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Iloprost: an adjunctive approach to chronic viral hepatitis treatment

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Summary Chronic viral liver disease may evolve to cirrhosis. The medical treatment to slow down this passage is based on anti-viral and anti-fibrotic properties of interferon. Recently, we evidenced significant increase of portal vein flow velocity and volume after a prostacyclin analog (iloprost) infusion in subjects without and with chronic viral hepatitis. On the basis of these results and considering both the pathophysiology of viral liver disease and the mechanism of action of iloprost in portal microcirculation, we hypothesize that it may be of some efficacy in chronic liver disease ameliorating the portal hemodynamics and producing an anti-oxidant liver effect.

Introduction

Chronic liver disease is the tenth leading cause of death among adults in the United States and accounts for approximately 25,000 deaths annually, or approximately 1% of all deaths [1]. Population-based studies indicate that 40—45% of chronic liver diseases are alcohol related, 35—40% HCV-related and about 20% HBV-related, resulting in an estimated 12,000, 8000—10,000 and 5000 deaths each year, respectively, [1]. Chronic viral hepatitis is worldwide spread with about 170 million people affected by chronic hepatitis C virus infection and 350 million people being chronic carriers of the hepatitis B virus [2]. In a substantial proportion of cases, after 10—30 years of increasing fibrogenesis, chronic hepatitis evolves to cirrhosis, that, in turn, may give rise to hepatocarcinoma [3,4].

To slow down the evolution of chronic viral hepatitis these patients are treated with interferon

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(IFN) whose target is the reduction of viral replication and fibrosis.

Instead, no treatment exists to ameliorate the liver hemodynamics acting on the portal circulation and to diminish the oxidant effects on the liver of the chronic ischemic injury and inflammation.

Therefore, after an elucidation on the mechanisms that promote liver cirrhosis and on the pharmacology of prostacyclin and analogs, we hypothesize that the better portal microcirculation perfusion after the administration of iloprost (an analog of prostacyclin) might be useful in preventing the evolution of chronic viral liver disease.

Chronic hepatitis and mechanisms of progression to cirrhosis

Chronic liver disease starts when a liver injury (viral hepatic infection, alcohol assumption or others) promotes a cascade pathway in which hepatic stellate cells are activated so that profibrogenic mediators, such as transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF), are secreted [5–8]. This increased production of inflammatory cytokines causes the phenotypic modification of Ito cells into myofibroblasts that, in turn, produce profibrotic cytokines. The chronic increase of TGF-β and PDGF and that of other proinflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 [9], determine connective tissue deposition and progressive distortion of the lobular and microvascular hepatic architecture, thus causing hypertension in the portal microcirculation. Moreover, the simultaneous imbalance between hepatic vasodilator and vasoconstrictor mediators favors the increase of the liver sinusoidal microcirculation resistance. In fact, in healthy subjects, the homeostasis of the portal circulation is regulated by a balance between vasodilator and vasoconstrictor mediators whose function is to maintain a normal intrahepatic resistance. Targets of these mediators are the cells of the portal microcirculation, mainly the hepatic stellate cells (HSC), which modulate the vascular intrahepatic resistance through a mechanism of contraction and relaxation, depending on the type of signal received from vasoconstrictor or vasorelaxant substances [10,11]. In this context, hepatic sinusoids may undergo a process of constriction caused by the activation of the HSC [12–15] which takes place through the opening of calcium channels [16]. Once activated, they are capable of maintaining for a long time the sinusoidal resistance that causes portal hypertension [17], and their stable activation favours the progressive development of hepatic fibrosis [16]. Therefore, not only fibrogenesis but also the local increase of several vasoconstrictive substances (endothelin, vasopressin, angiotensin) and the decrease of vasodilating mediators (mainly prostacyclin) [10–15] are responsible for hepatic sclerosis and portal hypertension. Furthermore, endothelin was also shown to induce the ischemic necrosis of hepatocytes by causing the hepatic artery constriction [18].

The logical consequence is that, in an attempt to oppose the portal microcircle and hepatic artery vasoconstriction, several vasoactive substances, such as nitric oxide and glucagon, are peripherally synthesized inducing a powerful splanchnic vasodilatation. Finally, a hyperdynamic circulation develops that results in an increased portal blood flow [19,20].

