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Research Article

Human mesenchymal stem cells towards non-alcoholic steatohepatitis in an immunodeficient mouse model

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ABSTRACT

Non-alcoholic steatohepatitis (NASH) is a frequent clinical picture characterised by hepatic inflammation, lipid accumulation and fibrosis. When untreated, NASH bears a high risk of developing liver cirrhosis and consecutive hepatocellular carcinoma requiring liver transplantation in its end-stage. However, donor organ scarcity has prompted the search for alternatives, of which hepatocyte or stem cell-derived hepatocyte transplantation are regarded auspicious options of treatment. Mesenchymal stem cells (MSC) are able to differentiate into hepatocyte-like cells and thus may represent an alternative cell source to primary hepatocytes. In addition these cells feature anti-inflammatory and pro-regenerative characteristics, which might favour liver recovery from NASH. The aim of this study was to investigate the potential benefit of hepatocyte-like cells derived from human bone marrow MSC in a mouse model of diet-induced NASH. Seven days post-transplant, human hepatocyte-like cells were found in the mouse liver parenchyma. Triglyceride depositions were lowered in the liver but restored to normal in the blood. Hepatic inflammation was attenuated as verified by decreased expression of the acute phase protein serum amyloid A, inflammation-associated markers (e.g. lipocalin 2), as well as the pro-inflammatory cytokine TNFα. Moreover, the proliferation of host hepatocytes that indicate the regenerative capacity in livers receiving cell transplants was enhanced. Transplantation of MSC-derived human hepatocyte-like cells corrects NASH in mice by restoring triglyceride depositions, reducing inflammation and augmenting the regenerative capacity of the liver.

Abbreviations: α-SMA, alpha-smooth muscle actin; ALT, alanine aminotransferase; DAB, 3,3′-diaminobenzidine; ECL, enhanced chemiluminescence; GS, glutamine synthase; hMSC, human mesenchymal stem cells; HRP, horse radish peroxidase; LCN2, Lipocalin 2; MCD, methionine- and choline-deficient; MTTP, microsomal triglyceride transfer protein; NAFLD, non-alcoholic fatty liver diseases; NASH, non-alcoholic steatohepatitis; SAA, serum amyloid A; TNFα, tumor necrosis factor alpha

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Introduction

Overweight and adipositas increasingly become major problems of the health care systems of "western lifestyle" countries. They are often associated with non-alcoholic fatty liver disease (NAFLD) [1,2]. If accompanied by inflammation it develops into steatohepatitis (NASH), one of the most abundant forms of chronic liver diseases. NASH is highly associated with metabolic diseases like obesity, type 2 diabetes and the metabolic syndrome [3]. Hepatic inflammation, massive lipid accumulation concomitant with hepatocyte apoptosis and necrosis as well as fibrosis are the histological hallmarks [2–5]. The disease may progress into liver cirrhosis and finally hepatocellular cancer, and is itself a cardiovascular risk factor [6]. About 14% of patients suffering from NASH end up in liver failure with liver transplantation as the only therapeutic option [1]. However, orthotopic liver transplantation carries risks associated with the surgical procedure and the post-transplant immunosuppression, causes high costs and in addition is hampered by a serious donor organ shortage. Hence, feasible alternatives are needed [7–9]. Hepatocyte transplantation has long been regarded as a therapeutic way out. Yet, primary human hepatocytes may only be obtained from cadaveric donors and are often of marginal quality [7,10]. Applying specified differentiation and culture conditions, adult mesenchymal stem cells (MSC) derived from bone marrow or adipose tissue proved to be an alternative cell source to generate hepatocyte-like cells [11,12]. Besides their multiple differentiation potential, MSC feature immune-modulatory, anti-inflammatory and -apoptotic as well as pro-proliferative characteristics [13–17]. Hence, we hypothesized that hepatocyte-like cells generated from MSC might attenuate the pathological changes associated with NASH. Indeed, inflammation, lipid accumulation, fibrosis and regeneration improved in an immunodeficient mouse model of diet-induced NASH upon transplantation of human bone marrow-derived MSC.

