Original Article

Antifibrotic effect of adipose- versus bone marrow-derived mesenchymal stem cells in chronically infected mice with *Schistosoma mansoni* after Praziquantel treatment

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ABSTRACT

Background: Despite the effective infection treatment with Praziquantel (PZQ), hepatic fibrosis represents the leading cause of schistosomiasis-related morbidity and mortality. Mesenchymal stem cells (MSCs) have emerged as a potential therapeutic candidate for schistosomiasis-induced hepatic fibrosis.
Objective: To evaluate and compare the effect of bone marrow (BM) derived MSCs and adipose (AD) derived MSCs in ameliorating liver fibrosis in *S. mansoni* chronically infected mice treated with PZQ.
Material and Methods: *S. mansoni* chronically infected mice were treated with PZQ on the ninth week post-infection (pi), followed after four weeks by BM- or AD-MSCs administration. In addition to the two treated groups, three control groups (uninfected, infected untreated, infected and PZQ-treated) were employed. Mice were sacrificed two- and four-weeks post-MSCs treatment (wpt). The antifibrotic effect of MSCs was assessed by measuring liver function tests, hepatic tissue histopathological examination, and alpha-smooth muscle actin (α-SMA) immunohistochemical staining.
Results: Both types of MSCs significantly attenuated *S. mansoni*-induced liver damage four wpt compared to PZQ-treated control group. This was apparent in marked improvement of liver functions and overall hepatic histopathological changes with significant reduction of collagen content and α-SMA positive cells. However, the best results were achieved following AD-MSCs administration with statistically significant differences compared to BM-MSCs.
Conclusion: It was concluded that AD-MSCs are a potentially more potent therapeutic candidate than BM-MSCs in combating hepatic fibrosis in chronic murine schistosomiasis *mansoni*.

Keywords: adipose; antifibrotic therapy; bone marrow; hepatic fibrosis; praziquantel; *S. mansoni*; stem cells.

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INTRODUCTION

Hepatic fibrosis is a reparative process initiated in response to sustained and chronic liver injury[1]. Hepatosplenic schistosomiasis is a prominent etiological factor in the development of hepatic fibrosis, contributing significantly to the burden of disease and mortality in underdeveloped nations, especially in Africa[2]. Several studies employed the chronic schistosomiasis *mansoni* mouse model as an experimental setup to clarify the fibrogenic process[3,4].

The persistent stimulus formed by *Schistosoma* eggs trapped in hepatic tissue portal venules triggers an inflammatory-immune response resulting in a granulomatous reaction[5]. This response is characterized mainly by T helper-2 cytokines (IL-4, IL-5, and IL-13), activated macrophage and eosinophils[6]. Upon stimulation, in response to liver insult, hepatic stellate cells (HSCs) become activated and differentiated into collagen-producing myofibroblast-like cells, the main cells responsible for hepatic fibrosis[7]. Subsequently, collagen deposition, especially types I and III, takes place to heal the damaged tissues with unbalanced levels of matrix metalloproteinase (MMP) to tissue inhibitor of metalloproteinase (TIMP)[8]. This eventually terminates in liver fibrosis and portal hypertension that characterize chronic and advanced stages of infection[9].

Schistosomiasis chemotherapy by PZQ causes effective eradication of mature worms with subsequent reduction of deposited eggs[10]. Nevertheless, no specific therapy is currently being directed to reverse the persisting hepatic fibrosis despite successful schistosomiasis treatment with PZQ. A Chinese cohort study (1995-2019) reported the persistence of hepatic fibrosis in schistosomiasis *japonicum* patients despite repeated PZQ treatment and established parasitological cure[11].

Interestingly, MSCs have gained much attention in treatment of a variety of diseases due to their potential for self-renewal and differentiation[5]. Following systemic infusion, they have the ability to migrate into sites of inflammation and injury[12].

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Notably, MSCs are present in fetal tissues as well as in several adult tissues such as bone marrow and adipose tissue[13]. For years, research mostly focused on BM-MSCs as a potential therapeutic candidate for hepatic fibrosis[14]. Increasing evidence verified that BM-MSCs enhances hepatic regeneration, restores liver functions, and decreases fibrosis in a schistosomiasis-induced fibrotic liver model[4]. Meanwhile, AD-MSCs proved to be an attractive source of stem cells with regenerative properties as those of BM-MSCs. Additionally, they have the advantages of source abundance and minimal harvesting invasiveness[15]. Transplantation of human AD-MSCs was linked to a reduction of fibrosis in a murine model with carbon tetrachloride (CCl_4)-induced liver injury[16]. However, AD-MSCs have yet to be tested in a chronic schistosomiasis murine model. Given the previous success of BM-MSCs as a potential therapy of schistosomiasis-induced liver fibrosis, it is worthwhile to evaluate and compare the effect of BM- and AD-MSCs in ameliorating liver damage and fibrosis in *S. mansoni* chronically infected mice treated by PZQ.

