Use of Bone Marrow-Derived Mesenchymal Stem Cells in Improving Thioacetamide Induced Liver Fibrosis in Rats

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Abstract Liver fibrosis, is one of big problems usually ends with cirrhosis which considered a life threatening disease as the only way of treatment is the liver transplantation, this study aimed to find a new way for fibrosis treatment by the use of bone marrow isolated Mesenchymal stem cells (MSCs). Thioacetamide (TAA) was used for fibrosis induction in male Sprague Dawely (SD) rats which divided into two random groups: group infused with TAA for fibrosis induction and group as control negative group. MSCs were isolated from bone marrow of twenty five (4–5) weeks male SD rats, and labeled with fluorescent material (PKH26) to confirm the homing of cells. After fibrosis induction, rats were divided into four subgroups to study the effect of MSCs injection in fibrosis treatment. After 4 weeks from MSCs administration, all rats were sacrificed. Liver tissue were collected for histopathological and immunohistopathological studies. In comparison with control groups, the treated groups with MSCs showed improvement in the amount of deposited collagen which decreased compared to control positive group. So MSCs can be used to replace liver transplantation in the treatment of fibrosis.

1 Introduction

Liver fibrosis is the healing response of the liver to chronic injury. Subsequent of repeated injury, the liver undergoes a tissue remodeling and ends with cirrhosis. It is characterized by excessive accumulation of extracellular matrix, with the formation of scar tissue encapsulating the area of injury [1]. The key finding was the identification of activated hepatic stellate cells (HSCs) as the major source of ECM in the fibrotic liver. Activation of HSCs is currently considered the critical common step in liver fibrosis. Although liver transplantation is a good alternative treatment, there are limited available donor livers for hundreds of millions of patients worldwide. So, it is

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very important to investigate appropriate therapies for the disease by different treatments. Mesenchymal stem cell (MSC) which is an adult type of stem cells is considered as an attractive cell source for regenerative medicine. MSCs have the capacity to differentiate into hepatocytes in vitro and in vivo. Further, MSCs administration could repair injured liver by reducing inflammation, collagen deposition and remodeling. So MSCs could not only have a possibility to repair acute damaged tissue but also have potentiality of reducing chronic fibrosis [2].

2 Materials and Methods

Preparing for BM-derived MSCs in rats as mentioned by [3, 4].

Fibrosis induction by using TAA Sixty male SD rats were acclimatized for 7 days and randomly divided into two groups, Group (A): include 50 rat, Injected intraperitoneally with 200 mg\kg sterile thioacetamide (TAA) (Sigma, USA) with 5 % concentration twice weekly for 3 months [5]. Group (B): include 10 rats, as control negative group. The progression of fibrosis was demonstrated by scarification of three animals from group (A) and one from group (B) per month; in addition the mortality rate was 8 % in group (A). After 3 months, group (A) was subdivided into subgroups as described into Table 1. Then all animals were sacrificed after 1 month.

Clinical examination and Animal weighting the animals were regularly observed, and recording of any abnormal clinical signs. The animals were fastened overnight before the injection and the scarification time. Weighting with recording body weight before injection were done every week [6].

Postmortum and Histopathological Examination the sacrificed animals were subjected to careful postmortem examination. Liver tissues were fixed in 10 % buffered neutral formalin. Samples were routinely processed as paraffin embedded sections at 2–4 μ thickness; the prepared slides were stained by H&E, Masson trichrome stain and immunohistochemistery staining.

Fluorescent Microscope Examination Fluorescent microscope used for detection of marked stem cells in liver tissue to ensure homing of cells which were labeled with PKH26 [6].

Table 1 Shows the experimental design (effect of cells after TAA)

Main group	Sub-group	Animals no.	Treatment
(A)	(1)	10	Injected with TAA for 3 months only and treated with cells
	(2)	10	Injected with TAA for 4 months and treated with cells
	(3)	10	Control positive group
	(4)	7	Injected with TAA for 3 months only
(B)	(5)	7	Control negative group

Immunohistochemical Staining Immunohistochemical staining was performed to evaluate staining intensity of alpha-smooth muscle actin (α-SMA) [a protein marker for cirrhosis] in different groups by method described by sigma Aldrich manual [4, 6].

Statistical Analysis One way and two way (ANOVA) tests were used in the findings of the body weight evaluation. Significant Difference (SD) test was used to evaluate the significance between groups when ANOVA is significant. The significance level was set as P value ≤ 0.05 significant. Statistical analysis was Performed using SPSS version 16 [7].

