Research Article

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Resistin and visfatin in steatotic and non-steatotic livers in the setting of partial hepatectomy under ischemia-reperfusion

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Background & Aims: This study examined whether the regulation of resistin and visfatin could reduce damage and improve regeneration in both steatotic and non-steatotic livers undergoing partial hepatectomy under ischemia-reperfusion, a procedure commonly applied in clinical practice to reduce bleeding.

Methods: Resistin and visfatin were pharmacologically modulated in lean and obese animals undergoing partial hepatectomy under ischemia-reperfusion.

Results: No evident role for these adipocytokines was observed in non-steatotic livers. However, obese animals undergoing liver surgery showed increased resistin in liver and plasma, without changes in adipose tissue, together with visfatin downregulation in liver and increment in plasma and adipose tissue. Endogenous resistin maintains low levels of visfatin in the liver by blocking its hepatic uptake from the circulation, thus regulating the visfatin detrimental effects on hepatic damage and regenerative failure. Indeed, the administration of anti-resistin antibodies increased hepatic accumulation of adipocyte-derived visfatin, exacerbating damage and regenerative failure. Interestingly, treatment with anti-visfatin antibodies protected steatotic livers, and similar results were obtained with the concomitant inhibition of resistin and visfatin. Thus, when visfatin was inhibited, the injurious effects of anti-resistin antibodies disappeared. Herein we show that upregulation of visfatin increased NAD levels in the remnant steatotic liver, whereas visfatin inhibition decreased them. These later observations suggest that visfatin may favour synthesis of NAD instead of DNA and induces alterations in amino acid metabolism-urea cycle and NO production, overall negatively affecting liver viability.

Conclusions: Our results indicate the clinical potential of visfatin blocking-based therapies in steatotic livers undergoing partial hepatectomy with ischemia-reperfusion.

Introduction

In clinical situations, partial hepatectomy (PH) under ischemia-reperfusion (I/R) is usually performed to control bleeding during parenchymal dissection [1]. More than 20% of patients coming to liver resection have some degree of steatosis, usually related to obesity [2,3]. Hepatic steatosis is a major risk factor for liver surgery since it is associated with an increased complication index and postoperative mortality after major liver resection [4].

Under pathological conditions, adipose tissue has appeared as a highly active endocrine gland, secreting adipocytokines such as resistin and visfatin [5]. However, resistin and visfatin are also expressed in liver under obesity conditions and during fibrosis [6,7].

Increased levels of resistin have been reported in patients with cirrhosis, correlating with the severity of the disease [5,8]. Opposite effects of resistin on myocardial injury have been reported [9].

Visfatin, also known as nicotinamide phosphoribosyltransferase (Nampt), is critical for the synthesis of nicotinamide adenine

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Abbreviations: PH, partial hepatectomy; I/R, ischemia-reperfusion; Nampt, nicotinamide phosphoribosyltransferase; NAD, nicotinamide adenine dinucleotide; TNF, tumor necrosis factor; IL, interleukin; *Ob*, obese; Ln, lean; BrdU, bromo-deoxyuridine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HGF, hepatocyte growth factor; TGF, transforming growth factor; MPO, myeloperoxidase; MDA, malondialdehyde; LDL, Low-density lipoprotein; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; GDH, glutamate dehydrogenase; CPT2, carnitine palmytoil transferase-2; MCAD, medium chain acyl-CoA dehydrogenase; FGF21, fibroblast growth factor-21; SOD2, superoxide dismutase-2; GPX1, gluthathione peroxidase-1; SCD1, stearoyl-CoA desaturase-1; FAS, fatty acid synthase; COXI, cytochrome c oxidase subunit I; COXIV, cytochrome c oxidase subunit IV.

dinucleotide (NAD) [10]. Interestingly, a potential role for visfatin in the pathogenesis and progression of non-alcoholic fatty liver disease has been reported [11]. Circulating visfatin levels are increased in inflammatory disorders [12] and its inhibition decreases several pro-inflammatory cytokines in cultured cells and during endotoxemia [13]. However, there are conflicting results regarding the role of visfatin since other reports indicate that elevated levels of visfatin in obesity have hepatoprotective effects [14], or even are not associated with inflammation [15]. Moreover, it is unclear if visfatin exerts its actions through the modulation of pro-inflammatory cytokine and/or its NAD biosynthetic activity [16,17].

Considering the current knowledge, we herein examined the generation of resistin and visfatin in steatotic and non-steatotic livers undergoing PH under I/R. We also characterized adipose tissue as predominant source of both adipocytokines in PH under I/R. Finally, we investigated whether modulating resistin and/or visfatin could protect steatotic and non-steatotic livers against damage and regenerative failure following PH under I/R.

