Therapeutic potential effect of bone marrow-derived mesenchymal stem cells on chronic liver disease in murine *Schistosomiasis Mansoni*

Mohamed H. Hegab¹ · Somia H. Abd-Allah² · Maha S. Badawey¹ ·
Ayman A. Saleh³ · Ashraf S. Metwally¹ · Ghada M. Fathy¹ · Soad M. Nada¹ ·
Sara A. Abdel-Rahman¹ · Amira A. Saleh¹ · Amal fawzy² · Mohammed Abu El-Magd⁴

Received: 15 March 2018 / Accepted: 11 April 2018 / Published online: 23 April 2018
© Indian Society for Parasitology 2018

Abstract Some reports have shown that mesenchymal stem cells (MSCs) therapy could ameliorate chemically-induced hepatic fibrosis. This research assesses the therapeutic action of bone marrow mesenchymal stem cells (BM-MSCs) on chronic diseased liver in *Schistosoma mansoni* infected mice. All infected female mice divided into three groups, one group (15 mice) treated with oral praziquantel (PZQ), second group (15 mice) received intravenous injection of BM-MSCs and third group (15 mice) treated with both MSCs + PZQ. Two control groups (15 mice each) subdivided into one infected and second healthy one. BM-MSCs were obtained from bones of both femur and tibia of male mice (30 mice), then cultured and characterized morphologically by detection of CD105 by flow cytometer. Liver tissues for all groups were examined histopathologically. Measuring of the collagen 1 gene expression was done by real-time PCR and immunohistochemical study to detect stem cells differentiation for detection of MSCs engraftments in liver tissue. MSCs treatment caused marked improvement and regression of fibrosis, and prevents deposition of collagen and reduced

the expression of collagen 1 gene in infected mice on their liver tissues, especially when used with PZQ in mice treatment. It can be concluded that, MSCs is a good therapeutic method for liver fibrosis caused by *S. mansoni* infection.

Keywords MSCs · *Schistosoma mansoni* · Praziquantel · Collagen 1 gene · OV-6

Introduction

S. mansoni is used to explain the fibrogenic process and its mechanism as an example of hepatic fibrosis. In schistosomiasis, the parasite eggs are the main stimulant to inflammatory-immune response when trapped into portal venules forming granulomatous reaction (Oliveira et al. 2008).

The pipe-stem's periportal hepatic fibrosis is caused mainly by granulomatous reaction which is similar to fibrosis found in severe hepatosplenic form of the disease in human. This pipe-stem fibrosis causes cumulative occlusion of portal veins results in portal hypertension. The important cause of severity, complication and causes of death in schistosomiasis is due to accumulation of connective tissues in organs affected (Wynn et al. 2004). Liver fibrosis usually appears as areas of chronic inflammation with extracellular matrix (ECM) deposited in periovular granulomas and distributed at the periphery of portal vein system with different rates. Hepatic fibrosis that affects about 20 million individuals, leads to liver failure and death (Raque and Dessein 2001; Andrade 2009). The tremendous gap between the organs offered for transplantation and those needed is the major limitation of liver replacement therapy (Haridass et al. 2008). Also, the

✉ Sara A. Abdel-Rahman
sarameram@yahoo.com

¹ Department of Parasitology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

² Department of Biochemistry, Faculty of Medicine, Zagazig University, Zagazig, Egypt

³ Department of Animal Wealth Development, Genetics & Genetic Engineering, of Veterinary Medicine, Zagazig University, Zagazig, Egypt

⁴ Department of Anatomy & Embryology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt

problems resulting from major surgery and long-term immunosuppressive therapy, which are the risk factors of liver transplantation; do not occur without serious side effects. Alternative or adjuvant therapies for hepatic fibrosis are needed (Dahlke and Schlitt 2003).

Cell transplantation programs may provide a hopeful technology to replace organ transplantation, also mesenchymal stem cells (MSCs) documented as hopeful technique for cell therapy and have been applied successfully in treatment of many diseases. MSCs have been used for about 10 years, from experimental to clinical trials (Elkhafif et al. 2010; Wel et al. 2013).

The present work was carried out upon the experimental schistosomiasis to explain the capacity of BM-MSCs and their effect in the therapy of chronic liver disease

Materials and methods

This study was performed in 2 collaborating laboratories in Egypt; Parasitology Department and Stem Cell Research Laboratory in Medical Biochemistry Department, Faculty of Medicine, Zagazig University.

Experimental mice

A total number of 105 laboratory-bred parasite free Swiss albino mice weighing about 25 gm, 75 female mice for study groups while 30 male mice selected as donors of BM-MSCs.