3 Results

Isolation and culture of rat bone marrow derived MSCs at the third day of bone marrow incubation, elongated adherent cells were observed by microscopical examination. Extensive proliferation of cells was observed at the sixth day till the ninth day, at the following 3 days a relatively homogenous culture of the elongated cells was obtained, the population of cultured cells reached confluence within 2 weeks (70–80 % confluence). The cells appeared as fibroblasts in the morphology.

Clinical examination and animal weighting Animals of group (3) appeared lethargic, weak, dull, depressed with rough hair. The body weight changes are described in Tables 2 and 3.

Postmortem Examination Liver of group (3) appeared pale in color with numerous small nodules on the surface giving micronodular appearance and firm in consistency. Two animals from group (4) had liver with slightly nodular appearance and hard texture.

Histopathological Examination Group (A) at the end of the third month, revealed severe cytoplasmic vacuolation, individual cell necrosis of coagulative type and enlarged nuclei of hepatocytes. Proliferation of fibroblasts, few strands of collagen stained blue with MTS were bridging between central veins to surround

Table 2 Shows the mean body weight of group (A) and group (B) [mean ± SD] through the first 3 months

Month group	First month	Second month	Third month
Group (A)	148.50 ± 8.6^{bA}	113.99 ± 9.5^{cdB}	71.78 ± 5.4^{fB}
Group (B)	$155.16 \pm 2.^{defA}$	176.76 ± 3.7^{bcA}	185.74 ± 48.5^{abA}

Means with different letters (a, b, c, d, e, f) and (A, B) within the same column are significantly different at P value ≤ 0.05

Table 3 Shows mean ± SD of body weight in groups (1–5) at the end of the experimental study

Group	Group(1)	Group(2)	Group(3)	Group(4)	Group(5)
Mean ± SD	157.35 ± 5.3^{a}	113.96 ± 3.2^{a}	75.14 ± 6.2^{b}	113.92 ± 9^{a}	207.64 ± 2^{a}

Means with different letters (a, b, c, d, e, f) are significantly different at P value ≤ 0.05

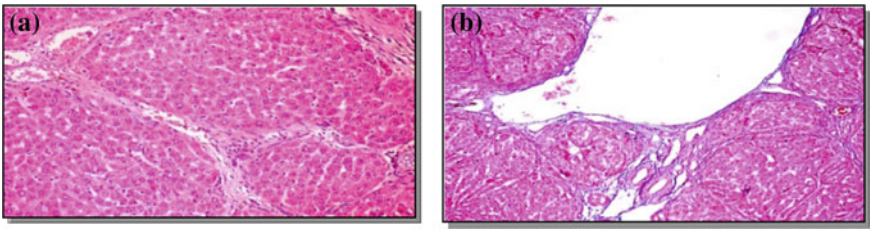

Fig. 1 **a** Liver of group (1) shows few strands of collagen with slight proliferation of fibroblasts between the hepatic lobules (H&E Stain X 200). **b** Shows few strands of collagen which stained *blue* in the portal area (MST Stain X 200)

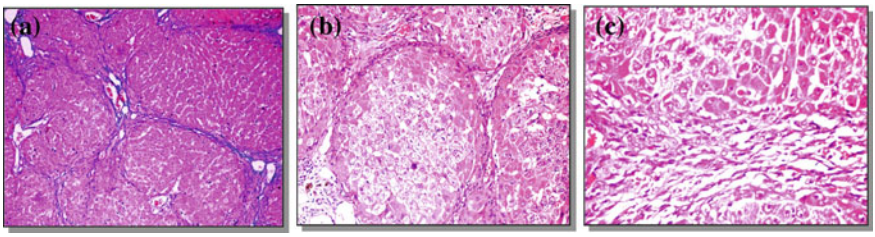

Fig. 2 **a** Liver of group (3) showing regenerative nodules appeared surrounded by *blue* stained collagen bundles (MST Stain X 200). **b** A regenerative nodule surrounded contain cells with vacuolar degeneration and most of cells were necrosed (H&E Stain X 400). **c** Showing apoptotic body (H&E Stain X 400)

