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Vulnerability of vascular endothelium in lipopolysaccharide toxicity: effect of (acyl) carnitine on endothelial stability

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THE literature presented illustrates that lipopolysaccharide (LPS), from bacterial cell walls, induces tumour necrosis factor (TNF) synthesis in macrophages. TNF affects a number of cell types, amongst which are endothelial cells, within a few hours. Its injection has been shown to produce all symptoms of the toxic syndrome. In the present communication the vulnerability of endothelial cells will be stressed. These cells require carnitine not only for fatty acid oxidation but also for membrane protection and repair. As endothelial cells lose carnitine during hypoperfusion, it is speculated that the supply of carnitine during the early phase of LPS toxicity in rats might delay or avoid loss of endothelial functions. Earlier it was observed that hearts from rats, injected 3 h previously with LPS, showed strongly increased interstitial fluid production compared to hearts from control rats, even when TNF was present during a 3 h *in vitro* perfusion. It showed that LPS *in vivo* generates factors other than TNF, such as platelet activating factor (PAF), that are responsible for the increased capillary permeability.

Key words: Carnitine, Endothelium, LPS, PAF, TNF

Introduction

The study of the pathophysiology of septic shock has gained momentum since the discovery that lipopolysaccharide (LPS) from the cell walls of Gram-negative bacteria, is mainly responsible for the toxic syndrome. LPS induces the synthesis of tumour necrosis factor (TNF) in macrophages, which has been isolated from these cells.[1] The structure of TNFα has been determined by cDNA cloning[2] and appeared identical to that of cachectin.[1] TNF causes cachexia and a state of shock.[3] *In vitro*, it is cytostatic or cytolytic for various tumour cell lines. It is not cytotoxic to normal cells.[3] TNF suppresses lipoprotein lipase,[4] an enzyme on macrophages and vascular endothelium of red muscle cells and fat cells for the hydrolysis of glycerides of plasma lipoproteins. TNF also affects other activities of endothelial cells, such as stimulation of procoagulant activity,[5] induction of phospholipase A_2[6] and inhibition of proteoglycan synthesis.[7] It enhances adhesion with neutrophils[8] and is also an angiogenic peptide.[9]

During studies with the Langendorff heart, it was observed that LPS acutely stimulates Ca^{2+} entry in cardiomyocytes, judged by the increase of contractility and endogenous lipolysis.[10] Later it was found,[11,12] that when LPS was bound to albumin during perfusion of Langendorff hearts, it was not able to stimulate contractility or lipolysis, so that *in vivo*, direct effects of LPS cannot be important for the toxic syndrome, but only after its induction of TNF and platelet activating factor (PAF) production.[13] That TNF plays a central role in the pathogenesis of septic shock has been shown by a number of groups.[14–17]

Involvement of Endothelium

From the brief introduction, it is clear that endothelial cells play an early and decisive role in the pathophysiology of shock. These cells are very vulnerable as they are directly exposed to the circulating blood, and moreover readily suffer from (imminent) ischaemia as will be discussed below.

The introduction mentioned the findings of others on adhesion of cells from the blood to endothelial cells as leucocytes and thrombocytes. This is accompanied by a rise of intra-endothelial Ca^{2+}, which stimulates phospholipase A_2. It results in arachidonic acid (and lysophospholipid) release from membrane phospholipids, which allows the synthesis of various autacoids. One of the endothelial prostaglandins is prostacyclin.[18] This PGI_2 only affects local phenomena, such as inhibition of platelet aggregation. When endothelial cells are not activated they do not cause platelet aggregation as they have a non-thrombogenic surface (a negatively charged glycocalyx containing