MATERIAL AND METHODS

This experimental randomized controlled study was conducted at the Center of Excellence, Stem Cell Unit, and the Medical Parasitology Department, Faculty of Medicine, Suez Canal University (FOMSCU) during the period from May 2020 to June 2021.

Study design: *S. mansoni* chronically infected mice were treated with PZQ and then subjected to MSCs administration. Mice were sacrificed two and four wpt and evaluated for fibrosis-related clinical, histopathological, and morphometric parameters compared to control groups.

Experimental animals: Seventy-eight male mice (Swiss Albino) of seven weeks age and 20±2 g in weight were employed in this study. The animals were provided by the Theodor Bilharz Research Institute (TBRI), Egypt, and were housed in the animal unit of FOMSCU. Five mice were used as donors for MSCs, three mice were used in a pilot study, while 70 mice were used for the study groups.

Mice infection: Experimentally infected *B. alexandrina* were used as a source of *S. mansoni* Egyptian strain cercariae, TBRI. A subcutaneous injection of 40±5 cercariae/mouse was used to infect the mice through loose skin over the upper back[17]. Infection was confirmed by the presence of *S. mansoni* eggs in mice stool examination 42 d pi[18]. Mice were then kept for eight weeks pi to establish chronic schistosomiasis and liver fibrosis[10].

Study groups: Seventy mice were randomly classified into five groups (14/each) as follows; group I: uninfected control; group II: infected untreated control; group III: infected and PZQ-treated mice (PZQ control); group IV: infected and PZQ-treated mice receiving BM-MSCs (PZQ/BM-MSCs); and group V: infected and PZQ-treated mice receiving AD-MSCs (PZQ/AD-MSCs).

Drug and MSCs administration: On the 9th wpi, groups (III-V) received PZQ (Sedico Pharmaceutical Co., Egypt) to eliminate the current infection. The PZQ tablets (600 mg) were administered as an aqueous suspension in 2% cremophor El, by intragastric infusion of 250 mg/kg/day for three days[10]. On the 13th wpi, control groups (I-III) were intravenously injected in their tail vein with 0.3 ml phosphate-buffered saline (PBS). Mice in groups IV and V intravenously received BM- and AD-MSCs ($1x10^6$ cells/mouse) suspended in 0.3 ml PBS, respectively.

Mice sacrifice: Half of each mice group (n=7) were euthanized by anesthesia overdose after two weeks, while the other half were euthanized after four weeks of MSCs administration.

Confirmation of hepatic fibrosis: During the experiment, a group of three concurrently infected mice treated similar to PZQ control group (not included in the study groups) were euthanized by the end of the 12th wpi. This group was employed to confirm the pathological existence of fibrosis in liver tissue using H&E stain, as well as to ensure infection clearance after PZQ treatment as assessed by hepatic portal system perfusion[10], before proceeding with MSCs treatment in the corresponding study groups.

Isolation and cultivation of MSCs: Donor mice's hindlimbs (femur and tibia) and visceral fat were used to extract bone marrow and adipose tissue, respectively. The BM- and AD-MSCs were cultured in Dulbecco's Modified Eagle's Medium containing 10% heat-inactivated fetal bovine serum and a Penicillin/Streptomycin/Amphotericin B (1%) mixture (Gibco, Thermo Fisher Scientific, US) at 37°C and CO_2 (5%). The cellular growth was evaluated daily under an inverted microscope, and culture media was changed every four days[19].

Morphological characterization of cultured MSCs: Using a phase-contrast microscope, the morphology of cultivated BM- and AD-MSCs was examined on days zero, two, six, nine, and eleven.

Labeling and transplantation of cultured MSCs: When MSCs reached ~80–90% confluence on day 11 post-cultivation, they were impregnated using an injectable ferumoxide solution containing 25 µg Fe/ml (Feridex®) and incubated for 24 h. Iron inside MSCs was later visualized in liver sections of euthanized mice using Prussian blue staining in order to ensure the incorporation of stem cells into liver tissue[20]. On day twelve post-cultivation, MSCs were harvested, PBS rinsed, quantified by an automated cell counter, and

assessed for viability by trypan blue[19] before being administered to mice in the corresponding treatment groups on their 13th wpi.

Parameters used in fibrosis assessment

Clinical parameters

- **Liver and spleen indices:** To study the impact of MSCs on improving the hepatic changes responsible for hepato- and/or splenomegaly, the liver and spleen indices were measured. For each mouse, both the total body weight and the organ weight (liver or spleen) were measured. Liver and spleen indices were then determined by calculating the ratio of organ weight to total body weight[21].
- **Liver function tests:** Blood (~ 1 ml) was withdrawn from mice via cardiac puncture and allowed to clot for 30 min. Serum was collected and kept at -20°C for subsequent valuation of albumin concentration and aminotransferases' levels. Following the method outlined by Savory *et al.*[22], serum albumin concentration was measured using Biuret reagent (Fisher Scientific, US) and was expressed as g/dl[22]. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured according to the method described by Winn-Deen *et al.*[23] using commercial kits (Fisher Scientific, US) and were expressed as IU/l.

