According to Brackett and coworkers [3], the process of OR^0 generation after endotoxin administration is not immediate but takes some ten minutes, thus suggesting the existence of an intermediate mechanism. Increased H_2O_2 concentration has been measured in the expired breath of patients with

acute respiratory failure (4). Finally, Vespasiano and coworkers (5) measured spontaneous O_2^- production by neutrophils taken from septic patients. Its low value was due to the exhaustion of OR^0 — generating mechanisns as a consequence of their overstimulation; the O_2^- production in fact recovered in direct proportion to the improvement in severity scores of the illness.

Recently, Galley and coworkers (6) measured xanthine oxidase activity in blood. It was absent in healthy subjects, but had significantly increased in septic ones, more in non-survivors than in survivors. Since xanthine-oxidase is an O_2^- producer; its presence might be interpreted as indicative of enhanced superoxide production.

Indirect signs of OR^0 overgeneration

The list of the biochemical markers of OR^0 overactivity during sepsis, septic shock etc., is long. Malondialdehyde and alkenales, alkanes in exhaled air, chemiluminescende, consumption of physiological scavengers etc. have been cited in laboratory animals and in humans (see 7).

Ethane in exhaled air, an atraumatic marker of lipid peroxidative reactions, increased after endotoxin injection in rats (8). Antioxidant deficiency has been observed in the bronchoalveolar fluid of patients with sepsis and acute lung injury (9—11). High blood levels of malondialdeyde were measured in shocked patients (12), where other investigators have measured conjugate dienes, vitamin E consumption (13, 14), vitamin C consumption (15), lipid peroxides (16). Increased cell membrane stiffness, a typical effect of OR^0 damage (17), has been reported during septic shock in rats or humans (8, 18).

An increase in malondialdehyde, hydroxyalkenales and other products of lipid peroxidation has been reported in erythrocytes and plasma from patients surviving a period of shock (18, 19).

Physiological anti-oxidant capacity was reduced in patients with acute respiratory insufficiency. Blood levels of catalase, superoxide dismutase (20) and ceruloplasmin (21) increased, while glutathione and vitamin E decreased (22). Moreover, the plasma concentration of hydroxynonenal (a very specific peroxidation product) was increased (23).

In conclusion, many indirect signs of OR^0 overgeneration and lipid peroxidation have been reported during sepsis and septic shock in both laboratory animals and humans.

DOES OR^0 INACTIVATION AFFECT THE EVOLUTION AND SEVERITY OF SEPSIS AND SEPTIC SHOCK?

This is a widely-explored field and experiments have been presented using scavengers, antioxidants, trappers etc. to modify the evolution of sepsis, septic shock and related conditions, which are mostly experimental but also clinical.

In our laboratory this approach has been adapted since 1984 on a laboratory model of lethal endotoxemia or trauma (24, 25).

Sping-trapping nitrones (PBN, POBN, DMPO) prevented and reverted traumatic and endotoxin shock in rats (*Tab. 1*) and prevented the microcirculatory troubles provoked by endotoxin (*Tab. 2*), the OR^0-induced stiffness of cell membranes and the exhalation of ethane, a by-product of lipid peroxidation (4).

Table. 1. Effect of spin-trapping nitrones (active or inactivated) on survival of rats after endotoxin or trauma. Survival of rats (survived/examined) after injection of *Escherichia coli* endotoxin (30 mg/kg i.p.) or trauma (rotating drum). Animals were pretreated i.p. with one of three spin-trapping nitrones (PBN, POBN and DMPO) or with the same nitrones which had been inactivated by exposure to light and air. Control experiments were performed using saline alone (from Novelli, 1992).

	PBN 150 mg/kg		POBN 100 mg/kg		DMPO 100 mg/kg		Saline
	Active	*Inactive*	*Active*	*Inactive*	*Active*	*Inactive*	
Endotoxin	9/10	0/10	8/10	0/10	8/10	0/10	1/10
Trauma	12/12	0/9	10/10	0/11	12/12	0/11	0/9

Table 2. Effect of spin-trapping nitrones on the microcirculatory derangements provoked by endotoxin. Non-quantitative effects of a lethal dose of *Escherichia coli* endotoxin on some variables of the microcirculation of the mesocecum of rats and on arterial pressure and pH. The modifications caused by each of three nitrones (PBN, DMPO, POBN) tested by i.p. injection or topical application to the mesocecum are summarized (from Novelli, 1992).

