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Original Article

Hepatic cell sheets engineered from human mesenchymal stem cells with a single small molecule compound IC-2 ameliorate acute liver injury in mice

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ABSTRACT

Introduction: We previously reported that transplantation of hepatic cell sheets from human bone marrow-derived mesenchymal stem cells (BM-MSCs) with hexachlorophene, a Wnt/β-catenin signaling inhibitor, ameliorated acute liver injury. In a further previous report, we identified IC-2, a newly synthesized derivative of the Wnt/β-catenin signaling inhibitor ICG-001, as a potent inducer of hepatic differentiation of BM-MSCs.
Methods: We manufactured hepatic cell sheets by engineering from human BM-MSCs using the single small molecule IC-2. The therapeutic potential of IC-2-induced hepatic cell sheets was assessed by transplantation of IC-2- and hexachlorophene-treated hepatic cell sheets using a mouse model of acute liver injury.
Results: Significant improvement of liver injury was elicited by the IC-2-treated hepatic cell sheets. The expression of complement C3 was enhanced by IC-2, followed by prominent hepatocyte proliferation stimulated through the activation of NF-κB and its downstream molecule STAT-3. Indeed, IC-2 also enhanced the expression of amphiregulin, resulting in the activation of the EGFR pathway and further stimulation of hepatocyte proliferation. As another important therapeutic mechanism, we revealed prominent reduction of oxidative stress mediated through upregulation of the thioredoxin (TRX) system by IC-2-treated hepatic cell sheets. The effects mediated by IC-2-treated sheets were superior compared with those mediated by hexachlorophene-treated sheets.
Conclusion: The single compound IC-2 induced hepatic cell sheets that possess potent regeneration capacity and ameliorate acute liver injury.

Abbreviations: BM-MSCs, bone marrow-derived mesenchymal stem cells; NF-κB, nuclear factor-kappa B; IL-6, interleukin-6; STAT-3, Signal Tranducer and Activator of Transcription 3; EGFR, epidermal growth factor receptor; *AREG*, amphiregulin; CBP, CREB-binding protein; DMSO, dimethyl sulfoxide; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCl_4, carbon tetrachloride; PCNA, proliferating cell nuclear antigen; *A1AT*, α1-antitrypsin; *CP*, ceruloplasmin; *TF*, transferrin; *APOE*, apolipoprotein E; *C4A*, complement C4A; *RBP4*, retinol binding protein 4; *C3*, complement C3; *hGAPDH*, human glyceraldehyde 3-phosphate dehydrogenase; *mActb*, mouse actin, beta; C5aR, complement C5a receptor; HB-EGF, heparin binding-epidermal growth factor-like growth factor; TGFα, transforming growth factor alpha; ERK, extracellular signal-regulated kinase; 8-OHdG, 8-hydroxydeoxyguanosine; SOD, superoxide dismutase; GPX, glutathione peroxidase; GSH, glutathione; TRX, thioredoxin; TRXR, thioredoxin reductase; PRX, peroxiredoxin; GRX, glutaredoxin; GR, Glutathione reductase; MDA, malondialdehyde; TNFα, tumor necrosis factor alpha; LXR, liver X receptor; HGFR, hepatocyte growth factor receptor; ChoREs, carbohydrate response elements; ChREBP, Carbohydrate-responsive element-binding protein; IL-1ra, interleukin-1 receptor antagonist.

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1. Introduction

Liver transplantation is the best therapeutic treatment for acute as well as chronic liver failure. However, liver transplantation cannot be successfully applied to all patients with liver failure because of donor scarcity [1]. Although hepatocyte transplantation has been performed as an alternative or a bridge approach to liver transplantation, the results of hepatocyte transplantation are not satisfactory [1]. Stem cell therapy is regarded as an attractive therapy for liver disease [2,3]. In particular, mesenchymal stem cells (MSCs) are emerging as a promising cell source for treatment of acute liver failure and cirrhosis [3,4].