Infection with *S. mansoni*

Female mice were infected by injection of 100 cercariae subcutaneously in each mouse. They were classified into:

Group I: (15 mice) control infected-non treated and injected with phosphate buffer saline (PBS).

Group II: infected (45 mice) which subdivided into 3 subgroups:

Group IIa: (15 mice) Received praziquantel (PZQ) orally).

Group IIb: (15 mice) Received BM-MSCs.

Group IIc: (15 mice) Received both PZQ and BM-MSCs.

Group III: (15 mice) healthy control female mice.

MSCs preparation from BM

Six week's old male mice femurs and tibiae were used for bone marrow collection by flushing them. By using of 10% fetal bovine serum (FBS)—(LonzaBioproducts, Belgium) with Dulbecco's modified Eagle's medium (DMEM 1.0 g/

L glucose lonzaBioproducts, Belgium). The isolation of nucleated cells with a density gradient was done by using Ficoll/Paque (Pharmacia). Then, they were resuspended in supplemented culture medium with 10% penicillin–streptomycin–amphotericin B mixture (LonzaBioproducts Belgium). Cells were left from twelve to fourteen days at 37 °C in 5% humidified CO₂ till the development of large colonies, (80–90% confluence). PBS was used in washing culture twice and then cells were detached using 0.25% trypsin in 1 mM Ethylene diamine tetra acetic acid (EDTA) (LonzaBioproducts, Belgium) at 37 °C for five minutes. Then suspension was centrifuged and counting viable and non-viable cells by hemocytometer followed by subculturing of the viable cells which was determined via trypan blue passage. The adhesiveness and fusiform appearance were the characters of cultured MSCs Detection of CD105 by flowcytometer which is known as a marker for MSCs (Calabro et al. 2003).

Pkh₂₆ labelling of stem cells

Using red fluorescent dye PKH₂₆ (Sigma) as in manufacture's guides. MSC were isolated and washed with serum-free medium triple after the third passage. Cells were incubated at 1×10^7 cells in 1 ml of diluents C plus dye, and then left at 25 °C for five min., two ml of FBS was added to stop the reaction. Cells were added to four ml of complete medium and washed for three times. Cells were added to 1×10^6 cells in complete medium and utilized within thirty min. For testing viability of PKH₂₆-labelled primary BM-MSCs, they were re-cultured. Fluorescent microscope was used to examine the liver specimens of mice treated with the labelled cells (subgroups IIb and IIc).

Transplantation of cells

The transplant process included a male injection with non-fractionated BM-MSCs into infected female mice on their 8th week post-infection in a dose of 1×10^6 cells/mouse suspended in 0.3 ml (PBS).

Sacrifice of mice

Scarification of all mice groups were done 4 weeks after transplantation. Their livers were isolated under aseptic condition.

PCR detection of male-specific sry gene in female mice liver

PCR was used to confirm the region of sex determination on Y chromosome male (Sry) gene in recipient female mice (Fang et al. 2004). Liver tissue homogenate of the

mice in each group was used to prepare Genomic DNA using G-spin™ total DNA Extraction Mini kit (iNtRON Biotechnology, Korea) protocol. Primer sequences for Sry gene (forward primers: 5-TGGGACTGGTGA-CAATTGTC reverse GAGTACAGGTGTGCAGCTCT-3) were designed according to Schiffmann et al. (1995). Phenol and chloroform were used for the extraction of genomic DNA from the mice liver. PCR was applied in a volume of 25 µl which consists of 2 µl (30 ng) of genomic DNA, 0.2 µmol/l of each primer and 10 µl of Tag PCR Master Mix (BIORON). The system of amplification was as follows: 94 °C for 10 min., then 35 cycles of 94 °C for 1 min., 56 °C for 45 s. and 72 °C for 45 s., then 72 °C for 7 min. utilizing heated lid thermal cycler. PCR products (400 bp) were isolated using 1.5% agarose gel electrophoresis.

Liver histopathology and immunohistochemistry parameters

Fixation of Liver specimens in 10% formalin, preparation of paraffin blocks and sectioned at 4 µm thickness were done. Sections were stained with hematoxylin and eosin (H & E), Masson trichrome (MT) and picrosiruis-red (SR) to study the hepatic morphological changes and hepatic fibrosis (Elkhafif et al. 2008). This study focused on tracing the homing of the transplanted BM-MSCs and their differentiation into newly formed hepatocytes (Pan 2010), applying immunohistochemistry technique using monoclonal antimouse anti-OV-6 antibodies for detection of newly formed hepatocytes.

Mean egg granuloma diameter

The liver specimens were prepared for pathological examination and the measurement of the diameter of the largest twenty egg granulomas was done in each group and calculated according to Bartley et al. (2006). Only circular granulomas were considered.

