NITRIC OXIDE IN SEPSIS FROM DEFICIENCY TO OVERPRODUCTION

C. PĂUN, A. CIOCĂNEA

The Emergency Hospital of Piteşti

Abstract: *The presence of NO in blood is important to regulate the vasomotor tonus and the blood flux, and to inhibit the adhesion of leukocytes and of plakets and to modulate the coagulation activity. The proofs suggest that sepsis is associated with: rapid lowering of the ecNOS function with altered relaxation derived from endothelium; increase of the iNOS function which is delayed with a few hours, associated with a modified response of the smooth muscle to vasoconstrictors and vasoplegia.*

Keywords: *nitric oxide, severe sepsis, overproduction deficiency.*

Rezumat: *Prezenţa în sânge a NO este importantă pentru reglarea tonusului vasomotor, a fluxului sanguin pentru inhibarea adeziunii leucocitare şi plachetare şi pentru modularea activităţii coagulării. Dovezile sugerează că sepsisul este asociat cu: scăderea rapidă a funcţiei ecNOS cu relaxare derivată din endoteliu alterată, o creştere întârziată cu câteva ore a expresiei NOS asociată cu un răspuns modificat al muşchiului neted la vasoconstrictori şi vasoplegie.*

Cuvinte cheie*: oxid, nitric, sepsis sever, deficienţă de nefroproducţie*

The Nitric Oxide (NO) is physiologically produced by ecNOS (endothelial NO constitutive syntax). Its presence in the blood flux is important not only for it regulates the vasomotor tonus and the blood flux, but it also inhibits the adhesion of leukocytes and of plakets, as well as it modulates the coagulation activity.

Sepsis is associated with a rapid lowering of the ecNOS function with altered relaxation derived from endothelium, with a delayed (of few hours) increase of the inducible NOS function (iNOS), associated with an alteration of the response of the smooth muscles to vasoconstrictors and vasoplegia.

The use of the NOS inhibitors restores the response to vasoconstrictors and vasoplegia but it cannot restore the relaxation derived from endothelium: the normalization of the blood pressure is associated with an increased risk of the flux lowering, with the increase of the adhesion of leukocytes and of plakets and the increase of the coagulation activation and organs injury.

The clinical research reported the benefic effects of the iNOS inhibition on the homodynamic status but also the undesirable effects, such as: the increase of the pulmonary artery pressure and the augmentation of the organs injuries, as well as the increase of mortality. The negative effects of the NOS inhibitor regarding the septic shock may be related to the absence of the improvement of the ecNOS function, as well as other NO proprieties suppressed during sepsis, such as: the bacterial activity, the modulation of the activated coagulation and the inhibition of the adhesion of leukocytes and of plakets.

Endothelium, which could be found in the circulatory system, is a very specialized tissue, involved in the modulation of the immune responses and the increase of the vascular cells and in the regulation of the haemostatic, inflammatory and vasoactive agents' level (1). The endothelium releases the tissue hormones (autacoids) which decisively affect the vascular tonus and the placket function. Many of the relaxing autacoids derived from endothelium were described and characterized – for example: prostacyclin, and the strong vasodilatator compound – the nitric oxide (NO). NO is synthesized in picomolar concentrations (Figure no.1) of the arginina-L amino acid of NO synthesis (NOS), which is constitutively present in the endothelial cell, named the NOS endothelial constitutive enzyme (ecNOS).

NO is diffused in the smooth muscle and induces relaxation through the simulation of the soluble guanylate cyclase (GC) and an increased concentration of monophosphatic cyclic guanosine (2).

NO: no more than a relaxing agent derived from endothelium.

The relaxation derived from endothelium and /or the production of NO derived from endothelium may be used as an indicator of the endothelial cell function. For example, the response of relaxation of the isolated vascular rings in vitro, regarding the picomolar concentration of acetylcholine is dependent of the presence and integrity of the endothelial cells (3).

A calcium influx to the endothelial cells results from the Ach receptors occupation. The calcium influx may be directly achieved by the calcium ionophor A23187. The calcium-calmodulin complex produces the ecNOS activation. In physiological conditions, besides the NO basic release of NO from the endothelial cells, the

increased production of NO is the result of stress and / or of the receptors activation.

Another enzyme may synthesize NO: its presence is induced upon 4-8 hours after the cell exposure to inflammation stimuli. The activity of this enzyme, the so-called inducible NOS (iNOS) is calcium-dependent, so it is not stimulated by stress or antagonist agents. Its presence was identified in different tissues and especially in the vascular tree. NO is produced in nanomolar concentrations of iNOS (4).