	Microcirculation Vasomotion			Capillary flow					
	30	60	120	30	60	120	BP	pH	Outcome
Endotoxin	↑	↓	↓↓	↓	↓	↓↓↓	↓↓	↓↓	Diet
Endotoxin + i.p. nitrones	=	=	=	=	=	=	=	=	Survived
Endotoxin + topical nitrones	=	=	=	=	=	=	↓↓	↓↓	Died
i.p. nitrones	=	=	=	=	=	=	=	=	Survived
topical nitrones	=	=	=	=	=	=	=	0	Survived

These same nitrones have been reported to affect favourably mortality after large doses of endotoxin in rats (26, 27) and to protect the myocardium against reprefusion-induced arrhythmias in rats (28—30).

Other reports from our laboratory have demonstrated a protective action of large doses of reduced glutathione (500 mg/kg) against endotoxin or traumatic shock in rats (31). Reduced glutathione is a potent physiological intracellular antioxidant that was effective in improving systemic and pulmonary hemodynamics during acute respiratory insufficiency in humans (personal unpublished data).

The multiple organ dysfunction syndrome (MODS) can also be viewed as a consequence of continuous, prolonged OR^0 generation. MODS can be provoked in rats by sterile intraperitoneal zymosan in parafin, which causes prolonged complement activation (32); the mortality of this model of MODS was lessened in a dose-dependent manner by large doses of reduced glutathione or by spin-trapping nitrones. Antioxidants also significantly prevented pulmonary interstitial edema (as assessed by computerized tomography) and ethne exhalation (33, 34).

In a similar model of intraperitoneal zymosan injection in rats, the degree of lung and liver peroxidation as assessed with malondialdehyde was correlated to the severity of the illness and with mortality (35). Many years ago, Bitterman and coworkers (36) reported that intravenous superoxide dismutase improved the damaging effects of OR^0 in a model of superior mesenteric artery occlusion shock in rats. The iron-chelator deferoxamine, an inhibitor of OR^0 reactions through iron chelation, exerted an impressive protective effect in early sepsis following cecal ligation and puncture in rats (11). Dimethyl sulfoxide, a hydroxyl scavenger that readily penetrates cell membranes, induced significant beneficial modifications of the course of endotoxemia in rats (37).

N-acetyl-cysteine improved acute lung injury (38) and septic shock (39) in some patients, but available data are few and debatable (see below).

In conclusion, there is evidence that drugs which inhibit OR^0 reactions exert a beneficial action on the evolution of experimental sepsis and septic shock.

MAY THE PATHOPHYSIOLOGY OF SEPSIS AND SEPTIC SHOCK BE VIEWED AS A CONSEQUENCE OF OR^0 OVERGENERATION?

The basic pathophysiological disorder of sepsis, septic shock and MODS is a diffuse impairment of oxygen consumption and tissue perfusion, mostly focused on microcirculatory and capillary dysfunction. The question is whether such disorders can be viewed as consequences of OR^0 overactivity.

Capillary function and permeability have been studied in our laboratory on the mesocecum of rats with fluorescein-labeled albumin injected i.v. and polarized microscopy. In the absence of any stimulus the capillaries and their walls were outlined by the fluorescence but after a lethal dose of i.v. endotoxin fluorescent albumin progressively leaked out of capillaries and invaded the

interstitium. This increase in capillary permeability was prevented by nitrones or glutathione (*Tab. 3*), thus confirming that it was due to overgeneration OR⁰ (40) whose main source is located inside the neutrophils which are activated and which adhere to the post-capillary veins (41).

Table 3. Measure of capillary permeability as assessed by counting the leakage points of fluorescent albumin on the mesocecum of rats. Experiments were performed on a) endotoxin alone, b) endotoxin preceded by reduced glutathione 250 or 500 mg/kg i.p., c) endotoxin preceded by the spin-trapping nitrone PBN (from Novelli, 1993).

	Leakage points at various intervals				
	0.00 min.	10 min.	20 min.	30 min.	40 min.
Endotoxin	0	3 ± 1	7 ± 3	15 ± 6	∞
Endotoxin + GSM 50 mg/kg	0	0	0	1 ± 2	1 ± 2
Endotoxin + GSM 250 mg/kg	0	0	1 ± 3	3 ± 4	5 ± 3
Endotoxin + PBN	0	0	1 ± 2	1 ± 1	2 ± 1

This finding might be considered analogous to the early increase in pulmonary interstitial water observed by Suffredini and coworkers (42) after intravenous injection of small doses of endotoxin in healthy volunteers. Recently, protein extravasation from the capillaries was demonstrated to be the first step in experimental multiple organ failure provoked by intraperitoneal sterile zymosan (43). An increase in xanthine oxidase activity associated with increased free-radical concentrations and evidence of free radical damage and increased lactate concentrations have all been reported in patients with sepsis and organ dysfunction as compared with both healthy subjects and non-infected critically-ill patients (6).

In conclusion, the overall picture of sepsis ans septic shock can be viewed as a consequence of OR⁰ generation not only in laboratory animals but also in humans.