Mean egg granuloma number according to Ali and Hamed (2006)

For each mouse the number of liver granulomas was determined in five randomly selected microscopic field/liver sections at low power field magnification. Three liver sections were examined for each mouse. The mean number of egg granulomas/liver section was calculated.

Morphometric analysis

The collagen content in the liver specimen stained with Picro-Sirius red was evaluated for the percentage areas of

collagen using computerized cells Image analysis (Junqueira et al. 1978).

Immunohistochemical technique for detection of OV-6

The transplanted BM-MSCs in the liver were detected by staining OV-6 cells with biotinylated anti-OV-6 antibodies followed by universal diaminobenzidine tetra-hydrochloride (DAB) immunostaining detection kit. The standard avidin–biotin immunoperoxidase technique was used (Hsu and Raine 1981).

Quantitative RT-PCR analysis for collagen 1 gene in mice

The isolation of total RNA was done from liver using an RNeasy Mini Kit by digestion of DNase according to the manufacturer's guides (Qiagen, #74104). The reverse transcription of RNA was performed by using Quantiscript reverse transcriptase. Then, quantitative real-time PCR analysis was processed with a (Rotor-Gene Q2 plex, Qiagen) with SYBR green master mix (Roche Diagnostics), with GAPDH as an internal reference gene. The mice liver collagen gene expression was normalized to mice GAPDH. The following primers were used for analysis: mice collagen 1 gene, 5'-ACGGCTGCACGAGTC-3' and 5'-GGCAGGCGGGAGGTCTTT-3' mice GAPDH, 5'-TGTGTCCGTGGT GGGATCTGA-3' and 5'-CCTGCTTCACCACCTTCTTGA-3'. The following thermal cycling conditions were used for the 2 genes: initial denaturation at 94 °C for 3 min. 35 cycles of amplification (DNA denaturation at 94 °C for 40 s., annealing at 57 °C for 1 min., extension at 72 °C for 1 min.) and final extension at 72 °C for 10 min.

Statistical analysis

Collected Data were arranged, summarized and then analyzed by SPSS-PC + (SPSS). The difference between the groups was determined by statistical method (1-way analysis of variance) and Tukey's Honest Significant Difference according to Snedecor and Cochran (1980). Data were expressed as mean ± SD and significance was declared at $P < 0.05$.

Ethical consideration

The mice were maintained according to the research protocols following the recommendations of the National Institutes of Health (NIH) guidelines for animal experimentation. The experimental protocol was approved by the

ethical committee of the Faculty of Medicine, Zagazig University, Sharkia, Egypt.

Results

Isolation, expansion of BM-MSCs and characteristics of MSCs

The obtained BM-MSCs were detected by their adhesiveness, fusiform shape, and fibroblastic appearance after expansion. Flow cytometer analysis (Fig. 1) demonstrated that CD105 was expressed on the cell surface with no expression of hematopoietic lineage marker CD34 and CD45.

Incorporation of Transplanted BM-MSCs in livers of *S. mansoni*-infected mice

The detection of labelled MSCs (Fig. 2) with the red fluorescent dye PKH26 in liver samples of BM-MSCs treated mice and also, by PCR Sry gene (Fig. 3) that used the Y chromosome marker was expressed in male mice from which BM-MSCs was isolated. It was also detected in the liver of the infected group treated with MSCs, not in the infected control group.

Effect of BM-MSCs treatment on number and diameter of liver granuloma and Collagen deposit inhibition

Histopathological studies of liver tissues showed that there was an improvement in the histopathological finding of the liver which demonstrates obvious decrease in the mean

Fig. 2 Liver section from group IIb showing PKH26 labeled cells appearing as bright dots within liver tissue (Fluorescent Microscope, $\times 200$)

diameter and number of granuloma (Fig. 4). There was a significant decrease in fibrous tissue in *Schisto*/MSCs group in comparison with the infected group by using Sirius red stain morphometric study (Fig. 5, 6).

Also, MSCs have a significant antifibrotic effect which illustrate that exhibited marked periportal fibrosis in hepatic lobules. The qualitative observation obtained by measurement of liver tissues mRNA expression of collagen type 1 supported real time PCR. Specifically, collagen 1 mRNA in MSC group was significantly less (1.6 ± 0.25) than in control infected group (3.9 ± 0.18 , $P < 0.001$). The level was significantly less in the PZQ + MSCs group (1.32 ± 0.04) than in the PZQ group (2.7 ± 0.15 , $P < 0.001$) (Fig. 7)

Fig. 1 Flow-cytometry analysis of cell surface markers in mesenchymal stem cells (MSCs)

