Role of Vascular Nitric Oxide in Experimental Liver Cirrhosis

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Abstract: One of the most important features of liver cirrhosis is the splanchnic and systemic arterial vasodilation, related to an increase in vascular capacity and an active vasodilation. This arterial vasodilation seems to be the consequence of the excessive generation of vasodilating substances, which also contributes to a lower than normal pressor response to circulating nervous or humoral substances. The following review analyzes the mechanisms responsible for the vascular hyporesponse to vasoconstrictors observed in the experimental models of liver cirrhosis. It has become increasingly clear that, among the great variety of substances studied, nitric oxide (NO) seems to be one of the main contributors to this vascular alteration, since elimination of the endothelium or inhibition of its synthesis corrects it. The mechanism by which NO interferes with the contractile apparatus in smooth muscle cells seems to be related to a direct effect on calcium entry from the extracellular space and release from the internal stores.

Key words: endothelium, smooth muscle cells, nitric oxide, liver cirrhosis, vasoconstrictors, vasodilation, portal hypertension.

INTRODUCTION

One of the most important features of liver cirrhosis, either human or experimental, is the splanchnic and systemic arterial vasodilation. Although its origin is not completely established, it is thought that, as well as factors such as the increase in vascular capacity, there is also an active vasodilation as a consequence of the excessive generation of vasodilating substances, many of them of a local origin. Among the great variety of substances involved, in this review we will analyze the role of nitric oxide (NO) as one of the main contributors to the arterial vasodilation of liver diseases.

NO IN THE VASCULAR WALL

NO is formed from the aminoacid L-arginine [1-3], due to the action of several isoforms of the enzyme NO synthase (NOS). The endothelial isoform or NOS3 is expressed constitutively in the endothelial cell where it produces NO in a continuous manner, albeit in small amounts, in response to physical factors (shear stress) or chemical mediators (vasoconstrictor hormones, for instance). On the contrary, isoform NOS2 or inducible is induced in the vascular wall and cells by endotoxins and cytokines, among others, and produces big amounts of NO although in a short period of time. This contributes to the vasodilation characteristic of the inflammatory process.

The vasodilation produced by NO requires the production of a second messenger, cyclic GMP (cGMP), as it can be demonstrated after the inhibition of guanylate cyclase with methylene blue. In turn, cGMP vasodilates by means of the modulation of several protein kinases such as the inhibition of myosin light chain kinase, which reduced the activity of myosin or through the control of the intracellular calcium levels [2]. Although cGMP seems to be the main mediator of the effects of NO, it is now known that NO can also affect vascular tone through the direct activation of potassium channels, both ATP-sensitive and calcium-dependent. Then, the opening of potassium channels would hyperpolarize the cell, a mechanism that would contribute to the vasodilation induced by cGMP.

ROLE OF NO IN THE VASCULAR ALTERATIONS OF EXPERIMENTAL LIVER CIRRHOSIS

The three most frequently used animal models of liver cirrhosis and portal hypertension are the carbon tetrachloride inhalation model (CCl4), the model of bile duct ligation (BDL) and the partial ligation of the portal vein model (PWL). All these three models share many features with the human disease, but the role of NO is different in each one. Unfortunately, there are not too many studies in human patients, thus the reader should be aware that all the experimental studies mentioned in this paper have to be treated with caution, before extrapolating data or conclusions directly to the human subject [4]. These three models have important differences between them, which we will not deal with here, but it is important to note that they show what is probably the central pathogenetic event in liver cirrhosis. These three experimental models show the typical hyperdynamic circulation characteristic of the human disease, that is, elevated cardiac output and low vascular resistances with normal (in the pre-ascitic phase) or clearly low arterial pressure (in the ascitic phase) [4-5].

Role of NO in the Hyperdynamic Circulation

Several experiments have demonstrated clearly that the acute administration of inhibitors of the synthesis of NO to animals with cirrhosis or portal hypertension increases blood pressure in a dose-dependent form [6-8]. Moreover, this
pressor effect is always greater in the cirrhotic animals than in their respective controls, which suggests that the cirrhotic animals have an increased production of NO. Furthermore, the chronic treatment of the cirrhotic animals with these NO synthesis inhibitors also elevates blood pressure and reduces cardiac output and hypervolemia, which indicates that NO is an important mediator of the hyperdynamic circulation [8-10]. Other studies have also shown that there is an increased release of NO from the endothelium of mesenteric arteries, and this has been shown to occur even before the splanchic hyperdynamic circulation is completely established [11], therefore well in advance of the development of the systemic hyperdynamic circulation. These data clearly suggest that NO plays a primary role in the development of this arterial vasodilation. Although the NOS isoform involved seems to be mainly the endothelial type, there is some evidence of the involvement of the inducible isoform, specially in the PVL model [12-13].