However, this mechanism of compensation causes only splanchnic vessel vasodilatation and hyperdynamic circulation without opposing the portal microcircle vasoconstriction, thus worsening the portal hypertension [21].

Pharmacology of prostacyclin and its analogs

Prostanoids, which consist of prostaglandins and thromboxane, are metabolites of the arachidonic acid under the action of ciclooxigenase. Prostaglandins contain a cyclopentane ring with two side chains, α and ω, attached to the ring.

Based on the ring modifications, they are classified into the types (prostaglandins) from A to I (PGA-I).

PGI2 is the prostacyclin physiologically produced by human organism: discovered in 1976 [22], it is an antiplatelet, arterial vasodilator substance.

Subsequently, a prostacyclin (epoprostenol) was synthesized which has a short half-life (about 2–3 min) and from it many PGI2 analogs were derived that show chemical modifications consisting of addition or removal of chemical groups to the base molecule. They are a great number, have the same properties of the PGI2 but a greater degree of chemical and metabolic stability and a higher plasma half-life [23–25].

All PGI2 analogs, as well as PGI2 itself, bind to the cellular receptors of PGI2 (IP) [26], and increase the intracellular level of cyclic adenosine monophosphate (cAMP), the mediator of their action. Recently, other receptors were found to be involved in the interaction with PGI2 analogs; in fact, peroxisomal proliferator-activated receptors

(PPARs) are a class of nuclear receptors that use PGI2 and analogs as endogenous ligands to modulate specific cellular functions [27].

Literature reports that PGI2 and its analogs are effective both as antiplatelet drugs — in pulmonary hypertension, pulmonary thromboembolism and peripheral arterial diseases [28] — and as gastroprotective agents [29]. Other studies have shown their effect on fibroblast migration, thus suggesting an antifibrotic role [30]. Synthetic analogs of PGI2, such as iloprost, are inhibitors of platelet aggregation and potent arterial vasodilators, have a fibrinolytic activity in patients with peripheral arterial disease, limit neutrophilic migration in damaged tissues and also have a cytoprotective action [31].

They are effectively used in the treatment of primary pulmonary hypertension [32], Raynaud's phenomenon secondary to scleroderma [33], severe chronic ischaemia of the lower limbs in patients at risk of amputation and when a surgical or angioplasty intervention is contraindicated and, finally, thromboangiitis obliterans (Buerger's disease) at an advanced stage of critical ischaemia when revascularisation is contraindicated.

Furthermore, PGI2 and analogs have an effect on the renal circulation in patients with systemic sclerosis and renal failure [34].

The PGI2 analogs have shown hepatic cytoprotective as well as antiapoptotic effects [35—40] (Table 1). They also express an antifibrotic activity inhibiting fibroblast migration [30], preventing lymphocytes T from producing TNF-α [41,42] and slowing down the production of connective tissue growth factor (CTGF) [43].

Iloprost in chronic viral hepatitis

As stated above, in healthy subjects there is a correct balance between vasodilators and vasoconstrictors which maintains the portal flow homeostasis, whereas in chronic viral liver disease the increase of vasoconstrictors produces an imbalance and thus an altered portal hemodynamics resulting in portal hypertension.

On the basis of this regulatory pathway of the portal hemodynamics, we evaluated the efficacy of the prostacyclin analog iloprost in liver circulation. In fact, recently, we demonstrated an increase of portal flow velocity and volume after the administration of iloprost in subjects with and without chronic hepatitis [44,45]. Previous studies showed the capability of iloprost to increase the hepatic blood flow, and the spanchnic blood flow together with the splanchnic oxygen delivery in patients with septic shock [46], and in hyperdynamic porcine experimental endotoxemia [47], respectively.

