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### **Bile Acids and Portal Hypertension**

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#### **ABSTRACT**

The recent discovery of bile acid (BA) receptors and a better delineation of the multiple roles of BAs in relevant biological processes have revamped BA research. The vasoactive actions of BAs were recognized more than three decades ago but the underlying mechanisms of the BA-induced vasorelaxation are now being clarified. Recent evidence shows that the BA receptors FXR and TGR5 are expressed in endothelial cells and may have important effects on both systemic and portal circulation. The availability of genetically engineered mice with ablation of BA receptors and the development of BA receptor agonists has allowed to explore the modulation of FXR and, in a lesser extent, of TGR5 in the setting of portal hypertension (PHT) with promising results. In this review, we summarize recent data on how BA-dependent pathways influence several processes that impact in PHT and the preclinical data showing that pharmacological modulation of those pathways may hold promise in the treatment of PHT.

**Key words.** Bile acids. Hepatic flow. Portal hypertension. Cirrhosis. Farnesoid X receptor. Fibrosis. Cirrhosis. Bacterial translocation. Intestinal permeability. Inflammation.

#### **PREAMBLE**

Portal hypertension (PHT) develops mainly as a consequence of chronic liver disease and is responsible for the development of the main complications of cirrhosis leading to death or liver transplantation. In recent years, there has been significant advances in the understanding of the pathophysiological basis of PHT, which mainly results from an increased hepatic vascular resistance related to several factors including a fixed mechanic component related to distorted hepatic microarchitecture secondary to sustained fibrogenesis and functional factors such as endothelial dysfunction, hepatic stellate cells (HSC) contraction and increased portal inflow.<sup>2,3</sup> Endothelial dysfunction involves a variety of molecular mechanisms causing peripheral vascular hyporeactivity with increased endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO) generation in the splanchnic and systemic circulations and reduced eNOS-derived NO production in the intrahepatic vascular bed, which results in increased hepatic vascular resistance.<sup>3</sup>

To date, the only drug-class used to treat PHT are nonselective beta-blockers, which are of limited utility and have recently been shown to be potentially detrimental in advanced cirrhosis.<sup>4,5</sup> In that context, additional therapeutic targets are being explored with focus in those factors involved in the functional component of PHT including NO, endothelin, vascular endothelial growth factor (VEGF) and other angiogenic molecules.<sup>6</sup> In addition, bile acid (BA)-based treatment of PHT may be also considered in light of recent data showing that BAs, through the activation of dedicated BA receptors, may influence relevant pathways involved in PHT development and progression.<sup>7,8</sup> In the present review, we summarize the current information on the role of BAs in PHT and the perspectives on the potential use of BAs in PHT treatment.

## BILE ACIDS AS VASOACTIVE COMPOUNDS: OLD AND NEW CONCEPTS

Experimental observations published more three decades ago showing that obstructive jaundice associates to a reduced total peripheral resistance and hypotension suggested that BAs might act as vasoactive agents. <sup>9,10</sup> In that line, Lautt and Daniels <sup>11</sup> showed that intravenous administration of taurocholic acid caused a vasodilation of mesenteric and hepatic arteries in cats. *In vitro* studies replicated the vasodilatory effect of BAs in aortic rings and

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showed that lipophilic BAs were more potent.<sup>10</sup> The mechanisms of BA-induced vasodilation remained unclear or hypothetical until recently when several reports showed that BAs could influence vasoactive pathways in the endothelium through various pathways including modulation of NO production and inhibition of endothelin-1 release.<sup>12</sup>

The discovery that BAs are relevant signaling molecules that exercise a myriad of biological functions (i.e. regulation BA synthesis, transport, and detoxification as well as both lipid and carbohydrate homeostasis) have revolutionized the BA field. BA exercise many of these functions through specific membrane and nuclear receptors with FXR (farnesoid X receptor) and TGR5 (takeda G-protein associated receptor, also known as GPBAR1) being the best characterized. 13 Of note, recent work has demonstrated that the vasodilatory effects of BAs in the systemic and splanchnic circulation are in part mediated by activation of TGR5 that is expressed in both endothelial cells and liver sinusoidal endothelial cells (LSECs).<sup>12</sup> Recent work has also shown that the mechanism of BA-vasodilation involves the regulation of eNOS and cystathionine-lyase (CSE), an enzyme that catalyzes L-cysteine producing hydrogen sulfide (H2S), a gaseous vasodilator. A role of BAs in modulating calcium-activated potassium (BKCa) channels in vascular smooth muscle cells has been also proposed.12

In addition to the vascular effects of BAs, some reports suggest that BAs can also modulate cardiac function by reducing heart rate and reduced cardiac contractility although the bases of these effects remain unclear. Since heart expression of FXR and TGR5 is negligible cardiac effects of BAs do not seem to be mediated by these receptors. BA-modulation of channel conductance and calcium dynamics in sino-atrial and ventricular cardiomyocytes has been proposed.<sup>14</sup>

