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Editorial

Simvastatin – From cholesterol to nitric oxide, from ischemic heart disease to portal hypertension

Even though changes in the intrahepatic vascular architecture resulting from cirrhosis represent the primary factor responsible for the increased vascular resistance that leads to portal hypertension, there is currently no doubt that other vascular factors are also involved, which may be modified with drugs (1). We are referring to intrahepatic vascular tone, which is increased in liver cirrhosis (2).

In this respect, the anatomical-functional complex formed by the sinusoidal endothelial cells (SECs) lining the lumen of sinusoids, and the hepatic stellate cells (HSCs) surrounding the endothelial cells plays a very important role in the regulation of intrahepatic blood flow (3,4). The physical proximity of both cell types accounts for the close relationships between them (5). HSCs, in addition to producing components of the extracellular matrix (ECM) display also some contractility and respond to SEC signals determined by the characteristics of blood flow within the sinusoids. When blood flow is ongoing, laminar, unidirectional or pulsed, SECs release nitric oxide (NO), which acts on neighboring HSCs and keeps them in an inactive, relaxed state with no ECM production (6-8). In contrast, when the intrahepatic vasculature is distorted, as occurs in liver cirrhosis, blood flow through the sinusoids is no longer continuous, and turbulence arises that has an impact on sinusoidal endothelial function, which in turn affects neighboring HSCs. Under such conditions sinusoidal endothelial dysfunction develops, which manifests as a decrease in NO production (9,10), an increase in NO consumption from its reacting with superoxide anions to form peroxynitrite (11), and an increase in vasoconstrictor factors such as thromboxane A2 and endothelin-1 (12). As a consequence, vascular tone increases, together with intrahepatic sinusoidal resistance.

Among the functional changes SECs undergo as a result of sinusoidal blood flow, the transcription factor KLF2 (*Krupple-like factor 2*) plays a fundamental role (13,14). The mechanisms by which the passage of blood through the sinusoids increases the genetic and proteinic expression of KLF2 are poorly understood; however, the MEK5 (*mitogen-activated protein kinase kinase-5*), ERK5 (*extracellular signal-regulated kinase 5*), and MEF2 (*myocyte enhancing factor-2*) pathways seem to be involved (14). This is a protein in a class of factors where zinc atoms bound to several cysteine residues determine their molecular morphology (*zinc-finger proteins*). All this family of factors has affinity for CACCC or GC-rich sequences in DNA. The gene and protein expression of KLF2 is particularly high in endothelial cells, whence it controls normal vascular development (13,15) and protects against inflammation, thrombosis, vasoconstriction, leukocyte adhesion, oxidative stress, and death by apoptosis (14,16,17). All these effects are exerted by augmenting gene expression for antithrombotic molecules (thrombomodulin) (18) and vasodilator molecules [CNP *(C-type natriuretic peptide*), eNOS (*endotelial nitric oxide synthase*) (14)], and inhib-

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iting gene expression for proinflammatory (17) and leukocyte adhesion molecules [VCAM-1 (*vascular cell adhesion molecule-1*), selectin E (17)]. In order to acquire full enzymatic activity, eNOS, which is directly responsible for the synthesis of the potent vasodilator NO, requires phosphorylation to serine 1197, which is carried out by the serine kinases PI3K (*phosphatidylinositol-3-kinase*) and Akt (19). In some situations, as is the case with liver cirrhosis, KLF2 expression in SECs in increased but eNOS enxymatic activity remains low. This may be accounted for by inadequate eNOS phosphorylation (20). The latter is likely due to the fact that the expression of VEGF (*vascular endothelial growth factor*), which activates the PI3K/Akt pathway (21), is not increased in liver cirrhosis (20).

Considering that intrahepatic vascular pressure is partially reversible and that NO plays a key role in its regulation, NO donor drugs, including nitrites, have been logically used for the management of portal hypertension (22). However, the experience derived from its use in patients with cirrhosis has shown that their effects are not liver-selective and, in addition to reducing portal pressure, also induce systemic vasodilation and a decrease in mean blood pressure, which may impair renal function. Because of this, drugs have been sought to selectively reduce intrahepatic vascular resistance without significantly changing systemic vascular resistance. Such drug would be ideal for the management of portal hypertension, particularly if it could concurrently reduce ECM deposition around liver sinusoids. The latter would improve liver function by favouring metabolite exchange between sinusoidal blood and hepatocytes. Such exchange is seriously compromised in liver cirrhosis because HSC proliferation and activation (23) enhance ECM deposition and angiogenesis, and induces so-called sinusoidal capillarization. Liver vasculature-selective NO donors have been developed, but have not been used in patients as yet.

Amongst drugs considered for the management of liver cirrhosis-associated hepatic hypertension statins stand out, most particularly simvastatin (SVST). These drugs inhibit the enzyme 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase, which plays a role in the synthesis of cholesterol. This is why these drugs reduce blood LDL-cholesterol levels and are successfully used in the treatment of hypercholesterolemia and the prevention of cardiovascular accidents (24). Furthermore, both experimental and clinical studies have shown in recent years that they decrease sinusoidal endothelial dysfunction as present in liver cirrhosis (25), and increase *eNOS* gene expression, mRNA stability (26), and enzymatic activity, and NO availability in the liver (25). They also reduce the resistance of blood through the liver and portal pressure without modifying splanchnic or systemic hemodynamics (27,28). It is on these grounds that statins seem to possess a number of the characteristics that ideal drugs should have for the management of portal hypertension. Namely, they improve liver perfusion and reduce clinically significant portal pressure while having no impact on mean blood pressure and systemic vascular resistance. Furthermore, clinical observations have shown that these drugs also improve liver function (28).