The increase of portal hematic flow together with the microcircle vasodilatation might be useful in subjects with chronic hepatitis, improving the hepatic perfusion and favoring a decrease of microvascular hepatic hypertension. This activity clearly

Table 1 Main evidences supporting the hepatic cytoprotective and antiapoptotic activity of iloprost

First author's name, year	Model	Route of administration	Effect
Changani KK, 1999 [35]	Stored pig livers	Addition in the preservation solution	Increase of viable hepatic bioenergetics
Harada N, 1999 [36]	I/R injury of rat livers	i.v. periprocedural treatment	Reduction of hepatic damage and leukocyte activation; inhibition of the decrease in hepatic tissue blood flow
Bozkurt S, 1997 [37]	CCl4-induced rat liver injury	i.v. pretreatment for 18 weeks	Hepatoprotection and reduction of histamine levels
Okboy N, 1992 [38]	I/R injury of rat livers	i.v. periprocedural treatment	Histopathologic improvement; decrease in tissue MDA and increase in tissue GSH levels
Nasseri-Sina P, 1992 [39]	Pracetamol-induced toxicity in hamster hepatocytes *in vitro*	Addition in the medium culture	Reduction of the loss of cell viability
Bursch W, 1989 [40]	CCl4-induced rat liver injury	i.v. periprocedural treatment	Reduction of the aldehyde-positive liver section area and of the liver MDA content

I/R, ischemia/reperfusion; i.v., intravenous; CCl4, carbon tetrachloride; MDA, malondialdehyde; GSH, glutathione.

reflects a direct effect on HSC. In fact, iloprost has been shown to counteract the HSC contraction and to induce their relaxation in vitro [12]; this effect was mediated by a substantial elevation of intracellular cAMP levels. Interestingly, it has also been evidenced that cAMP inhibits the growth itself of human HSC in vitro [48]. Therefore, since HSC stable activation, through the production of TGF-β and PDGF and other proinflammatory mediators, is the principal mechanism leading to hepatic fibrosis, the capability of iloprost to counteract their stable activation must be underscored. This effect of iloprost gives rise to important perspectives on the possibility of preventing the hepatic fibrotic response to these proinflammatory mediators. Furthermore, as mentioned, experimental studies on the rabbit demonstrated that the exogenous administration of endothelin is able to determine the ischemic necrosis of hepatocytes [18]. Subjects with chronic viral liver disease have increased serum levels of endothelin which can elicit similar deleterious activities on the liver. Iloprost can be considered to prevent the ischemic effects of this molecular agent by its capability to antagonize the hepatic vasoconstrictive effect of endothelin [12].

In addition, it is also known that reactive oxygen species (ROS) play a crucial role in the induction and in the progression of liver disease, independently of its etiology, and directly influence hepatic fibrogenesis. They are involved in the transcription and activation of a large series of cytokines and growth factors that, in turn, can contribute to further ROS production. ROS are derived from activated Kupffer cells, recruited inflammatory cells, and HSC under some circumstances [49]. Exogenous ROS have been shown to enhance HSC expression of the collagen gene [50], and HSC genetically engineered to generate high levels of intracellular oxidants overexpress collagen [51].

As has been observed by several researchers, iloprost displays anti-oxidant effects. In fact, iloprost and prostacyclin analogs were found both to decrease the tissue levels of malondialdehyde and to increase the hepatic content of glutathione, the most powerful anti-oxidant agent, in several studies on the ischemia-reperfusion injury in the rat livers [38]. An improvement of the mitochondrial function in combination with superoxide dismutase (SOD) and catalase was also observed [52]. Furthermore, it was found that another PGI2 analog (OP2507) suppressed hepatocyte death in a model of superoxide-induced hepatocyte injury in vitro [53]; the increase of the intracellular cAMP levels followed by suppression of intracellular calcium elevation was involved in the cytoprotective effect. Moreover, the antioxidant properties of prostacyclin and analogs are potentiated in vivo by the inhibition of inflammatory cell recruitment and by the induction of PMN apoptosis in the liver [54].

Finally, iloprost benefit in chronic viral hepatitis might also derive from its capability to directly inhibit the production of proinflammatory-profibrotic cytokines by leukocytes [40,41] and fibroblasts [42], and to down-regulate the mitogen activated protein kinases of macrophages [55].

Taken together, these observations elucidate the possible role of iloprost in preventing the biochemical and biological alterations leading towards liver cirrhosis and thus suggest further therapeutic perspectives in patients with chronic viral hepatitis. Acting on the HSC, iloprost seems able to modulate both the portal microcirculation and the synthesis of collagen, thus inhibiting the development of portal hypertension and decreasing the progression of liver fibrosis. Furthermore, iloprost and analogs display beneficial effects also modulating the inflammatory and oxidant response of other cells that play a role in the progression of chronic liver disease (Fig. 1).