Fig. 3 Agarose gel electrophoresis of PCR product (402 bp) for Sry gene, Marker: DNA ladder, Lane 1: male control positive; Lane 2: Infected female control negative; lanes 3–4: positive female received MSCs (Group IIB); lane 5: positive female received PZQ + MSCs (Group IIC); Lane 6: negative female not received MSCs (Group IIA). The sizes of marker fragments and specific PCR products are indicated on the side of the figure

Detection of OV-6 in hepatic tissues using immunohistochemical technique (Fig. 8, 9, 10)

The presence of OV-6 in liver specimen of normal healthy mice, *S.mansoni* infected mice (control infected) and infected mice transplanted with BM-MSCs was detected immunohistochemically using monoclonal antimouse OV-6 antibodies as an indicator for the formation of new hepatocytes (hepatocyte-like cell) differentiate from BM-MSCs. Normal healthy control, infected control mice showed negative expression of OV-6 in hepatocytes and granuloma cells.

At treatment with BM-MSCs combination with PZQ, hepatocytes stained with brown color appeared indicating detection of newly formed hepatocytes. The staining of cells occurred lodged between mouse hepatocytes and around granulomas either singly or in clusters

Fig. 4 Mean granuloma number and diameter in studied groups

Discussion

Schistosomiasis is a widely spread chronic helminthic infection causing death of many people yearly. The granulomatous liver affection caused by *Schistosoma* considered the most frequent disease among Egyptians. It is characterized by disarranged proliferation of hepatocytes, excessive scarring and excess collagen deposition ends by liver failure. So it could be selected as an experimental model of chronic liver disease which allows follow up of the migration of stem cell to damaged parts and their contribution to repair tissues and host survival (Ghanem 2010).

In the current study, stem cell transplantation was tried as a possible anti-fibrotic therapy and compared with the traditional anti-schistosomal chemotherapy praziquantel drug (PZQ) which routinely given as a treatment of schistosomiasis either experimentally or in human, PZQ was given as a single or dual therapy with MSCs to find its effect upon PZQ.

We recorded that dual therapy greatly increase the effect of PZQ for repairing liver fibrosis induced by infection suggesting that MSCs can be used in the improvement of the treatment of liver fibrosis induced by *S. mansoni*. Also the transplanted MSCs have been properly homed in the affected liver. This was proved by detection of Sry gene of the Y chromosome of the injected MSCs of donor male mice within livers of the *Schistosoma*-infected female mice by PCR and also, by detection of cells labelled with PKH26 dye inside the liver by fluorescent microscope.

Homing of injected MSCs within injured liver was reported by many authors Zhao et al. (2005), Abdel Aziz et al. (2007), Elkafif et al. (2010), El-Mahdi et al. (2014), Anan et al. (2016) and Fikry et al. (2016).

Also, it was found that affected liver of *S. mansoni* infection elicits the migration of BM-MSCs, which could reach the granuloma areas. Thus, the liver injury is essential for attraction of the MSCs to the liver through upregulation of cytokines like stem cells factors,

Fig. 5 Liver section of mice infected group I; group IIb and group IIc showing the number of *S. mansoni* granulomatous reaction surrounded by pink red dense collagen bundle (Sirius red stain, $\times 200$)

group I showing the large number of *S. mansoni* granulomatous group IIb showing less number of *S. mansoni* granulomatous group IIc showing the least number of *S. mansoni* granulomatous reaction

hepatocytes growth factor (HGF) and matrix metalloproteinase (MMPs) and many of these cytokines and chemokines are chemoattractant (Zhang et al. 2008). Herrera et al. (2007) have explained that, chemotactic factors can be released from injured tissues to recruit MSCs and the numbers of MSCs homing to the liver are independent to the route of MSCs infusion. To investigate the differentiation potential of MSCs extracted from bone marrow in regeneration of injured liver tissues induced by *S. mansoni* chronic infection. In our study, there was significant expression in group IIb treated with MSCs while negative reaction for OV-6 marker was noticed in liver specimen of either normal uninfected control group or-infected but untreated group I.

Moreover marked expression of OV-6 antibody in liver section of group IIc treated with BM-MSCs combined with PZQ than other groups. These feedbacks coincide with studies of Oliveira et al. (2008) and El-Mahdi et al. (2014). In addition, El-Mahdi et al. (2014) reported that MSCs infusion could generate new hepatocytes that improve the function of the rest hepatocytes.

Oval cells are hepatic precursors of hepatocytes and cells of bile duct (Fausto and Campbell 2003). Wang et al. (2003) showed that oval cells can be stimulated to differentiate into hepatocytes and respond to liver injury to promote hepatic regeneration. It was concluded that bone marrow cells could be transformed into hepatocytes after severe hepatic injury. The occurrence of severe hepatic injury may lead to rapid migration of the stem cells of the bone marrow and their transformation into hepatocytes inside the liver (Tokcaer-keskin et al. 2009). In this work, the injected BM-MSCs could transmigrate to the damaged tissue and might transformed into hepatocytes-like cell to initiate liver regeneration.