glycoproteins and proteoglycans, enriched with heparan- and chondroitin sulphates). Upon surface damage platelets adhere and aggregate. The anti-aggregatory effects of PGI_2 is due to increase of cAMP in platelets which lowers their intracellular Ca^{2+} level, which makes the platelet insensitive to activation for aggregation. After exposure to TNF (and/or PAF) activation of cyclooxygenase rapidly takes place.[19] Whereas PGI_2 is the most potent anti-aggregative agent, platelets make an aggregation promoting thromboxan, TXA_2, from arachidonic acid. The ratio TXA_2/PGI_2 therefore is an important determinant for aggregation. Endothelium derived relaxing factor (EDRF, i.e. the free radical NO), increases cGMP in platelets, which like cAMP decreases its Ca^{2+} level and inhibits aggregation. Other endothelial compounds of great interest are endothelins-1, -2 and -3. They are vasoconstrictors, consisting of 21 amino acids and derived from 'big-endothelin'.[20] As there is no stock of the endothelins in the cells, it is the converting enzyme that determines their activity. ET-1 is the strongest vasoconstrictor and ET-3 the weakest, but the strongest aggregator of thrombocytes. Increased levels of endothelins have been found in septic shock, myocardial infarction and diabetes. Activation of endothelial cells (e.g. by thrombin) induces the synthesis of platelet activating factor (PAF or 1-alkyl-2-acetyl-glycero-3-phosphorylcholine).[21] *In vitro*, it could activate platelet aggregation. Whether this also holds for *in vivo* situations is not certain as it remains within the cells. PAF, like lysolecithin,[22] affects the cell surface.

Vulnerability of Vascular Endothelium and Loss of Carnitine

Earlier, it was observed that the vascular surface continuously produces lysophospholipids during cell free Langendorff perfusion of control rat hearts.[23] This process is stimulated by stress hormones like glucagon and noradrenaline Therefore, continuous repair of the phospholipid bilayer by (activated) fatty acids is required. Hence the well-known stabilizing effect of palmitoylcarnitine might partly be due to this process and partly to the ability of this amphiphilic compound to affect membrane fluidity. This stabilization of the cell surface is even more urgent when tissues are subject to hypoperfusion when the equilibrium between synthesis and breakdown is disturbed. Endothelial cells, in particular, are sensitive to hypoperfusion as they suffer from their high content of xanthine dehydrogenase, which upon conversion to xanthine oxidase may generate (damaging) oxygen free radicals.[24,25]

Effect of Carnitine Supply on Endothelial Functions

Endothelial cells catalyse carnitine dependent fatty acid oxidation,[26] so that they are able to generate long-chain acyl CoA and acyl carnitine. That endothelium is most vulnerable to hypoperfusion, as mentioned above, may result in preferential loss of low molecular components, like carnitine, from these cells. Indeed, during reperfusion after ischaemia of Langendorff rat hearts, a loss of carnitine from a non-myocyte compartment was observed, probably vascular endothelial cells.[27] It may explain the restoration of flow regulation by carnitine addition in imminent ischaemia, as recently reviewed.[27,28] Oxygen free radicals might be involved in the loss of carnitine from endothelium, as observed after reperfusion following ischaemia.[27] Therefore, carnitine supply may prevent the loss of flow regulation by endothelium in imminent ischaemia. Such a situation may be the early stage of endotoxin shock when capillary flow in many organs becomes endangered as can be demonstrated *ex vivo*, after injection of rats with LPS.[11] After 3 h the animals showed signs of dysthermia, lethargy and diarrhoea. Yet their hearts, tested *in vitro* during Langendorff perfusion, did not show contractile failure or lower lipoprotein lipase activity, indicating intactness of the cardiomyocytes. However, the microcirculation was affected, judged by an increase of cardiac interstitial fluid production from $1.08 \pm 0.19\%$ (control, $n = 6$) to $12.6 \pm 2.7\%$ (LPS rats, $n = 6$) of the total effluent, as described previously.[11] The *in vitro* perfusion of hearts from control animals for 3 h with TNF affected neither lipoprotein lipase activity[11] nor interstitial fluid production.[11] The latter is in agreement with the conclusions reached by Langeler *et al.*[29] based upon studies with an *in vitro* model of endothelial monolayers. Therefore, it is not likely that TNF directly affects increased permeability, but another product of LPS toxicity, such as PAF. Preliminary experiments indicated that 10 nM PAF increased interstitial fluid secretion during 3 h Langendorff perfusion of hearts from control rats to $17.3 \pm 2.9\%$ ($n = 3$) of the total effluent. Effects of carnitine and derivatives upon this phenomenon may be expected since Van Hinsbergh *et al.*[30] observed that propionyl carnitine decreases intracellular Ca^{2+} levels in thrombin challenged endothelial cells in culture. PAF has also been shown to increase intracellular Ca^{2+} after specific binding to isolated endothelial cells,[31,32] so that carnitine and carnitine derivatives may be expected to antagonize PAF effects.

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