Material and methods

Animals and experimental design

Immunodeficient male Pfp/Rag2$^{-/-}$ mice (Taconic; Ejby, Denmark) were housed under standard conditions. At the age of 12–14 weeks animals were randomly divided into two main experimental groups (Supplemental Fig. 1). After 7 days of diet customisation, one group (+NASH, $n=14$) was fed exclusively with a methionine-choline-deficient (MCD) diet (MP Biomedicals, Eschwege, Germany) for 35 days while the control group received a standard rodent diet for the whole period of time (−NASH, $n=10$). After the total feeding period of 42 days all animals underwent 1/3 partial hepatectomy and each of these main groups were subdivided into two groups either receiving human MSC (+hMSC) or PBS instead (−hMSC). All +NASH mice received the MCD diet until the end of the experiments. Blood and liver samples were collected 1 and 7 days after cell transplantation. Livers were stored at −80 °C until further analyses. All animal experiments were approved by the regulatory authorities of the City of Halle, Germany, and the animal protection commissary of the University of Halle-Wittenberg, Germany.

Biochemical analyses

The activity of serum alanine aminotransferase (ALT) and serum triglycerides were measured by the Central laboratory of the University Hospital and Clinics of Halle using standard analytics. Hepatic triglycerides were determined as described previously [18].

Semi-quantitative RT-PCR

Snap-frozen liver samples were used for extraction of total RNA according to the procedure as described [18]. The expression of the housekeeping gene cyclophilin A was used to normalise the expression of the mRNA species of interest. Primer pairs and corresponding experimental settings to analyze expression of serum amyloid A (SAA), tumor necrosis factor alpha (TNFα), alpha-smooth muscle actin (α-SMA) and collagen type I were as described [18] and the Image J 1.42 software (National Institute of Health, Maryland, USA) was used for quantification.

Western blot analyses

Liver tissue lysates were prepared using RIPA buffer containing 20 mM Tris–HCl (pH 7.2), 150 mM NaCl, 2% (w/v) NP-40, 0.1% (w/v) SDS, 0.5% (w/v) sodium deoxycholate and the Complete™-mixture of proteinase inhibitors (Roche, Mannheim, Germany). Equal amounts of cellular protein extracts or supernatants were diluted with Nu-PAGE™ LDS electrophoresis sample buffer with DTT as reducing agent, heated at 95 °C for 10 min, and separated in 4–12% Bis-Tris gradient gels and 10% Bis-Tris gels, using MOPS or MES running buffer (Invitrogen, Darmstadt, Germany). Proteins were electroblotted on nitrocellulose membranes, and equal loading was confirmed with the Ponceau S stain. Subsequently, non-specific binding sites were blocked in TBS containing 5% (w/v) non-fat milk powder. All antibodies (Supplemental Table 1) were diluted in 2.5% (w/v) non-fat milk powder in Tris-buffered saline. Primary antibodies were visualized using horseradish peroxidase conjugated anti-mouse-, anti-rabbit- or anti-goat IgG (Santa Cruz Biotech, Santa Cruz, CA) and the SuperSignal chemiluminescent substrate (Pierce Thermo Fisher, Bonn, Germany). The anti-MTTP antibody was visualized on X-ray films using the ECL reagent (GE Healthcare, Buckinghamshire, UK) and quantified with the Image J 1.42 (National Institute of Health, Maryland, USA).

Histological analyses

Histology was performed using paraffin- or cryo-sections. Changes in liver architecture were assessed by haemalaun-eosin and lipid accumulation and steatosis by Sudan III staining. Fibrosis was assessed by staining with Sirius red in combination with polarisation microscopy [18] or by probing with a specific antibody directed against collagen type I and α-SMA immunofluorescence staining as described [19,20]. The expression of HepPar1, glutamine synthase (GS) and cell proliferation marker Ki-67 was analyzed by immunohistochemical staining on paraffin sections. Slices for the detection of HepPar1 and GS were processed as described [18]. For detection of Ki-67 an additional avidin-biotin block was used to avoid non-specific background reactions. The anti-Ki-67 (1:200; Abcam ab66155, Cambridge, UK) antibody was added in a 1% BSA solution and slices were incubated overnight at 4 °C in a humidified incubation box. 0.5% Of the