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Improvement of portal venous pressure in cirrhotic rat livers by systemic treatment with adipose tissue–derived mesenchymal stromal cells

SANDRA BRÜCKNER[1,*], ALEXANDER ZIPPRICH[2,*], MADLEN HEMPEL[1], ANTJE THONIG[2], FABIAN SCHWILL[3], MARTIN RODERFELD[3], ELKE ROEB[3] & BRUNO CHRIST[1]

[1]*Department of Visceral, Transplantation, Thoracic and Vascular Surgery, Applied Molecular Hepatology Laboratory, University Hospital Leipzig, Leipzig, Germany,* [2]*First Department of Internal Medicine, Martin-Luther-University Halle, Halle (Saale), Germany, and* [3]*Department of Gastroenterology, Justus-Liebig-University, Giessen, Germany*

Abstract

Background aims. Portal hypertension is the main cause of complications in cirrhosis caused primarily by extensive fibrosis. Both anti-fibrotic and pro-fibrotic properties of mesenchymal stromal cells (MSCs) have been described in various animal models of liver fibrosis. Therefore, the impact of MSCs on portal hypertension and fibrosis should be investigated in an animal model of liver cirrhosis. *Methods.* The effect of systemic treatment with adipose tissue–derived MSCs, predifferentiated into hepatocytic cells, was investigated in a rat model of liver cirrhosis induced by chronic inhalation of carbon tetrachloride. *Results.* Chronic intoxication with carbon tetrachloride increased the portal venous pressure, which was significantly attenuated by the treatment with MSCs. Consistent with the increase in portal and sinusoidal resistance in the cirrhotic liver, the splenic weight increased, which was again attenuated by the MSCs. The cells had no impact on the spontaneous improvement of liver dysfunction after cessation of treatment with carbon tetrachloride. However, fibrosis was significantly improved as assessed by image quantification of collagen stained with Sirius red. However, hydroxyproline was unchanged indicating that fibrillary collagen content was not affected. That was in line with the finding that the activation of hepatic stellate cells, mainly contributing to excess collagen production in liver cirrhosis, was not affected by the MSCs. The expression of metalloproteinases and their inhibitors did also not change. *Discussion.* It is suggested that hepatocytic differentiated MSCs improved portal blood flow in the cirrhotic liver rather by the physical reestablishment of liver architecture than by biochemical repair.

Key Words: *cell transplantation, liver cirrhosis, mesenchymal stromal cells, portal hypertension, rat model*

Introduction

Cirrhosis is the end-stage of many chronic liver diseases and is characterized by the damage of hepatocytes followed by marked changes in the liver architecture mainly due to deposition of high amounts of fibrotic tissue [1]. The consequences of these changes are loss of metabolic functions and the increase in the intrahepatic vascular resistance. The latter accounts for the main complications of cirrhosis, such as varices and ascites [2]. An ideal treatment of cirrhosis should therefore increase the metabolic capacity and attenuate the pathological increase in portal pressure.

The increase in the intrahepatic vascular resistance is composed of a static and a dynamic component [3]. The static part is mainly the consequence of higher amounts of fibrotic tissue surrounding the sinusoids, which causes reconstruction of the natural basement membrane in the space of Disse, followed by narrowing of the sinusoids. Together with the collapse of sinusoids and enlargement of hepatocytes, these static components are responsible for approximately 70% of the elevated intrahepatic vascular resistance. The remaining 30% are the dynamic part. In cirrhosis, there is an imbalance between vasoconstrictors and vasodilators favoring vasoconstriction [4]. In this

*These authors contributed equally to this work.
Correspondence: **Bruno Christ**, PhD, Department of Visceral, Transplantation, Thoracic and Vascular Surgery, University Hospital Leipzig, Liebigstraße 21, Leipzig, D-04103, Germany. E-mail: bruno.christ@medizin.uni-leipzig.de

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setting, the underlying mechanism is the higher amount of endothelin causing vasoconstriction of the intrahepatic circulation [5]. On the other hand, the decrease of vaso-relaxing factors, which is also reflecting endothelial dysfunction, has been shown in numerous studies in cirrhosis. The main vasodilator is nitric oxide, which in cirrhosis is decreased in the intrahepatic circulation [6]. Having these two mechanisms in mind, a decrease of the intrahepatic vascular resistance could be attained by the decrease of fibrotic tissue and/or improvement of the endothelial dysfunction.