Role of NO in the Vascular Hyporesponsiveness to Vasoconstrictors

Another important cardiovascular alteration observed in the experimental models of liver cirrhosis or portal hypertension is a lower pressor response to the vasoconstrictor influences, either humoral or neuronal [13-14]. It is known that in liver diseases there is activation of the renin-angiotensin and sympathetic nervous systems, and of vasopressin, three of the most powerful endogenous vasoconstrictors. In most cirrhotic patients and experimental animals, in spite of this activation and that they show elevated levels of angiotensin II, vasopressin and catecholamines, arterial hypotension is very frequent and the administration of these vasoconstrictors to cirrhotic animals and patients produces a lower than normal pressor response. This vascular hyporesponsiveness has been observed both in awake and in anesthetized animals and in different tissues and vessels in vitro. As we shall see, most of the works performed indicate that NO is the main pathogenetic mechanism involved.

In anesthetized BDL rats, the pressor response to endothelin 1 was lower than in the controls and the inhibition of NO production improved this response partially [15]. In aortic rings obtained from BDL animals, the response to phenylephrine is reduced and the inhibition of NO synthesis with L-NAME and the elimination of the vascular endothelium improved the response and almost corrected the alteration [16-17]. This result suggests that a greater production of NO, of an endothelial origin, interferes with the contractile mechanism in the smooth muscle. Interestingly, when aminoguanidine, a preferential inhibitor of the inducible NOS isoform was administered, the defective response to phenylephrine of the cirrhotic rings was not improved [17]. Also in rats with BDL, the vasodilator response to sodium nitroprusside, an NO donor, was lower than that observed in the control rats [18], which would be indicative of an alteration in the muscle layer. However, the vasodilator response to acetylcholine that releases endothelial NO in aortic and mesenteric artery rings from rats with BDL was completely normal, which suggests that not all the mechanisms that release NO are altered in this model of biliary cirrhosis [17-18].

In the experimental model of prehepatic portal hypertension (PVL), the systemic administration of methoxamine also revealed an important pressor hyporesponsiveness that could be reversed with the use of an inhibitor of NO production [19]. Not only in this model, but in the isolated and perfused mesenteric vascular bed, the pressor responses to norepinephrine, vasopressin and potassium chloride were lower than in the control animals. Again, the inhibition of NO synthesis potentiated and normalized the altered pressor responses of the PVL animals [20]. These data have been partially confirmed by other laboratories [21-23], which also demonstrated that the main source of NO is the endothelial layer [24-25]. In this model, the participation of other factors different to NO [26], such as potassium channels, has also been reported [27]. Moreover, in these latter experiments [27], it was also demonstrated that the role of NO is carried out by two different mechanisms. One is the well-known mechanism of vasodilation induced by the formation of cGMP and the other is through a direct activation of potassium channels, which would vasodilate by inducing the hyperpolarization of the smooth muscle. It seems, however, that the role of potassium channels is small and that NO is the main mediator of the vascular hyporesponsiveness [27]. These results are also in agreement with studies that observed a greater activity of the constitutive NOS isoform in mesenteric arteries of animals with PVL [28-29], as well as with experiments demonstrating a greater release of NO from mesenteric arteries of animals with PVL, in response to increases in blood flow and shear stress, basic stimuli for the release of endothelial NO [30].

In sharp contrast to these homogeneous responses in the mesenteric vascular bed among different laboratories, the use of aortic rings from animals with PVL has yielded contradictory results. Thus, normal, lower or even increased responses have been reported with the application of vasoconstrictors [31-34]. Also, the enzymatic activity of NOS has been described as normal [29] or elevated [28]. It is likely that these differences could be due to the different methodologies employed or to normal variations in the aortic enzymatic activity depending on the evolution of the disease.

In the CCl4 model, there are few studies published but the above mentioned results are valid. Then, it has been described that angiotensin II and norepinephrine produce less pressor effect in these cirrhotic animals than in their controls [35-37].