These effects of statins on liver sinusoids are also linked with factor KLF2 (29). SEC exposure to these drugs, e. g., to SVST, increases KLF2 and all the target genes for this transcription factor (20,30). With this, statins improve endothelial dysfunction and all its consequences (apoptosis), and reduce the effects of proinflammatory cytokines on the endothelium (16). These endothelial effects of statins are not independent of their action on HMG-CoA reductase, as they may be fully suppressed by mevalonate (6,16). The latter is a metabolite in the cholesterol synthesis pathway that directly results from HMG-CoA reductase activity, and is the precursor of several isoprenoids, including GGPP (*geranyl-geranyl pyrophosphate*) and FPP (*farnesyl*

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pyrophosphate). The latter are incorporated into cell membranes where they bind GTP-binding proteins such as Rho, Rac and Ras. Once these proteins have been isoprenylated, they activate signaling pathways leading to NFkB overexpression, and hence increased inflammatory phenomena, and *KLF2* gene repression, which enhances said inflammatory phenomena, the synthesis of reactive oxygen species (ROS), and cell proliferation (16,31). Upon inhibiting HNG-CoA reductase, which blocks Rho activation, statins increase KLF2 expression and decrease inflammatory phenomena, ROS production, and cell proliferation.

Statins, in addition to inducing HSC relaxation and reducing sinusoidal resistance to blood flow through the liver, have also shown their ability to diminish liver fibrosis in patients with chronic hepatitis C (32). This reduced fibrosis may help improve liver function in patients with cirrhosis, probably by decreasing ECM deposition in liver sinusoids (28). The mechanisms by which statins may behave as antifibrogenic agents are poorly understood. In the present issue of *Revista Española de Enfermedades Digestivas (Spanish Journal of Digestive Diseases),* Miao et al. (33), using a human immortalized HSC line (LX2 cells), show that SVST reduces or inhibits TGFβ (*transforming growth factor-beta*) effects on the activation, proliferation and migration of LX2 cells to a significant extent, while reducing the production of several proinflammatory factors (NF-κB) and proangiogenic factors, including HIF-1α (*hypoxia-inducible factor-1*α) (34) and VEGF. The authors of this study confirm in LX2 cells what other authors previously demonstrated using other cell lines (20,30), namely that SVST increases the expression of transcription factor KLF2 and decreases the expression of its angiogenic target genes. While the authors do not demonstrate it, as they performed no KLF2 gene inhibition or silencing studies, SVST most likely inhibits the fibrogenic and proangiogenic response related to TGFβ by increasing KLF2 expression. These *in vitro* studies in HSC cultures show that SVST may exert its effects on KLF2 by directly targeting these cells, regardless of their effects on SECs. However, studies with SEC and HSC joint cultures have shown that treating SECs with SVST inactivates neighboring HSCs, which stop producing ECM (30). In these inhibiting effects of SECs on HSCs, NO and cGMP seem to play a role, in addition to KLF2 (30,35).

Doubtless, the effects of SVST on HSCs also depend upon its ability to inhibit HMG-CoA reductase (36), to keep Rho inactive (37), and to increase factor KLF2. Indeed, KLF2 overexpression as a result of treating cirrhotic rats with SVST, or of infecting them with KLF2 gene-carrying viruses, is followed by a marked decrease in hepatic fibrogenic activity, manifested by a decrease in type-I collagen production and in α-smooth muscle actin expression (5), a marker of HSC activation. In these animals HSC inactivation slows their proliferation, increases apoptosis and stops ECM production (5,38). Antioxidative effects represent another mechanism through which SVST might reduce HSC activity (5). The role of oxidative stress as profibrogenic factor is well known (39-41). Both in HSC cultures and cirrhotic rats KLF2 has been seen to induce Nrf2 nuclear translocation (42), which increases the genetic expression of antioxidant proteins such as hemeoxygenase-1 and NADPH dehydrogenase quinone-1, which decrease superoxide anion levels (5).

To conclude, simvastatin, which as all statins was initially conceived to fight hypercholesterolemia, has been seen to possess much wider effects, as it may correct the sinusoidal endothelial dysfunction present in liver cirrhosis and selectively reduces intrahepatic vascular resistance without compromising systemic circulation. Finally, it can inactivate hepatic stellate cells, thus reducing liver fibrosis extent, and reduce the extracellular matrix barrier between sinusoidal blood and liver cells. From all

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the above, we may state that, from a theoretical viewpoint, statins represent a drug class that certainly meets the requirements demanded of ideal drug candidates for the management of portal hypertension. However, such theoretical hopes must be ratified by sufficiently extensive, controlled, randomized studies specifically designed to find out whether statins may decrease portal hypertension to a clinically significant degree, and whether these benefits are reached with minimal adverse effects.

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