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Augmenter of liver regeneration improves therapeutic effect of hepatocyte homotransplantation in acute liver failure rats

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ABSTRACT

Hepatocyte transplantation (HCT) is an available option on treatment for acute liver failure (ALF). However, short-term survival of engraftment and immunological rejections of recipient are major obstacles. Augmenter of liver regeneration (ALR) has cytoprotective and immunoregulatory effects in liver injury, and has been used in many experimental applications. In the present study, we investigated the potential effect and mechanism of recombinant human ALR (rhALR) on ALF rats treated with intraperitoneal HCT. ALF rats induced by D-galactosamine (GalN) were studied in vivo, and were intraperitoneal injected with or without hepatocytes and rhALR 24 h after the induction. Animal survival, serum and ascites liver enzymes, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were assessed. Histological examination was performed, and liver regeneration, apoptosis and immunological responses were identified by immunohistochemistry assay. Our results showed that rhALR promoted hepatocytes regeneration, attenuated liver injury and suppressed immunological responses. The ascites liver enzyme, serum and ascites pro-inflammatory cytokines (TNF-α, IL-1β), liver histological injury, apoptotic hepatocytes and activated immunocytes were significantly reduced in ALF rats treated with rhALR and HCT compared with those without rhALR. The proliferative and mitotic hepatocytes were markedly increased, and overall survival improved with rhALR. The administration of rhALR improved survival and promoted liver recovery in HCT treatment for ALF, which was associated with the role of proliferative promoter and immunosuppressor. This study suggests that co-treated with rhALR and HCT can provide a promising strategy for the treatment of ALF.

1. Introduction

Acute liver failure (ALF) is a catastrophic clinicopathological condition with a high mortality rate [1]. Orthotopic living donor liver transplantation is the most effective treatment receiving increasing attention in recent years [2,3]. However, the lack of available livers to use for orthotopic liver transplantation (OLT) is one of the major obstacles [4,5]. Considering the vigorous regeneration capacity of the hepatocytes, several techniques could ideally serve as a bridge to transplantation or liver regeneration in ALF patients during the waiting time [6]. According to recent research and clinical results, the utilization of hepatocyte transplantation (HCT) has been proved safe and supportive [7,8].

HCT has a number of potential advantages compared with OLT [9]. There is lower risk of morbidity and mortality in the less invasive surgical approach. Importantly, the procedure overcomes the limitation of liver source. Enough hepatocytes can be isolated from one donor liver to provide for several recipients on the waiting list. In contrast to whole liver, hepatocytes can be cryopreserved and stored according to a specified protocol until needed; this progress makes HCT especially available and attractive for patients with ALF without a whole organ [10]. Besides the cell source, the anatomical site for cell implantation is another factor of the treatment [11]. Various sites have been investigated by different research teams, but the conclusion is still controversial. The spleen is considered as the most privileged site for HCT, but the risks associated with coagulation disorder and portal vein thrombosis are obvious [12]. On the other hand, peritoneal cavity is a promising site as it can be accessed by minimally invasive approach and allows a large number of cells [13]. Recently, HCT into the peritoneal cavity has been used in patients with ALF, but survival time of the engraftment and immunological rejection are still major problems.

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Augmenter of liver regeneration (ALR), a vital cytokine of growth factor, originally identified in the soluble fractions of hypertrophic rat livers, was purified from weanling rat liver, and its gene cloned in rat, mouse and human [14]. The mitogenic and anti-atrophic activities of native and cloned ALR were demonstrated in partially hepatectomized rats and dogs with portacaval shunt [15]. ALR also inhibits the lytic activity of hepatic natural killer cells, which is critical for unabated liver regeneration [16]. Moreover, ALR not only enhances liver regeneration and hepatocytes proliferation as well-known, but also has been demonstrated an anti-apoptosis effect on hepatocytes recently [17].

In this study, we explored whether recombinant human ALR (rhALR) could improve the therapeutic effect of intraperitoneal HCT in ALF rats by promoting regeneration and suppressing excessive immune-inflammatory responses.

2. Materials and methods

2.1. Animals

Syngenic male Sprague–Dawley (S–D) rats (10–12 weeks of age, weighing 170–230 g) were maintained at the Animal Center of Chongqing Medical University and used as the experimental animals for HCT. Rats were placed in cages within a temperature-controlled room with a 12-hour light/dark cycle and ad libitum access to food and water in the animal facilities. All animal procedures were conducted in accordance with the guidelines approved by the China Association of Laboratory Animal Care.