the hepatic cells giving pseudolobulation appearance. The epithelial lining bile ducts showed hyperplasia with destructed corrugated basement membrane. Oval cells and Kupffer cells were highly activated. In between hepatocytes of Group (1) Liver, few strands of collagen were detected with slight proliferation of fibroblasts in five animals (Fig. 1a). In another four animals only few strands of collagen which stained blue with MTS were detected in the portal area (Fig. 1b). One animal showed complete pseudolobulation. In Group (2), only one animal showed few strands of collagen in the portal area. Six animals showed few strands of collagen between hepatocytes and bridging between central veins. The other three animals showed complete pseudolobulation as hepatic lobules were completely surrounded with thin collagen bundles. Liver of Group (3) showed multilobular cirrhosis, regenerative nodules appeared surrounded by collagen bundles stained blue with MTS (Fig. 2a). Each nodule contained cells with vacuolar degeneration and most of cells were necrosed (Fig. 2b). Others showed hyperatrophy with karyomegaly of the nucleus which appeared enlarged when compared with normal ones. Apoptotic bodies were observed as seen in (Fig. 2c). The main lesion in Group (4) was the vacuolation; few fibroblasts were proliferated in the portal area and in between hepatocytes. In four cases, few strands of collagen stained blue with MTS were observed bridging between central veins.

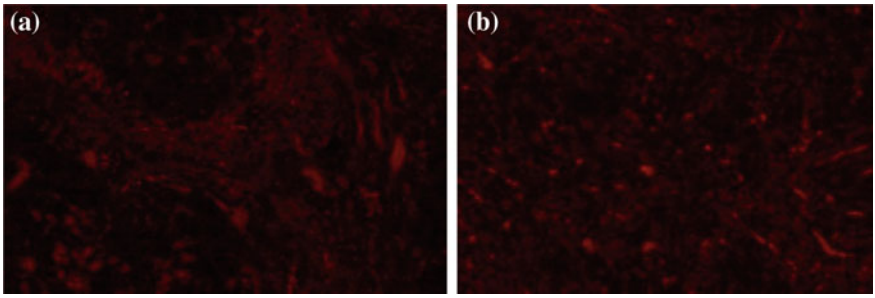

Fig. 3 **a** Unstained liver sections of rats treated with labeled PKH26 MSCs showing strong *red* autoflouresence between regenerative nodules and around blood sinusoids (200x) and **b** Showing diffuse pattern of strong *red* autoflouresence (200x)

Fluorescent microscope examination PKH26 stained cells showed strong red autoflouresence in unstained liver sections of animals treated with MSCs around blood sinusoids, in the fibrotic areas and between regenerative nodules in some animals (Fig. 3a). In other animals, PKH26 stained cells showed diffuse pattern as seen in (Fig. 3b).

Immunohistochemistery staining showed intense staining patterns in group (3), the intensity of α-SMA staining in TAA/MSCs groups [group (1) and group (2)] was lower when compared to control positive group.

4 Discussions

Animals treated with TAA appeared lethargic, weak, dull, depressed with rough hair [6]. Significant decrease in the body weight in animals treated with TAA could be attributed to the toxic effect of TAA decreasing the levels of nutrient absorption, energy utilization, and metabolic efficiency [8–10, 13]. While animals treated with MSCs showed significant increase in the body weight that could be contributed to the effect of MSCs. Micronodular appearance liver could be contributed to the development of collagen bundles which surround the regenerative nodules and cause pressure on the blood vessels, resulted in ischemia and decrease of the blood supply. So the color appeared pale grossly [4, 10].

The histopathological changes in the present study due to the administration of the TAA could be due to the metabolic conversion of TAA to free radical products: (thioacetamide sulfoxide) and (thioacetamide-S,S-dioxide) which attack microsomal lipids leading to their peroxidation, and production of reactive oxygen species (ROS), such as the hydrogen peroxide, super oxide anion and the hydroxyl radical. that result in liver injury and cirrhosis development [3, 8, 9, 11].

After treatment with MSCs, animals showed remolding of the collagen fibers which could be lysed by metalloproteinase (MMPs) more particularly the MMP2

that promote the degradation of (ECM) which have been secreted by MSC. The treatment effect of MSCs was higher in group (1) than (2). That as TAA injection was stopped in group (1) for 1 month during treatment with cells that was in agreement with [12].

PKH26 labeled MSCs showed strong red auto fluorescence after transplantation into rats, confirming that these cells were actually seeded into the liver tissue and differentiated into healthy cells replaced the damaged ones either hepatocytes or cholangiocytes which agreed with the work of [3, 4].

α-SMA expression showed intense staining patterns in cirrhotic animals, the intensity of α-SMA staining in TAA/MSCs groups were significantly less which indicate reductions in α-SMA expression reflecting a reduction in the number of activated HSCs by MSCs transplantation [4].

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