The high-output path of NO during inflammation was considered as a very important aspect of the pathophysiology of sepsis, especially of the circulatory abnormalities. Due to the fact that iNOS requires a certain period of time in order to be present in the inflammable tissue, its activity frequently accompanies the "late" sepsis.

This late stage of NO excess is however preceded by a stage of the "early" sepsis, where a state of nitrosopenia could be identified. This deficiency of NO cannot fight against vasoconstriction, having a high risk of tissue hypoperfusion and organs injuries.

The inhibition of the NO synthesis may occur similarly during ischemia and reperfusion, as it also occurs in the initial stages of sepsis. For example, it was proved that the intestinal endogen NO was reduced by 90% after ischemia and reperfusion (6). This was associated with an increased permeability in the small intestine of felines, which took place mainly during reperfusion (6). The involved mechanism seems to be an increased production of oxygen free radicals during reperfusion.

Permeability was increased when the inhibitor of NOS. N (G) – nitro – L- arginina methyl ester (L-NAME) was infused and diminished when the precursor L-arginine was infused, suggesting a decisive role for the NO remained in the oxygen free radicals and preventing the gut injuries. The proofs showed that the intestinal release of NO was prevented one hour after the exdotoxins (LPS) infusion at mice (this effect was dose-dependent).

At the same time, the production of the oxygen free radical, the peroxide of hydrogen (H_2O_2) was also increased (dose-depended, too). When L-NAME was administered to the control group, the NO synthesis decreased, but it did not change the production of H_2O_2.

When L-NAME was administered to the LPS – treated animals, the NO production was hugely reduced; the H_2O_2 production was also hugely reduced, suggesting again the protection given by the remained NO against the production of H_2O_2 during severe endotoxemy and against the negative effect of NOS inhibition, regarding this situation. .

Early sepsis and NO deficiency

Endothelium-dependent abnormal vascular relaxation was recognized in sepsis multiple conditions. Many researchers (8-10) proved a relaxation induced by the reduced ACh in the vascular isolated rings from the large arteries drew from the septic animals. Besides the anatomic injuries, such abnormalities identified to these blood vessels may be the result of the following mechanisms:

- Alteration of the surface receptors of the endothelial cells,
- Changes regarding the ways of transforming the signal (receptor to ecNOS),
- Altered function and /or altered density of ecNOS,
- Changes of the ways bringing about the NO release,
- Mechanisms participating in the subsequent degradation.

Zhou and his collaborators (11) proved a decrease of ecNOS density regarding a model of mice with peritonitis in endothelial cells which can be compatible with the observations in vitro, proving a negative adjustment of ecNOS mRNA and/or degradation of the pro-inflammatory cytokines, such as TNF-α, as well as of bacterial endotoxins (12,13). For example, regarding the use of the biologic activities of blocking the TNF – α, Wang and his collaborators (14) prevented the dysfunction of the vascular endothelial cells and the anatomic injuries which occurred on a septic shock model.

The authors recently observed (10) that the endothelium-dependent vascular relaxation was significantly reduced after the LPS injection in small dose, at the rabbit. One single and unlethal LPS injection (0,5 mg/kg) was associated with the loss of the relaxation induced by ACh, which was maintained for a period of minimum 5 days. The recovery was marked usually on the 21^{th} day. Interesting was the fact that this altered endothelium-dependent relaxation was associated with an increased expression of the tissue factor, the major contributor to the coagulation activation.

Regarding this model, the authors also noticed that the relaxation induced by the A23187 ionophor was not altered, suggesting that the prevention of the relaxation derived from endothelium during sepsis is more related to the changes of the transmitting ways than to the ecNOS dysfunction. These results confirmed those of Parker (8), who made similar observations on a canine model of sepsis.

More important, the research made on isolated perfused, rabbit hearts (15) and on mouse's mezenterium (17,18) suggested that similar mechanisms are operating regarding microvascularity.

On a model of mouse extensor muscle, *longus digitorum* by intravital microscopy, it was proved that (19) sepsis is associated with a decrease of the tissue perfused capillary density, with an increased heterogeneity of the perfusion and the increased inter-capillary medium distance. So, sepsis seems to affect the ability of microcirculatory of distributing the red blood cells. The decreased ability of extracting oxygen was long reported to patients and on models of animals, in the sepsis case (20).

Regarding the healthy volunteers, even a short exposure to endotoxins or cytokines prevents the endothelium-dependent relaxation for many days (21, 22). This effect was named endothelial blockade.