For these reasons we like to hypothesize some beneficial effect of iloprost in combined therapy with the already consolidated treatment of chronic viral hepatitis (IFN and ribavirin or lamivudine).

A purpose of combined therapy in patients with chronic viral hepatitis

It is known that the combined treatment with IFN and ribavirin or lamivudine produces both antiviral and immunomodulatory effects, and decreases the inflammation and fibrosis process thus preventing the evolution to cirrhosis, as shown by several multicenter studies and trials [56–58].

The association of IFN and ribavirin or lamivudine with iloprost might offer a better preventive action in patients with chronic viral hepatitis. At least three reasons support this medical hypothesis. First, IFN and ribavirin or lamivudine in association with iloprost are complementary treatments, the former producing an antiviral, immunomodulatory and anti-fibrotic action, the latter determining the inactivation of the HSC and a consequential anti-fibrotic action. Second, the better perfusion of the liver due to iloprost might slow down the production of oxidant mediators by reducing the local ischemia. Third, iloprost might facilitate the IFN and ribavirin or lamivudine action by improving the hemodynamics of the usually poorly perfused areas of the liver.

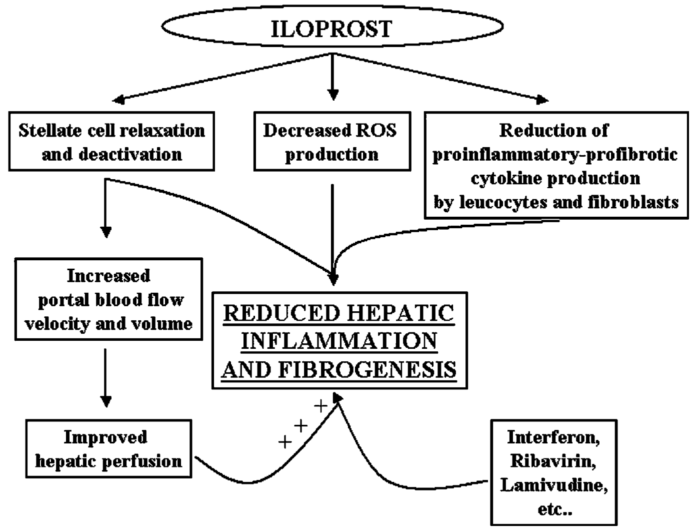

Figure 1 Beneficial effects of iloprost administration in chronic hepatitis.By relaxing and deactivating Ito cells, iloprost increases the portal blood flow velocity and volume, thereby improving the hepatic perfusion and favouring the arrival and activity of antiviral agents. Furthermore, iloprost directly counteracts reactive oxygen species (ROS) formation and proinflammatory-profibrotic cytokine production by leukocytes and fibroblast. All these properties contribute to reduced hepatic inflammation and fibrogenesis.

In the studies we are conducting, iloprost is administered intravenously and the therapeutic response (the change in hemodynamic parameters and biological mediators) is tested immediately before and after the cycle of treatment. The promising results show a positive effect on the hepatic microcirculation, therefore documenting a beneficial short-term activity. However, since in viral chronic hepatitis the liver injury has a slow evolution over the years, and the acquired therapeutic regimens last for substantial periods of time, the purpose of a combined treatment requires the confirmation of a long-term iloprost activity or the possibility of an easier drug administration. Equally, when underscoring the capability of iloprost to counteract the HSC activation and thus the progressive development of fibrosis, it seems obvious that such activity needs to be maintained till the proinflammatory—profibrotic mediators act on the HSC. For these reasons, it is important to consider that iloprost can be administered orally by sustained release oral preparation for outpatient therapy [59,60], and studies testing this possibility in chronic liver disease are clearly awaited.

In conclusion, we propose that the different mechanisms and targets of action of IFN and ribavirin or lamivudine with those of iloprost make them compatible and complementary in treating subjects with chronic viral hepatitis, limiting or even avoiding the evolution of the chronic liver disease. Obviously, the possibility of a combined treatment of IFN, ribavirin or lamivudine and iloprost remains an attractive hypothesis that, however, needs further evaluations and studies.

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