Materials and methods

Experimental animals

Male homozygous obese (*Ob*) (400–450 g) and heterozygous lean (Ln) Zucker rats (350–400 g) and male Wistar rats (200–220 g), fed with a choline-deficient or standard chow diet for 10 days [18], were used. *Ob* Zucker and *Ob* Wistar rats showed severe macrovesicular and microvesicular fatty infiltration in hepatocytes (60–70% steatosis). All procedures were performed under isofluorane anesthesia. This study complied with European Union regulations on animal experiments.

Surgical procedure

The experiments in this study employed experimental models of partial (70%) hepatectomy with or without 60 min of ischemia, as well as 60 min of ischemia without hepatectomy [4,19]. Samples were collected 24 h after completion of the surgical procedure. The experimental groups based on the pharmacological modulation of visfatin and/or resistin, and the different biochemical and molecular analyses are described in Supplementary Materials and methods.

Results

Resistin and visfatin in steatotic and non-steatotic livers from Zucker rats undergoing PH + I/R

In Ln Zucker animals, resistin and visfatin levels in liver, plasma and adipose tissue of the PH + I/R group were similar to those found in the Sham group (Fig. 1A). This is consistent with the finding that pharmacological modulation of either resistin or visfatin (administering these adipocytokines separately or using antibodies against each cytokine) in Ln animals undergoing PH + I/R did not induce changes in either hepatic damage or liver regeneration parameters (data not shown).

In *Ob* Zucker animals, plasmatic and hepatic resistin mRNA and protein levels of the PH + I/R group were higher than in the Sham group (Fig. 1A), whereas resistin levels in adipose tissue were unmodified. In addition, a reduction in visfatin mRNA and protein levels was observed in steatotic livers of the PH + I/R group compared with sham, whereas visfatin levels in plasma and adipose tissue were increased.

Relevance of resistin and visfatin on hepatic damage and regenerative failure in steatotic livers from Zucker rats undergoing PH + I/R

Administration of anti-resistin antibodies (PH + I/R + anti-resistin group) resulted in hepatic damage exacerbation, an increase in transaminase levels, damage score values and the extension of necrosis areas compared with the PH + I/R group (Fig. 1B and Supplementary Fig. 1A). The number of Ki-67-positive hepatocytes (Fig. 1B and Supplementary Fig. 1B), BrdU-positive hepatocytes (Fig. 1B) and mitotic index (data not shown) in the PH + I/R + anti-resistin group were significantly lower than in the PH + I/R group. This decrease in proliferative cells was associated with low HGF and high levels of active TGF-β (Fig. 1B).

Administration of anti-visfatin antibodies (PH + I/R + anti-visfatin group) protected steatotic livers as indicated by the reduction in transaminases, number and extent of necrotic areas and damage score values compared with the PH + I/R group (Fig. 1B and Supplementary Fig. 1A). PH + I/R + anti-visfatin increased percentages of Ki-67-positive hepatocytes and BrdU-positive hepatocytes (Fig. 1B and Supplementary Fig. 1B) and mitotic index (data not shown) in steatotic livers compared with the PH + I/R group. This improvement was associated with high HGF and low TGF-β levels.

Relation between resisitin and visfatin in steatotic livers form Zucker rats undergoing PH + I/R

Fig. 1C shows that endogenous resistin does not modify the hepatic generation of visfatin but prevents its accumulation in the liver from the circulation. Indeed, anti-resistin antibody administration (PH + I/R + anti-resistin group) did not induce changes in the hepatic mRNA levels of visfatin but increased protein levels of visfatin in steatotic livers. Visfatin accumulation was associated with a decrease in visfatin levels in both adipose tissue and plasma compared with the PH + I/R group (Fig. 1C). Removal of adipose tissue in *Ob* animals by surgical interventions (PH + I/R + LPT group) resulted in diminished visfatin levels in plasma compared with the PH + I/R group. In addition, visfatin accumulation in steatotic livers after resistin antibody administration (PH + I/R + anti-resistin group) was not observed when adipose tissue was removed (PH + I/R + LPT + anti-resistin group). Analysis of intrahepatic localization of visfatin demonstrated that it is mainly expressed in hepatocytes, whereas non-apparent immunoreactivity for visfatin was confined to sinusoids and hepatic vessels (Supplementary Fig. 2). Visfatin staining was less evident in steatotic livers of the PH + I/R group compared with the Sham group. However, a more robust visfatin expression in hepatocytes was observed in steatotic livers of PH + I/R + anti-resistin and PH + I/R + visfatin groups when compared with the Sham of PH + I/R groups. Moreover, visfatin was mainly accumulated in hepatocytes of sham-operated *Ob* livers after visfatin administration (data not shown).

Effects of visfatin accumulation on damage and regeneration in steatotic livers from Zucker rats undergoing PH + I/R

Exogenous visfatin was administered to achieve hepatic visfatin protein levels comparable to the PH + I/R + anti-resistin group. Visfatin administration (PH + I/R + visfatin group) significantly exacerbated hepatic injury compared with the PH + I/R group