2.2. Hepatocyte isolation

Under general anesthesia, the S–D rats (Chongqing Medical University, Chongqing, China) weighing about 200 g, underwent abdominal "U" incision for hepatocyte isolation. Hepatocytes were isolated with an improved two-step semi-situ recirculating collagenase perfusion method as previously described [18]. After isolation, the cells were resuspended with DMEM medium (Sigma), and the cell purity was determined by hematoxylin and eosin (H&E) stain, then the cell viability was determined by trypan blue exclusion test. In our experiments, we only used isolated hepatocytes if the purity and viability exceeded 95% and 90%, respectively.

2.3. Experimental ALF rats model and transplantation experiments

Rats with ALF were induced by an intraperitoneal injection with 1.0 g/kg 10% D-galactosamine (GalN) [19]. On day 1 after the induction, rats with ALF were divided into the following four groups: (i) physiological saline (PS): keeping intraperitoneal injecting of PS for 6 days ($n=15$), (ii) HCT: intraperitoneal transplantation of 2×10^7 hepatocytes, and keeping injecting PS in the following 5 days ($n=15$), (iii) HCT and rhALR (HCT + rhALR): intraperitoneal transplantation of 2×10^7 hepatocytes and rhALR (50 μg/kg), and keeping injecting rhALR in the following 5 days ($n=15$), and (iv) rhALR: keeping intraperitoneal injection of rhALR (50 μg/kg) for 6 days without hepatocytes ($n=14$). All the solutions were intraperitoneal injected in a volume of 1 ml on day 1 including the hepatocytes, and 0.5 ml on the following 5 days. Rats were evaluated every 8 hours and killed if they appeared moribund. RhALR has been produced by our Laboratory of Institute for Viral Hepatitis (Chongqing Medical University, Chongqing, China) [20].

2.4. Survival study

All rats in each group were used for the survival study. Rats that had lived for more than 3 days after induction to ALF were considered to survive.

2.5. Collection of body fluid samples and tissue specimens

Rats were killed before natural death or on day 14 post transplantation. To detect the biochemistry and cytokines level of orthotopic and intraperitoneal hepatocytes, blood and ascites samples were collected on days 2 and 14 after transplantation. Orthotopic liver tissues and intraperitoneal transplanted hepatocyte islets were fixed and processed for histology and immunohistochemistry. The specimens of greater omentum wrapped the islets were excised and processed for immunology analysis.

2.6. Measurement of hepatic enzymes and cytokine levels after cell transplantation

The serum and ascites levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured with an automatic biochemical analyzer (Olympus, Japan). The serum and ascites levels of tumor necrosis factor (TNF-α) and interleukin-1β (IL-1β) were detected using commercially available ELISA kits according to the manufacturer's instructions (RD Biosciences, USA).

2.7. Histological examination

The tissue specimens obtained from the rats were harvested and fixed with 10% buffered formalin, embedded in paraffin and processed for staining with H&E and periodic acid Schiff (PAS).

2.8. Measurement of proliferation, apoptosis and cytoimmunity by immunohistochemical assay

Paraffin sections of hepatic and greater omentum tissues were prepared to determine the expressions of proliferating cell nuclear antigen (PCNA), CD68, CD4, and CD8 using immunohistochemical assay. The sections were incubated overnight with anti-PCNA, CD68 (1:100, AbD Serotec, UK), CD4 and CD8 antibodies (1:200, AbD Serotec, UK) as a

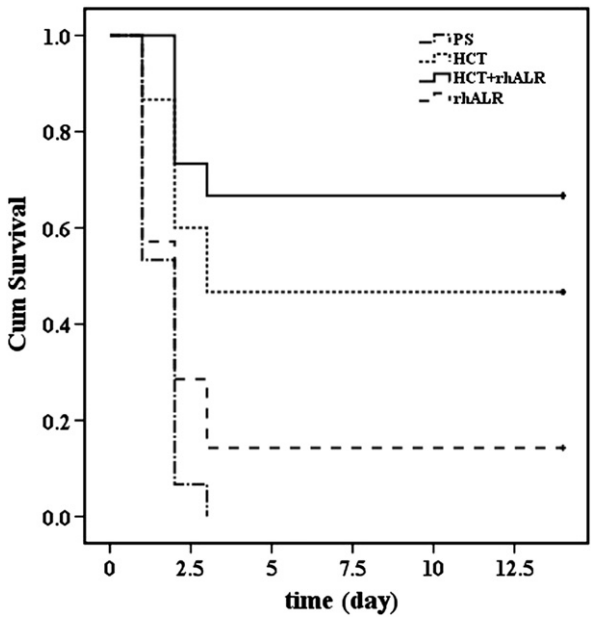

Fig. 1. Kaplan–Meier plot showing the survival of ALF after various treatments. PS, physiological saline ($n=15$); HCT, hepatocyte transplantation ($n=15$); HCT + rhALR, hepatocyte transplantation combined with rhALR ($n=15$); rhALR, injection of rhALR alone ($n=14$).