On the cellular level, the activation of hepatic stellate cells (HSCs) by inflammatory macrophages challenged by exposure to chronic nutritional, environmental or medical intoxication has been identified as a key event in the pathogenesis of liver fibrosis characterized by the onset of expression of α-smooth muscle actin (α-SMA) and massive collagen I production and deposition (see Fabregat *et al.*, Lee *et al.*, and Trautwein *et al.* for recent reviews) [7–9]. Myofibroblasts in the liver may originate from bone marrow mesenchymal stromal cells (MSCs), contributing to liver fibrosis [10–14]. Yet the anti-fibrotic features of MSCs have also been well documented in numerous experimental models of liver fibrosis and cirrhosis [15–19]. MSCs attenuated HSC activation as demonstrated by the alleviation of α-SMA and collagen I expression [18,20–22]. In addition to attenuating the activation of HSCs, MSCs also have an impact on inflammation in chronic liver disease, as demonstrated by the inhibition of inflammatory macrophages [23–25], which delineates the well-documented anti-inflammatory mode of action mediated by MSCs in general [26–29]. Although little is known about MSC-mediated effects at the molecular level on liver fibrosis and cirrhosis, it is now clear that these effects are related to paracrine mechanisms, rather than replacement of cirrhotic liver tissue, with MSCs integrating into the host parenchyma and taking over hepatocyte-specific functions. This supports the concept that MSCs, by attenuating inflammatory and fibrotic events in the liver, promote cellular and architectural self-restoration of the liver parenchyma [30–33].

The versatility of MSCs paved the way for clinical treatment of diseases with a pathophysiological background of progressive inflammatory degeneration of tissue integrity, both acute and chronic, including liver diseases [31,34–36]. Postsurgical acute organ failure of liver and kidneys might be ameliorated by MSCs through support of cardiovascular stability [37–41]. As noted earlier, liver cirrhosis is associated with massive tissue deterioration due to fibrotic alterations in conjunction with portal hypertension. It was thus the goal of this study to investigate in a rat model whether systemically administered MSCs might ameliorate hepatic hemodynamic complications associated with carbon tetrachloride–induced liver cirrhosis.

Methods

Animals

All animal experiments were approved by the federal state authority of Saxonia-Anhalt and complied with the Protection of Animals Act. Rats were housed under a 12-h light/dark cycle at an ambient temperature of 25°C and received a standard rodent diet. Wild-type male F344-Fischer rats served as donors of MSCs, which were isolated from peritoneal adipose tissue and differentiated into the hepatocytic lineage for 14 days (hepatocytic differentiated rAT-MSCs) essentially as described previously [42,43]. In brief, rAT-MSCs from rat peritoneal adipose tissue were plated at a density of 200–300 cells/cm^2 on fibronectin-coated dishes. They were grown for 10–14 days until 95% confluence. Starting with a demethylation step using 20 μmol/L of 5′-azacytidine, the hepatocytic differentiation protocol was initiated by the incubation in hepatocyte growth medium containing 20 μg/L EGF and 40 μg/L HGF [44] and continued for 14 days with a medium change every 4 days. Cells were not cultured for 28 days until final differentiation as described in Sgodda *et al.* [43] because we anticipated that a higher degree of differentiation might mitigate the migratory features of the differentiated MSCs, which could be needed for hepatic entry and integration upon systemic delivery via the tail vein. Rats of the same strain bearing a natural mutation in the CD26 gene were used as recipients allowing for the histochemical detection of wild-type donor cells in the CD26-negative host liver background [45].

Experimental schedule

Cirrhosis in the host animals (120 g) was induced by inhalation exposure of carbon tetrachloride (CCl_4) three times a week. In addition, phenobarbital (0.35 g/L) was administered with the drinking water, and treatment continued for 12 weeks to achieve decompensated cirrhosis with concomitant ascites [46,47]. Age-matched untreated rats served as controls. Thereafter, treatment with CCl_4 was terminated, and the cell transplantation experiments commenced. The animals were anesthetized (2% [vol/vol] Isofluran [Baxter], 2 L/min oxygen), and 1.5×10^6 hepatocytic differentiated rAT-MSCs were slowly injected into the tail vein using a 26-gauge cannula (BD Biosciences). Livers were harvested after another 5 days or after 3 and 10 weeks, respectively. Perfusion experiments for the measurement of the portal venous and sinusoidal pressure were carried out 3 weeks after cell transplantation.