Oliveira et al. (2008) reported that the number of oval cells in liver specimen of *S. mansoni*-infected mice increased 3–4 folds after transplantation of MSCs. This may be explained by the fact that oval cells can be originated from BM-MSCs or it is propale that the increases in oval cells replace areas of fibrosis and degradation with functional parenchymatous cells after BM-MSCs application. Also, Petersen et al. (1999), put emphasis on the

Fig. 6 Image analysis of mouse liver section of infected group I and group IIc showing computer delineation of the fibrous tissue in whitish coloration for the morphometric assessment. marked reduction of the deposited fibrous tissue in group IIc as compared to group I. (Sirius red, $\times 50$)

Fig. 7 Collagen 1 mRNA gene expression in studied groups

Fig. 8 Immunostain for OV6 antibody in a liver section of group IIa showing negative expression of OV6 monoclonal antibody (IHC, DAB, $\times 400$)

possibility that bone marrow may act, under certain physiopathological conditions as the progenitor of various types of liver cells. Also, Fang et al. (2004) reported that liver regeneration occurs through the affinity of stem cells to stimulate resident oval cells to proliferate and replace areas of fibrosis degradation.

This study showed that there was significant histopathological improvement of the liver including diminution in the number and diameter of granulomas and decrease in the fibrotic content. Our finding coincides with Abdel Aziz et al. (2007) who found that MSCs infusion cause marked decrease in hepatic collagen and histopathological improvement of the liver. El-Mahdi et al. (2014) revealed that BM-MSCs elicit histopathological improvement of the liver. Moreover, Ahmed et al. (2014) reported that BM-MSCs could improve the structure and function of the liver in experimental model of liver fibrosis. In this trial the degree of fibrosis was estimated by the quantitative morphometric determination of the collagen content in Sirius red stained liver sections.

The results revealed significant decreases in the collagen content after treatment with BM-MSCs alone or when combined with PZQ. In this regard, Ghanem et al. (2010) demonstrated that fibrosis developed during the chronic phase of granulomatous inflammation in murine schistosomiasis and it represented a protective function during infection by neutralization and sequencing egg antigens that can potentially damage host tissues.

The inhibitory effect on the collagen deposition due to inoculation of BM-MSCs may be due to an increase of fibrotic degradation rather than a decrease in fibrous tissue synthesis. In this study, the decrease in liver fibrosis after administration of BM-MSCs could be clarified by the inactivation of HSCs by BM-MSCs, an explanation

Fig. 9 Immunostain for OV6 antibody in a liver section of group IIb and group IIc showing moderate expression of OV6 monoclonal antibody as cytoplasmic brownish colour in hepatocytes like cells and

granuloma cells (arrows) (IHC, DAB, $\times 200$, $\times 400$) in group IIb and marked expression in group IIc

Fig. 10 Immunostain for OV6 antibody in a liver section of infected control group and normal healthy group showing negative expression of OV6 (IHC, DAB, $\times 200$)

supposed by Pulavendran et al. (2010), findings which indicated that BM-MSCs can prevent the activity of HSCs and cause apoptosis to heal fibrosis. In this study, the molecular results showed a significant decrease in gene expression of collagen type1 mRNA in mice received BM-MSCs (groups IIb and IIc) compared with control infected group I. These coincide with Di Campli et al. (2004) and Abdel-Aziz et al. (2007). Also, antifibrotic effects of MSCs have been previously documented by many authors Kollet et al. (2003), Sakaida et al. (2004), Zhao et al. (2005), Moriya et al. (2008), Mohsen et al. (2011), Xu et al. (2012),

Ahmed et al. (2014), Shao et al. (2014) and Fikry et al. (2016).

In the same context, Fang et al. (2004) and Oliveira et al. (2008) reported that treatment with MSCs in mouse model of schistosomiasis lead to decrease in fibrogenic cytokine (TGF) These authors stated that this cytokine is presumed as fibrosis promoting cytokine and its reduction denotes resolving of fibrosis. Also, Ali and Masoud (2012) recorded that BM-MSCs transplantation ameliorate liver fibrosis by enhanced expression of antifibrotic factor matrix metalloproteinases-9 (MMP-9) and albumin which is a hepatic

functional marker, reduced RNA expression of collagen 1 gene and reduced expression of (SMA which is a marker of activated hepatic stellate cell.

