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Directly injected native bone-marrow stem cells cannot incorporate into acetaminophen-induced liver injury

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ABSTRACT

The paucity of liver donation highlights the use of cell-based strategies for end-stage liver failure. We recently showed that *b*one *m*arrow-*d*erived *a*ggregates (BMDAs) can completely restore the hematopoietic system in gamma-irradiated mice. These aggregates are stem and progenitor cells in the *b*one *m*arrow (BM), composed of both hematopoietic and non-hematopoietic lineages. Furthermore, reports showed that resident BM cells migrate to the liver and integrate themselves into the tissue in small numbers. Hence, we hypothesized that direct delivery of BMDAs to the damaged liver might enhance the integration of BM cells in the liver because of its stemness property, intact BM architecture, the physical proximity of these niche-like structures to the damaged sites and the existence of liver paracrine factors. To this aim, we made an acute liver model by intraperitoneal injection of acetaminophen. Then, GFP-expressing BMDAs were intrahepatically injected. Despite the detection of GFP-expressing cells five days after intrahepatic injection, these cells were not detectable at days 15 and 60, indicating that the puzzle of BM cell integration in the liver still has more missing pieces other than stemness, physical proximity, and paracrine factors. Actually, it seems that even intact BM structures need further signals to be qualified for integration.

1. Introduction

Liver transplantation is the ultimate therapy for many patients who are suffering from end-stage liver failure. However, the paucity of liver donation along with long-term side effects severely hamper the solution [1]. Hence, there is an urgent need to implement cell-based therapeutic strategies to ameliorate the severity of the disease.

Reports have shown that resident *b*one *m*arrow *s*tem *c*ells (BMSCs) in mice and humans can migrate and participate in the liver's cellular structure in small numbers under physiological condition [2,3]. This phenomenon is routinely referred as Bone Marrow Hepatic axis (BM-Hepatic axis). These findings have led to the concept of transplanting different subpopulations of BMSCs such as *h*ematopoietic *s*tem *c*ells (HSCs) [4,5], *m*esenchymal *s*tem *c*ells (MSCs) [6,7] and *e*ndothelial *p*rogenitor *c*ells (EPCs) [8] to replenish damaged liver tissue. However, there is little evidence that these BMSC subpopulations have stable or long-lasting engraftment, nor could they make hepatocytes at a level of clinical significance [9]. Actually, this low efficacy has been mainly attributed to the loss of BM cell characteristics during their *ex vivo* or *in vitro* preparations [9]. Long-term *in vitro* expansion of MSCs or forcing HSCs to leave BM causes unnatural alterations in cell phenotypes [10], homing markers [11], proliferation [12] and unwanted self-differentiation [13]. These expansion procedures are inevitably done to increase the number of transplantable cells. Hence, non-efficacious engraftment of BMSC subpopulations in many reported studies might be originated from aforementioned changes during cell expansion.

Recently, we demonstrated that *b*one *m*arrow-*d*erived *a*ggregates (BMDAs) are able to completely reconstitute bone marrow's stromal and hematopoietic system in gamma-irradiated mice when peritoneally transplanted [14,15]. These aggregates are intact niche like structures in bone marrow, composed of primitive progenitor cells of both hematopoietic and stromal tissues.

Based on the aforementioned data, we hypothesized that it would be possible to promote the integration rate of BMSCs in the damaged liver by direct injection of BMDAs. Actually, intact BM architecture of the aggregates, their stemness state, their physical proximity to the damaged sites along with the existence of high concentrations of liver paracrine factors were rationally supported the hypothesis. To this aim,

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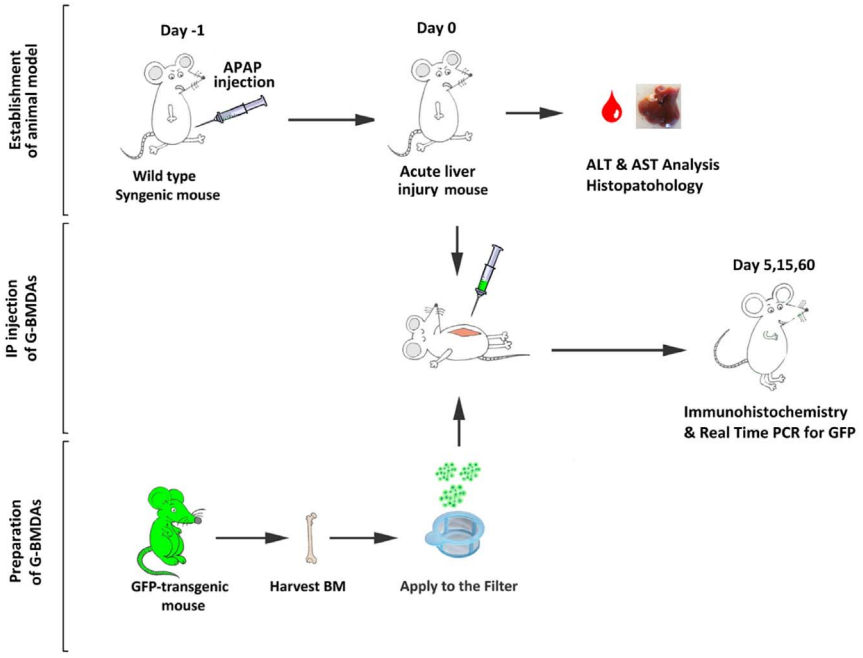