## ROLE OF BILE ACIDS RECEPTORS IN PHT PATHOPHYSIOLOGY

The availability of genetically engineered mice lacking FXR or TGR5 as well as the existence of agonists of BA receptors has allowed exploration of their role of in liver disease in general and PHT in particular. In fact, recent work has pointed out to a relevant role of several FXR-mediated pathways in biological processes that are important for PHT development and progression. FXR has been shown to influence hepatic fibrogenesis, <sup>15</sup> control hepatic and gut inflammation and regulate intestinal permeability and bacterial translocation. <sup>16</sup> These factors influence hepatic architecture and both tissue and vascular resistance. With regard to the latter, available data suggest that FXR also regulates vascular hepatic NO signaling by upregulat-

ing eNOS through up-regulation of dimethylarginine dimethylaminohydrolase 1 that in turn degrades asymmetrical dimethylarginine (eNOS inhibitor), thus increasing local NO production, which has been shown to be dysregulated in cirrhosis. <sup>12</sup> In addition, FXR seems to counteract local inflammatory responses, by reducing inducible isoform nitric oxide synthase (iNOS) and cyclooxygenase (COX)2 expression. <sup>17</sup>

Extrahepatic effects of FXR activation can also be relevant for PHT. In particular, FXR-induced prevention of gut barrier dysfunction and bacterial translocation through augmenting production of antimicrobial peptides such as angiogenin1 and RNase family member 4 by enterocytes<sup>18,19</sup> can contribute to ameliorate the leaky gut that has been shown to play an important role in PHT.<sup>20</sup> Indeed, clinical and experimental evidence suggest that bacterial translocation, endotoxemia and proinflammatory cytokines impair contractility of mesenteric vessels increasing portal pressure in cirrhosis.

With regard to TGR5-related effects influencing PHT, available evidence is more limited. LSECs express TGR5 and the increase in NO production by these cells after exposure to BAs has been shown to be TGR5-dependent.<sup>21</sup> In addition, the role of TGR5 in systemic BA-mediated control of vascular tone could indirectly impact on PHT through an increased mesenteric flow.<sup>12,14</sup>

Collectively, data summarized above suggest that modulation of FXR and TGR5 related pathways might be beneficial for PHT.

# PRECLINICAL AND CLINICAL DATA ON THE USE OF FXR AND TGR5 AGONISTS IN PORTAL HYPERTENSION

In the last few years, pharmaceutical industry had devoted significant effort in developing BA-based therapies aimed to treat different liver diseases including primary biliary cholangitis, primary sclerosing cholangitis and non-alcoholic steatohepatitis.<sup>22</sup> Pre-clinical work is now being translated into clinical trials with the first-in class FXR agonist obeticholic acid (OCA) being the first BA-receptor agonist approved by the FDA to treat primary biliary cholangitis.<sup>23</sup> Other agonists are in the pipeline and data on their effects in preclinical models is also emerging.<sup>7,24</sup>

With regard to PHT, preclinical work with OCA has shown that this FXR agonist restored intrahepatic eNOS levels and reduced PHT in experimental cirrhosis.<sup>25</sup> Also, it has been shown that FXR agonists are able to increase hepatic CSE, thus increasing local H2S production and reducing intrahepatic resistance.<sup>12</sup> Finally, OCA may also inhibit endothelin-1 (ET-1)-mediated contraction of HSC,<sup>26</sup> which has been suggested to contribute to the dy-

namic component of portal hypertension.<sup>3</sup> A preliminary attempt to translate the preclinical work into clinic was presented by Mookerjee, *et al.* that directly measure portal pressure in patients with alcoholic cirrhosis showing that OCA reduced PHT.<sup>27</sup>

Other FXR agonists are in development.<sup>24</sup> The nonsteroidal FXR agonist PX20606 has been recently shown to ameliorate PHT through improving sinusoidal dysfunction and promoting vascular remodeling in two different experimental models of PHT.<sup>7</sup> In this work, PX20606 showed multiple modes of action including reduction of hepatic fibrosis and inflammation and improvement of intestinal barrier.

With regard to modulation of TGR5 in PHT less information has been published. A recent paper by Renga, et al. showed that administration of BAR501, a semisynthetic bile acid derivative, which is a potent and selective TGR5 agonist, reversed endothelial dysfunction in carbon tetrachloride-induced cirrhosis mouse model.8 In this report, BAR501exerted genomic and non-genomic effects in liver endothelial cells resulting in decreased PHT in cirrhotic mice. The beneficial effects of BAR501 involved several pathways including an increase in liver CSE expression and activity, a higher expression of eNOS and as well as a reduction of ET-1 gene expression in LSECs through a mechanism involving a Akt-dependent phosphorylation of FoxO18. In addition to these observations, preliminary data from Klindt, et al. also showed that incubation of LSECs with a TGR5 agonist caused a significant reduction of ET-1 expression and secretion.<sup>28</sup> Unfortunately, the clinical development of TGR5 agonists is in a very early development and, due to the fact that TGR5 is widely expressed, unwanted effects has been observed in humans leading in some cases to discontinuation of TGR5-based trials.29

#### **PERSPECTIVES**

The beneficial effects of FXR agonists on PHT in experimental models acting through several distinct mechanisms plus the proof of concept study showing that FXR agonism does reduce PHT in humans suggest that exploiting FXR-related mechanisms may represent a promising and novel option to treat PHT. The ongoing clinical trials investigating FXR agonists in different liver diseases will shed light on the real magnitude of the antifibrotic effects of this drug-class as well as will provide human data regarding the effects FXR-based treatment on other factors that influence PHT such as intestinal permeability and vascular remodeling. Collectively, current data on BA-based therapy of PHT is encouraging but more human evidence is eagerly needed.

#### CONFLICT OF INTEREST

The authors declare not to have conflict of interests related to this scientific work.

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