Interference of NO with Calcium as the Mechanism Responsible for the Vascular Hyporesponsiveness in Cirrhosis

In summary, a greater NO production, mainly of endothelial origin, seems to be responsible for the lower pressor response to vasoconstrictors observed in the mesenteric vascular bed [21, 24-25, 38], kidney [39] and aortic rings from rats with liver cirrhosis or portal hypertension [40-42]. Recent studies from our laboratory have provided some insight into the mechanism of action by which the excess of NO affects the normal vascular response. Our data indicate that NO interferes with the mechanism that regulate the intracellular level of calcium [43]. In a group of experiments performed in the isolated and perfused mesenteric vascular bed of rats with BDL, we
analyzed the pressor response to the addition of calcium in vessels perfused with a zero-calcium Krebs. After perfusion with a high potassium Krebs to open voltage-dependent calcium channels, the addition of calcium induced a lower than normal pressor response in the vessels of the BDL animals than in their controls. In a similar way, the entry of calcium through the adrenergic receptor-operated channel was also lower in the BDL mesenteric beds. Also, the release of calcium from the internal stores was found to be defective in response to methoxamine, as well as the entry of calcium through store-operated channels, also called capacitative calcium entry. These three alterations were reversed and corrected after the inhibition of NO synthesis, which suggests that NO directly interferes with the mechanisms that control both the entry of calcium from the extracellular space and its release from the internal stores, thus making less calcium available for the contractile machinery [43]. To analyze directly this pharmacological evidence, we performed experiments directly in smooth muscle cells freshly isolated from the abdominal aorta of rats with BDL. The calcium levels were measured after loading with fura-2, a fluorochrome that binds to calcium, and its changes analyzed in a fluorescence microscope equipped with a highly sensitive photometer. The results obtained [44] confirmed these latter experiments [43]. The entry of calcium from the extracellular space and the release of calcium from the internal stores were clearly lower in the smooth muscle cells of the BDL rats. After inhibition of NO synthesis with L-NNA, a non-selective NOS inhibitor, these calcium responses improved greatly. A similar improvement was also observed when a selective inhibitor of inducible NOS, L-NIL, was used. In support of this finding, an increased expression of inducible NOS was clearly observed in smooth muscle cells freshly isolated from the abdominal aorta of the BDL rats (Fig. 1).

![Fig. (1). Western blot of iNOS in smooth muscle cells freshly obtained from the abdominal aorta of control and BDL rats.](image)

**Fig. (2).** Smooth muscle tone is regulated by cellular Ca\(^{++}\), which activates the Ca\(^{++}\)/calmodulin (CaM)-dependent enzyme myosin light chain kinase (MLCK), which leads to MLC phosphorylation and contraction. MLC phosphorylation is also regulated by MLC phosphatase. In cirrhosis, the excess formation of nitric oxide (NO) leads to relaxation of smooth muscle by stimulating the soluble guanylyl cyclase, which results in the production of cyclic GMP (cGMP) and the activation of cGMP-dependent protein kinase (PKG). PKG causes smooth-muscle relaxation by mechanisms that are still being defined, but which include a reduction in cytosolic Ca\(^{++}\) (by enhanced Ca\(^{++}\) export and storage and/or by reduced inositol trisphosphate receptor-mediated Ca\(^{++}\) mobilization) and dephosphorylation of myosin light chains (by activation of MLC phosphatase and/or by sequestration of MLCK in a phosphorylated form that is not readily activated by Ca\(^{++}\)/CaM). Other mechanisms by which NO seems to reduce vascular tone is through the interaction with Ca\(^{++}\) entry channels (receptor-operated (ROC) or store-operated (SOC), which are activated after the binding of the agonist to its membrane receptor or after the depletion of internal stores, respectively).
In summary, an elevated production of NO, originated both from the vascular endothelium and smooth muscle cells, participates in the lower calcium mobilization of the smooth muscle cells of the animals with BDL cirrhosis [44]. It is concluded that these alterations play an important role as the pathogenetic mechanism responsible for the arterial vasodilation and associated vascular abnormalities in liver cirrhosis (Fig. 2).

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the funding obtained from CICYT (SAF97-0176, SAF 2000-0176) and Fundación Séneca de la Comunidad Autónoma de Murcia [PB/14/FS/99 and PB/45/FS/02], both from Spain.

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