Fig. 1. Schematic view of the study design. Study design has three main parts consisting of 1) preparation of GFP-Bone Marrow Derived Aggregates "G-BMDAs" 2) establishment of acute liver disease model by the use of acetaminophen and 3) intra parenchymal injection of G-BMDAs into median lobe of liver tissue. Different experimental procedures in each segment of the study design are indicated in the picture.

acute liver disease model was primarily prepared by intraperitoneal injection of acetaminophen to wild-type C57BL/6 female mice. Subsequently, BMDAs from GFP positive male C57BL/6 mice (G-BMDAs) were prepared and then intrahepatically injected into the mouse model of acute liver disease. A schematic representation of the workflow has been shown in Fig. 1.

2. Materials and methods

2.1. Animals

C57BL/6 mice were purchased from Pasteur Institute of Iran (Tehran-Iran). GFP-transgenic mice were kindly provided by Masaru Okabe (Genome Research Center, Osaka University, Japan). Eight to ten week-old mice were used for this study. Animal care and experiments were based on the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All the protocols were approved by the ethics committee of Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran.

2.2. Establishment of acute liver disease animal model

C57BL/6 *female* mice (20–22 g) were used to make the model of acute liver disease. Briefly, after 12 h starvation 750 mg/kg acetaminophen **(**N-Acetyl-Para-Amino-Phenol (APAP) (Apotel-Uni Pharma-Greece**)** was intraperitoneally injected (n = 12). The damage to the liver was subsequently verified by sacrificing 9 survived mice after 24 h. To this aim, we measured the plasma amount of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) along with histopathological inspection of the liver tissue with H&E staining. ALT and AST were measured using Roche/Hitachi 902 (Roche Diagnostics). The degree of liver damage was validated by pathological inspection under a light microscope (Olympus, Hamburg, Germany).

2.3. Preparation of GFP-bone marrow-derived aggregates (G-BMDAs)

GFP-transgenic C57BL/6 *male* mice were scarified by cervical dislocation. As previously described, the distal end of the tibia and femur bones (the total of four bones from each mouse) were uncapped to drain the BM content by centrifugation at 600*g* for 1 min [12,16]. Then, the resulting cell plate of each 10 mice was washed with 5 mL heparinized phosphate buffered saline (PBS; Gibco-BRL, Grand Island, NY), and subsequently filtered through a cell strainer of about 20 mm **(**adapted by cutting a 40 mm nylon mesh (BD Biosciences, San Jose, CA) and putting two pieces on top of each other**)**. Filter-retained cell aggregates were resuspended in 5 mL PBS and then 0.5 ml of the cocktail was transplanted into each recipient mouse. Parallely, a small portion of the prepared BMDAs was cultured in DMEM +10% FBS to check the viability of the cells. Radial expansion of cells from BMDAs was indicative of their viability. This approach resulted in the transplantation of the BM content of one GFP mouse to one non-GFP recipient counterpart.

2.4. liver's intraparenchymal injection of G-BMDAs

Out of 42 female C57BL/6 mice 27 ones survived 24 h after the induction of damage (APAP induction). The survived mice were given either G-BMDAs (n = 18) or PBS (n = 9) as test and corresponding control, respectively. All operations were performed under anesthesia with 10 mg/ml ketamine and 2 mg/ml xylazine in PBS. A longitudinal 1 cm abdominal incision was made to expose the liver. 0.5 ml suspension of G-BMDAs was directly injected into four different points of the median lobe of the liver's parenchyma. Then, the animals were sutured in layers with Nylon Monofilament 4-0 (Monocryl Ethicon, SP Brazil) and allowed to recover in the cage. Control group was submitted to the same surgical procedure, although receiving 0.5 mL PBS.

2.5. GFP tracking

The mice with G-BMDAs injection (n = 6/time point) and its corresponding control (n = 3/time point) were sacrificed on 5,15 and 60 day time points post cell transplantation.

2.5.1. Immunohistochemistry

Immunohistochemistry was performed as previously described [17]. To increase the accuracy and sensitivity of our pathologic validation step, we processed 12 sections for each positive and negative tissue while all microscopic fields were carefully inspected by an expert investigator. Furthermore, to determine the integration potency of G-BMDAs in the liver parenchyma of APAP-induced acute liver failure, we analyzed serial sections of whole median lobe by