Thus, transplanted BM-MSCs may affect activated stellate cells by inhibiting them or by leading them to apoptosis through release of interleukin-10 (Dai et al. 2009). It was proved that the matrix metalloproteinase (MMPs) can be produced by MSCs, an enzyme able to break, the extra cellular matrix, which directly relieves hepatic cirrhosis (Hardjo et al. 2009). Recently, many reports have suggested that MSCs may act via paracrine secretion of factor which helps in organ repair. In this concern, Chang et al. (2009) reported also that MSCs paracrine expression of matrix metalloproteinases (MMPs) facilitated recovery from chemically induced liver damage in a rat model. Also, Li et al. (2009) stated that paracrine action mediated the antifibrotic effect of transplanted MSCs in a rat model of heart failure.

These data encourage the therapeutic role of MSCs in hepatic illness. The mechanisms following the improvement in liver fibrosis and liver functions observed after MSCs transplantation in various experimental models of chronic liver injuries were still a matter of researches. Prosser et al. (2006) declared that (a) the promotion of MSCs transdifferentiation into parenchymal hepatocyte could be induced by hepatocyte growth factor (HGF), which was produced by MSCs (b) Fibrogenesis of liver can be prevented by MSCs through secreting several cytokines like HGF, interleukin IL-6 and IL-10. (c) MSCs may disintegrate fibrosis directly.

Our results in group IIc were in agreement with Xu et al. (2012) who reported that PZQ with MSCs together enhanced the effect of PZQ for resolution of liver fibrosis induced by *S. japonicum*, proposing that MSCs can be applied as an adjuvant for treatment of schistosomiasis. Kinnaid et al. (2004) and Silva et al. (2005) found that MSCs could release arteriogenic cytokine such as vascular endothelial growth factor and participate in decrease of fibrosis with paracrine mechanisms rather than by cell incorporation. These findings suggested that MSCs themselves could not functionally rescue the recipients by substituting the damaged cells directly (Kanazawa and Verma 2003). Subsequently, the reduction observed in the number of liver granulomas after BM-MSCs treatment in this research, may be due to paracrine factors secreted by stem cells, affecting the parasite maturation and/or fecundity. In conclusion, our study showed that transplantation of BM-MSCs in mice with chronic liver disease caused by *S. mansoni* infection decreased liver fibrosis and help in the formation of newly formed hepatocytes and/or in the improvement of resident hepatocytes. Moreover, combining MSCs with PZQ can cause significant decrease in formation of collagen fibers and

inhibition of subsequent liver fibrosis and that such reduction was better than the administration of each drug alone. This data prove that MSC is a new and effective therapeutic technique for *S. mansoni* induced liver affection. However, the mechanism of the antifibrotic effect of MSCs needs more study and more researches to determine the precise mechanism.

Acknowledgements We thank Dr. Soheir Sayed Mahmoud, Professor of Parasitology in Theodor Bilharze Research Institute, for her valuable advices during animal experimentation. Further we thank Dr. Olfat Aly Hammam, Professor of Pathology in Theodor Bilharze Research Institute, Egypt for her active participation in histopathological and immunohistochemical parts of this work.

Author contribution MH Abdel-Hady and SM Nada shared in the study design and research topics. SH Abd-Allah initiated the research idea and shared in performing the laboratory work of obtaining BM-MSCs. MS Badawey shared in the study design, in performing the laboratory work, and reviewing the manuscript. AS Metwally, GM Fathy, SA Abdel-Rahman and Amira A. Saleh shared in the laboratory work, interpretation of the results, collecting references, wrote and reviewed the manuscript. Ayman A. Saleh and MAbu El-Magd shared in real-time PCR study to detect stem cells differentiation. A fawzy, shared in performing the laboratory work of obtaining BM-MSCs, cultured and characterization.

Compliance with ethical standard

Conflict of interest Authors declare that there is no any conflict of interests.