WHAT IS THE RELATIONSHIP BETWEEN OR⁰ OVERGENERATION AND OTHER MEDIATORS OF SEPSIS AND SEPTIC SHOCK?

The above findings demonstrate unbalanced OR⁰ overgeneration during sepsis and septic shock that might provoke the typical pathophysiology, and is parallel to the production of the commonly-accepted mediators.

1. Polymorphonuclear granulocytes have a pathogenetic rôle in the disturbances of sepsis and septic shock as a consequence both of mechanical obstacles to microcirculation and of OR⁰ production ("respiratory burst"). In fact, leukocyte depletion prevented pulmonary and cellular damage after endotoxin injection (44, 14).

Chaotic OR^0 generation from the granulocytes is the consequence of their activation by complement fractions which are in turn activated by the well-known mediators of sepsis and shock: proteases, lysosomal enzymes, coagulation by-products, bacteria, endotoxins, etc. The source of these mediators is the site of infection or inflammation. The intestine also transfers endotoxins and bacteria into the systemic circulation but at the same time it has been claimed to produce OR^0 (45).

2. Endotoxins directly stimulate granulocytes and monocytes to produce OR^0 (2, 46, 47).

3. The tumor necrosis factor (TNF), interleukins (IL-1, IL-6) endothelins and platelet-activating factor (PAF) and nitric oxide (NO) have been claimed as pathogenetic mediators of sepsis and septic shock. None of them however has a unique role in provoking these conditions and discussion is underway concerning their hierarchical relationship. In any case, endothelins stimulate granulocytes to produce OR^0 (50) as does IL-1 (51). PAF also activates OR^0 production by granulocytes through the complement. TNF is a stimulator of OR^0 production by granulocytes and monocytes (52, 53), but it also increases vascular endothelium susceptibility to peroxidative damage (54).

OR^0 overgeneration is thus the final step in the actions of all the mediators of sepsis and one of the mechanisms of their destabilizing action on the whole organism.

4. NO, that is a free radical itself and is linked to OR^0, is an important mediator of sepsis and septic shock (Szabo) (55). The inhibition of its production by i-NOS improved endotoxin and septic shock in both laboratory animals (46, 57) and humans (48, 58). Moreover, the spin-trapping nitrone phenyl-butyl-nitrone (PBN), which we have discovered prevented death due to endotoxin (25), is also a selective inhibitor of i-NOS induction in mice (59, 60).

In any case, NO reacts with superoxide to form peroxynitrite, another free radical whose cytotoxicity is higher than that of NO itself (Brunelli, 1995) (61) and which like all the OR^0 impairs contractile protein activity through disturbances of the calcium transport systems (62). Moreover, the cytokines released during sepsis (TNF, IL-1) are potent inducers of i-NOS and NO production.

It thus seems very difficult to distinguish between the roles of OR^0 and NO in the pathogenesis and pathophysiology of sepsis and septic shock, also because of the lack of drugs which specifically and selectively act on one or the other system.

FUTURE THERAPEUTIC POSSIBILITIES

The concept of protection by some antioxidant drug against the chaotic, damaging action of OR^0 seems simple and attractive. So far, however, no drug with an anti-oxidant activity has been proved to be effective in controlling acute OR^0-derived diseases like sepsis and septic shock (see 63—66).

Many drugs that inhibit OR⁰ sources or their consequences have been tested in animal models, and they may have favourably affected the evolution of sepsis and septic shock; no one has however been submitted to a controlled clinical trial.

In our patients, large doses of reduced glutathione improved acute lung injury due to sepsis (personal unpublished data), but these observations did not go beyond a preliminary, absolutely non-controlled study.

N-acetylcysteine has a well-established antioxidant profile (67) and was given to patients with acute lung injury by Bernard in 1991 (38) and to septic shock patients by Spies and coworkers (39). The latter was a prospective, randomized, doubleblind study on 58 patients these were given very large doses of N-acetylcysteine as a bolus, followed by infusion. The drug led to increased oxygen consumption in all the treated patients but gastric intramucosal pH, hemodynamics and survival improved in only half of them. The only difference between responders and non-responders to N-acetylcysteine was the interval between the onset of sepsis and the administration of the drug (non-responders: 61 hours; responders: 37 hours), thus suggesting that after a certain time-lag the damage becomes irreversible and no longer able to be influenced by simple OR⁰ inactivation.

CONCLUSION

There is evidence that the pathogenetic chain leading to sepsis and septic shock involves OR⁰ as final mediators of biochemical cell and tissue damage. Their pharmacological inactivation has been poorly evaluated in humans, but in any case it might be effective only in the earliest stages of sepsis, before the setting in of structural damage leading to irreversible oxygen consumption deficiency.

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