After the recovery of the acute insult, the endothelium may remain dysfunctional for a larger period of time, before the entire recovery. It was similarly proved (23) that the reactive hyperemia is attenuated to the patients seriously ill with septic shock in spite of the normal delivery of oxygen. The proposed mechanisms for explaining hyperemia to the septic patients may include the reduced vascular reactivity and/or microvascular obstruction which limits the number of the available capillaries. It is important to mention the fact that hyperemia may also explain vasoplegia and the reduced vascular contractility.

Late sepsis and the overproduction of NO: therapeutic consequences.

It seems difficult to reconcile the endothelium deficiency of NO with the well-known vasoplegia observed in the case of sepsis. The large number of clinical and experimental research emphasized results of the hindered contractile response to the vasoconstrictors agonists during the septic shock. In the 1980's, the idea that the NOS inhibitors could not restore the normal contractility towards those agonists came up (24, 25).

This effect was maintained in the ex vivo vessels whose endothelium was extracted from, suggesting that inflammation and sepsis bring about the expression of an inducible NOS in the cells of the smooth muscle, preventing the contractile responses (Fig. 3). The authors decided that these observed modifications may correspond to the pathophysiological characteristics of the clinical and experimental sepsis and so, they may be responsible for the profound vasoplegia and for the limited response to the normal endogen stimuli in organs, which regulate the blood flux distribution into the organs. The observance of the high circulating levels of nitrates/nitrites (NO stable decomposition products) to the septic patients, combined with the decrease of the vascular tonus suggested that NO was clearly involved in the pathophysiology of the shock (26,27).

Other negative effects of the excessive NO may react with the superoxide anion in order to form a peroxynitrite, which is an oxidant of molecules, such as: nucleic acids, lipids and proteins. It may also prevent the Poly (ADP-ribose) mitochondrial and active respiration having as a result the decrease of glycolysis, of the electrons transportation and of the ATP generation.

These observations lead to the use of an unspecified inhibitor of NOS, such as L-NAME and/or L-N^G-Monomethyl-L-arginine (L-NMMA) to the septic animals (28) and to the patients seriously ill (29). L-NMMA caused an increase of the blood pressure and of the vascular resistance and a decrease of the cardiac debit, allowing a decrease of the vasoconstrictor dosage (28, 29). The results of the studies made on animals were generally diverging (27, 28). The explanations offered included the differences between the animals and the used model, the type and the used dose of NOS inhibitor and especially the periods of time when the NOS inhibitors were administered. It is important to underline that the impossibility of improving the survival rate was observed to an animal model of chronic endotoxemy (29).

The reduced clinical research reported the benefic effects of the NOS inhibition or of the GC inhibition on the homodynamic status, although this treatment was associated with the increase of the pulmonary artery increase and the lack of mortality decrease (27, 28). In spite of the potential side effects and of the impossibility of improving the survival rate (28), the decision of performing large controlled research at random was taken, regarding the patients with septic shock.

A research of Stage II (312 patients) proved a large percentage of the patients whose shock was solved 72 hours after, regarding the L-NMMA group (30). But, the next stage of Stage III was interrupted after 797 patients (126 centres), because of the increased mortality rate within the treatment group in the third day (155 vs 27% regarding the treatment group) on the 14^{th} day (36% vs 52% within the treatment group) and on the 28^{th} day (49% vs 59% regarding the treatment group (31).

The use of the iNOS specific inhibitors was sustained in order to achieve better clinical results for patients. Although certain positive results were obtained as a result of the experimental research with aminoguanidine or S-Methylisothourea, none was tested more profoundly. If the inhibition activity of ecNOS is modified during sepsis, the use of the selective inhibitors of ecNOS has a limited interest as the NO blockade will let unbalanced the endothelial function (Figura 3) and the weakly perfused and oxygenated tissues.

There are proofs suggesting that iNOS may, to a certain extent, compensate the loss of the ecNOS function during sepsis (26, 32). Indeed, under a treatment favouring the ecNOS protection during the endotoxins injection, the authors proved that the iNOS expression was absent to the mice model (33,34).

More, there are certain concerns that the NO blockade may augment other potential problems, such as:

- The decrease of the bacterial activity related to NO,
- Necrophagous proprieties related to NO,
- The decrease of the activated coagulation modulation,
- The increase of the oxygen increase by the release of the mitochondrial respiration in areas with low perfusion and reduced oxygen delivery.

All these considerations may explain why it has been recently observed that the over-expression of the chronic ecNOS in the mice's endothelium had as results the resistance to the hypotension induced by LPS, pulmonary injuries and death (35). So, it seems better that ecNOS be favoured than inhibited during sepsis.