References

- Abdel Aziz M, Atta H, Mahfouz S, Fouad H et al (2007) Therapeutic potential of bone marrow-derived mesenchymal stem cells on experimental liver fibrosis. *Clin Biochem* 40(12):893–899
- Ahmed RH, Roshdy NK, Aref MI, Hasaan NM et al (2014) Effect of bone marrow-derived mesenchymal stem cells and umbilical cord blood-CD34+ cells on experimental rat liver fibrosis. *J Stem Cell Reser and Transplant* 2(301):1–6
- Ali SA, Hamed MA (2006) Effect of Ailan thus altissima and zizyphusspina-christi on bilharzial infestation in mice, Histological and histopathological studies. *J Appl Sci* 6(2):1437–1446
- Ali G, Masoud MS (2012) Bone marrow cells ameliorate liver fibrosis and express albumin after transplantation in CCl4-induced fibrotic liver. *Saudi J Gastroenterol* 18(4):262–267
- Anan H, Zidan R, Shaheen M, Abd El-Fattah E (2016) Therapeutic efficacy of bone marrow derived mesenchymal stromal cells versus Losartan in adriamycin-induced renal cortical injury in adult albino rats. *Cytotherapy* 18(8):970–984
- Andrade ZA (2009) Schistosomiasis and liver fibrosis. *Parasite Immunol* 31(11):656–1147
- Bartley PB, Ramm GA, Jones MK, Ruddell RG, Li Y, McManus DP (2006) A contributory role for activated hepatic stellate cells in the dynamics of *Schistosoma japonicum* egg-induced fibrosis. *Int J Parasitol* 36:993–1001
- Calabro L, Donsatti E, Bellomo G, Alonci A, Collizzi F, Sigalotti L, Altomonte M, Musolino C, Maio M (2003) Differential levels of soluble endoglin (CD105) in myeloid malignancies. *J Cell Physiol* 194:171–175