We previously reported that orthotopic transplantation of hepatic cell sheets derived from human MSCs ameliorated carbon tetrachloride (CCl_4)-induced acute liver injury [5]. These hepatic cell sheets were originally developed by fusion of two independent technologies: the first is the chemical agent that suppresses the Wnt/β-catenin signal enhancing hepatic differentiation of MSCs, and the second is cell sheets manufactured on thermoresponsive, polymer-coated culture dishes. We previously identified hexachlorophene, a Wnt/β-catenin signal inhibitor, as an inducer of hepatic differentiation in human MSCs [5]. However, in antiseptic use except for anti-bacterial ingredient of medical soap, this compound was forbidden by Food and Drug Administration (FDA), more recently [6] because of its toxicity [7]. To accomplish hepatic cell sheets in clinical use, we need to identify more effective small molecule compounds to permit clinical application. To resolve this issue, we previously screened a library of synthesized, novel, small molecules. And of many small molecule variants of Wnt/β-catenin signal inhibitors, we identified IC-2, a derivative of ICG-001, as a more effective inducer of human MSCs toward hepatic differentiation [8]. IC-2 induces the expression of hepatocyte differentiation markers in bone marrow-derived MSCs (BM-MSCs) (e.g., Albumin and tryptophan 2,3-dioxygenase) most strongly among the screened 23 compounds and induces not only gene expression but also hepatocyte function (e.g., urea production and glycogen storage) [8]. ICG-001 has been reported to specifically downregulate the Wnt/β-catenin signal pathway through inhibiting the interaction of β-catenin with CBP [9,10]. Moreover, ICG-001-derived compound PRI-724 has proven to have acceptable toxicity in a phase I clinical study [11,12]. We reported that hexachlorophene-treated BM-MSC sheets were superior in ameliorating acute liver injury compared to non-treated BM-MSC sheets [5]. In the present study, we assessed the potential of IC-2-treated cell sheets derived from BM-MSCs in comparison with hexachlorophene-treated sheets in an acute model of liver injury. Consequently, we found that engineered cell sheets using the single small molecule IC-2 have a strong protective effect on acute liver injury.

2. Materials and methods

2.1. Wnt/β-catenin signal inhibitors and cells

Hexachlorophene was purchased from Sigma–Aldrich (St. Louis, MO, USA). IC-2 was synthesized in-house. Both inhibitors were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in media was 0.1%. UE7T-13 human bone marrow-derived mesenchymal stem cells (BM-MSCs) were used [5,8]. In other experiments, primary MSCs were prepared using human bone marrow mononuclear cells purchased from Lonza Walkersville, Inc. (Walkersville, MD, USA) as previously described [8].

2.2. Production of Wnt/β-catenin signal inhibitor-treated hepatic cell sheets derived from mesenchymal stem cell sheets

Hexachlorophene-treated cell sheets were manufactured as previously described [5]. IC-2-treated cell sheets were prepared under the same conditions except for a starting cell density of 3.6×10^4 cells/cm^2. The final number of cells per sheet in following the IC-2-treatment was similar to that after hexachlorophene treatment (Supplemental Fig. 1). The concentration of inhibitor was 0.8 and 15 μM for hexachlorophene and IC-2, respectively. Both cell sheets were harvested from φ60-mm-temperature-responsive culture dishes (CellSeed Inc., Tokyo, Japan) at a temperature of 20 °C for 15–30 min. Primary BM-MSC sheets with or without IC-2 treatment were prepared as follows: $CD90^+/CD271^+$ MSCs were fractioned from human bone marrow mononuclear cells (Lonza Inc.) and expanded as previously described [8]. At passage number five, cells were plated onto φ60-mm-temperature-responsive culture dishes (CellSeed Inc.) at a density of 1.8×10^4 cells/cm^2. One day and 5 days after seeding, medium was replaced with DMEM (Life Technologies Corp., Carlsbad, CA) containing 10% fetal bovine serum (GE Healthcare UK Ltd, Little Chalfont, UK), 100 U/ml penicillin, and 100 mg/ml streptomycin with 30 μM IC-2. Primary BM-MSC sheets without IC-2 were prepared 4 days before harvest at the same cell density as IC-2-treated primary BM-MSC sheets.

2.3. Animal experiments

All experiments were conducted in accordance with the ethical approval of the Tottori University Subcommittee on Laboratory Animal Care. Eight-week-old NOD-SCID mice (CLEA Japan, Inc., Tokyo, Japan) were transplanted with a total of six cell sheets at two sites on the liver surface as previously described [5]. Control mice received sham laparotomy. Mice receiving carbon tetrachloride were administrated with 0.24 ml/kg (Wako Pure Chemical Industries Ltd., Osaka, Japan) dissolved in olive oil at 5% concentration by oral gavage at 1 day after transplantation. Two days after transplantation, blood samples were collected from the retro-orbital venous plexus under anesthesia with pentobarbital sodium (Kyoritsu Seiyaku Corp., Tokyo, Japan). On 2, 3, 4, and 8 days after transplantation, mice were sacrificed by exsanguination under anesthesia with pentobarbital sodium, and blood samples were collected from the inferior vena cava followed by liver resection. All mice were housed under pathogen-free conditions in a temperature-controlled room and illuminated (12 h daily) with *ad libitum* access to water and chow.

2.4. Biochemical tests

Blood samples were kept overnight on ice, and the serum was isolated by centrifugation at 2,000 g for 20 min. Serum aminotransferases and total bilirubin were measured as previously reported [5].

2.5. RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA from the liver was extracted with TRIzol reagent (Life Technologies Corp.) and subjected to reverse transcription using Superscript II (Life Technologies Corp.) with oligo$(dT)_{18}$ primers. RT-PCR was performed using gene specific primers and rTaq DNA polymerase (TOYOBO CO., Ltd. Osaka, Japan). Primers used in the present study were the same as described in our previous report [5].