- Chang YJ, Liu JW, Lin PC, Sun LY et al (2009) Mesenchymal stem cells facilitate recovery from chemically induced liver damage and decrease liver fibrosis. *Life Sci* 85:517–525
- Dahlke MH, Schlitt HJ (2003) Making hepatocytes from stem cells: where are you? *Liver Transpl* 9(10):1100–1101
- Dai L, Li H, Guan L, Ritchie G, Zhou J (2009) The therapeutic potential of bone marrow-derived mesenchymal stem cells on hepatic cirrhosis. *Stem Cell Res* 2(1):16–25
- Di Campli C, Piscaglia AC, Pierelli L et al (2004) A human umbilical cord stem cell rescue therapy in murine model of toxic liver injury. *Dig Liver Disease* 36:603–613
- Elkhafif N, Yehia H, Voss B, Hammam OA, Akl M, Mansy S, Mahmoud S, ElBendary O, El Fandy G (2008) Differentiation and homing of transplanted bone marrow cells in livers of murine schistosomiasis: Pilot study. *Aust J Basic Appl Sci* 2:1362–1372
- Elkhafif N, Voss B, Hammam O, Yehia H, Mansy S, Akl M, Boehm S, Mahmoud S, ElBendary O, El Fandy G (2010) Homing of transplanted bone marrow cells in livers of *Schistosoma mansoni*-infected mice. *APMIS* 118:277–287
- El-Mahdi MM, Mansour WA, Hammam O, Mehana NA, Hussein TM (2014) Ameliorative effect of bone marrow-derived stem cells on injured liver of the mice infected with *Schistosoma mansoni*. *Korean H Parasitol* 52(2):5–162
- Fang B, Shi M, Liao L, Yang S, Liu Y, Zhao RC (2004) Systemic infusion of FLK1-mesenchymal stem cells ameliorate carbon tetrachloride-induced liver fibrosis in mice. *Transplantation* 78:83–88
- Fausto N, Campbell JS (2003) The role of hepatocytes and oval cells in liver regeneration and repopulation. *Mech Dev* 120:117–130
- Fikry H, Abdel Gawad S, Baher W (2016) Therapeutic potential of bone marrow-derived mesenchymal stem cells on experimental liver injury induced by *Schistosoma mansoni*, A histological study. *Int J Stem Cells* 9(1):96–106
- Ghanem LY, Dahman U, Dirsch O, Nosseir MM et al (2010) Does granulocyte-colony stimulating factor administrations induce damage or repair response in schistosomiasis. *World J Hepatol* 2:434–441
- Hardjo M, Miyazaki M, Sakaguchi M, Massaka T et al (2009) Suppression of carbon tetrachloride-induced liver fibrosis by transplantation of a clonal mesenchymal stem cells line derived from rat bone marrow. *Cell Transplant* 18(1):89–99
- Haridass D, Narain N, Ott M (2008) Hepatocyte transplantation waiting for stem cells. *Curr Opin Organ Transplant* 13:627–632
- Herrera MB, Bussolati B, Bruno S, Morando L, Mauriello-Romanazzi G, Sanavio F, Stamenkovic I, Biancone L, Camussi G (2007) Exogenous mesenchymal stem cells localize to the kidney by means of CD44 following acute tubular injury. *Kidney Int* 72:430–441
- Hsu SM, Raine L (1981) Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabeled antibody (PAP) procedure. *J Histochem* 29:577–580
- Junqueira LC, Bignolas G, Brentani RR (1978) Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J* 11:447–455
- Kanazawa Y, Verma IM (2003) Little evidence of bone marrow-derived hepatocytes in the replacement of injured liver. *Proc Natl Acad Sci USA* 100(1):11850–11853
- Kinnaird T, Stabile E, Burnett MS, Shou M et al (2004) Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation* 109:1543–1549
- Kollet O, Shvitiel S, Chen YQ, Suriawinata J, Spiegel A (2003) HGF, SDF-1, and MMP-9 are involved in stress-induced human CD34+ stem cells recruitment to the liver. *J Clin Invest* 112:160–169
- Li L, Zhang S, Zhang Y, Yu B, Xu Y, Guan Z (2009) Paracrine action mediate the antifibrotic effect of transplanted mesenchymal stem cells in a rat model of global heart failure. *Mol Biol Rep* 36:725–731
- Moriya K, Yoshikawa M, Oujii Y, Saito K et al (2008) Embryonic stem cells reduce liver fibrosis in CCl4-treated mice. *Int J Exp Pathol* 89(6):401–409
- Mohsen S, Shams S, Ali G, Khan M et al (2011) Enhanced hepatic differentiation of mesenchymal stem cells after pretreatment with injured liver tissue. *Differentiation* 81:42–48
- Oliveira AS, Souza FSB, Ferreira CAG, Barreto SE et al (2008) Therapy with bone marrow cells reduces liver alterations in mice chronically infected by *S. mansoni*. *World J Gastroenterol* 14(38):5842–5850
- Pan Y, Dong S, Hao Y, Zhou Y, Ren X, Wang I, Wang Y, Chu T (2010) Ultrasonic-assisted extraction process of crude polysaccharides from Yinshi mushroom and its effect on hydroxyproline and glycosaminoglycan levels. *Carbohydr Polym* 81:93–96
- Petersen BE, Bowen WC, Patrene KD, Mars WM et al (1999) Bone marrow as a potential source of hepatic oval cells. *Sci J* 284:1168–1170
- Prosser CC, Yen RD, Wu J (2006) Molecular therapy for hepatic injury and fibrosis: where are we? *World J Gastroenterol* 12(4):409–415
- Pulavendran S, Vignesh J, Rose C (2010) Differential anti-inflammatory and antifibrotic activity of transplanted mesenchymal vs hematopoietic stem cells in carbon tetrachloride-induced liver injury in mice. *Int Immunopharmacol* 10:513–519
- Ranque S, Dessein A (2001) *Schistosoma mansoni* schistosomiasis. *Rev Prat* 51(19):2099–2103
- Sakaida I, Terai S, Yamamoto N, Aoyama K et al (2004) Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice. *Hepatology* 40:1304–1311
- Schiffmann R, Medin J, Ward J, Stahl S et al (1995) Transfer of the human glucocerebrosidase gene into hematopoietic stem cells of nonablated recipients: successful engraftment and long-term expression of the transgene. *Blood* 86:1218–1227
- Shao CH, Chen SL, Dong TF, Chai H et al (2014) Transplantation of bone marrow-derived mesenchymal stem cells after regional hepatic irradiation ameliorates thioacetamide-induced liver fibrosis in rats. *J Surg Res* 186:408–416
- Silva G, Litovsky S, Assad JA, Sousa AL et al (2005) Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density and improve heart function in a canine chronic ischemia model. *Circulation* 111:150–156
- Snedecor G, Cochran W (1980) Statistical methods. Iowa State University Press, Ames
- Tokcaer-keskin Z, Akar AR, Ayaloglu-Butun F et al (2009) Timing of induction of cardiomyocyte differentiation for in vitro cultured mesenchymal stem cells: a perspective for emergencies. *Can J Physiol Pharmacol* 87:143–150
- Wang X, Foster M, Al-Dhalimy M, Lagasse E, et al (2003) The origin and liver repopulating capacity of murine oval cells. *Proc Natl Acad Sci USA* 100(1):11881–11888
- Wel X, Yang X, Han Z, Qu F et al (2013) Mesenchymal stem cells: a new trend for cell therapy. *Acta Pharmacol Sin* 34:747–754
- Wynn TA, Thompson R, Cheever AM (2004) Immunopathogenesis of schistosomiasis. *Immunol Rev* 201:156–167
- Xu H, Qian H, Zhu W, Zhang X et al (2012) Mesenchymal stem cells relieve fibrosis of *Schistosoma japonicum*-induced mouse liver injury. *Exp Biol Med* 237(5):585–592
- Zhang W, Wulboomers XF, Van Osch H, Jansen JA (2008) Hard tissue formation in a porous HA/TCP ceramic scaffold loaded with stromal cells derived from dental pulp and bone marrow. *Tissue Eng Part A* 14:285–294
- Zhao DC, Lei JX, Chen R, Yu WH et al (2005) Bone marrow derived mesenchymal stem cells protect against experimental liver fibrosis in rats. *World J Gastroenterol* 11(22):3431–3440