Histologic and morphometric parameters: Mice liver samples were fixed in 10% phosphate-buffered formalin solution and subsequently processed into paraffin blocks. Serial sections (4 µ thick) were prepared for staining with H&E, and Masson's trichrome as well as alpha-smooth muscle actin (α-SMA) immunohistochemical staining.

- **Histopathological changes:** The H&E-stained slides were microscopically examined to calculate the mean granuloma number (MGN) and mean granuloma diameter (MGD) in each mice group. The number of granulomas was counted (x100 magnification) in all fields of liver sections of each mouse (3 sections/mouse). Then the MGN for each mouse and for each experimental group was calculated[24]. For measurement of MGD, the diameters of granulomas containing a centrally located *Schistosoma* egg were measured (x100). In each section, the largest ten granulomas were selected for measurement, and the MGD was computed for each mice group[25].
- **Assessment of collagen content:** Masson's trichrome staining was used to measure the area percentage of collagen content (Sigma-Aldrich, St. Louis, USA). The stain colors collagen fibers blue against a red background. For every mouse in each group, five randomly selected fields/section in five sections were examined (x40)[26]. Then the collagen area percentage was evaluated by separating the blue color in appropriate image analysis software (Image Pro Plus, Photoshop).
- **Assessment of α-SMA:** Since α-SMA represents a reliable marker of activated HSCs, its assessment using immunohistochemical staining provides an indicator for fibroblastic proliferation and fibrosis[27]. The α-SMA positive cells appear as spindle to oval cells with brownish cytoplasm. The assessment of immune-stained cells areas was evaluated using a semiquantitative method that determined the area percentage of the immune-stained cells[28].

Statistical analysis: The data were recorded into Excel and SPSS (version 19.0; SPSS Inc, Chicago, IL). The values were presented as mean ± standard deviation (SD). The percentage reduction in the treated mice groups was computed using the equation (C-T/C) ×100, where C represents the mean of the infected untreated control group, while T represents the mean of the treated mice group. Statistical ANOVA and Tukey's post-hock test were used to estimate differences among the experimental groups. Statistical significance was considered at $P<0.05$.

Ethical considerations: The study design and protocol were revised and approved by the FOMSCU Ethics Committee of Research (Approval number: 3193). Experimental animals were treated according to the international and institutional guidelines for animal experimentation.

RESULTS

Morphological characterization of cultured BM- and AD-MSCs: The AD-MSCs exhibited a greater proliferative potential and smaller cell size than BM-MSCs as examined from day zero through day eleven (Figure 1), although both exhibited homogenous population morphology, with a final result of fibroblast-like spindle-shaped cells (Fig. 1i, j).

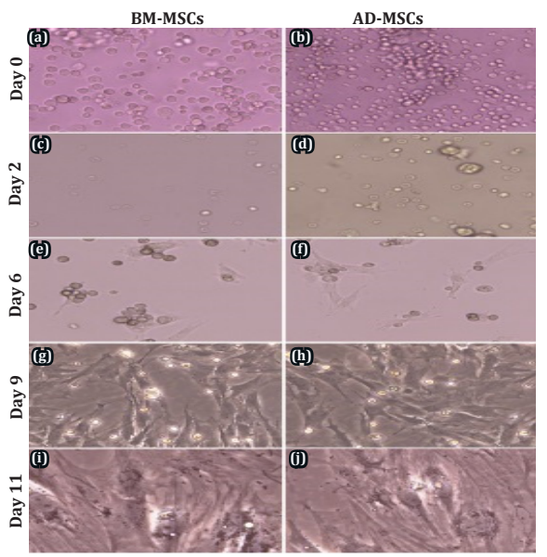

Fig. 1. BM- and AD-MSCs in culture at days 0, 2, 6, 9 and 11 showing smaller size and a greater number of AD- than BM-MSCs with no obvious difference in shape on the same examination day (Phase-contrast microscopy, x100).

Homing of transplanted MSCs in mice's livers: The injected MSCs were properly homed in livers of mice in groups treated with BM- and AD-MSCs. This was proved by the detection of incorporated Prussian blue stained ferumoxide-labelled MSCs within liver tissue of all MSCs-transplanted mice around and nearby the granulomatous lesions. The iron-positive cells were seen as oval cells with intracytoplasmic blue granules (Fig. 2).

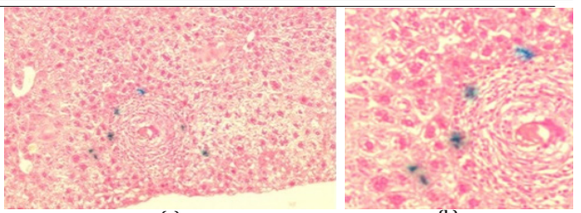

Fig. 2. Prussian blue-stained liver section showing ferumoxide-labelled MSCs with intracytoplasmic blue granules around and nearby *S. mansoni* granulomatous lesion **(a)** (x100); **(b)** magnified view (x200).

Effect of BM- and AD-MSCs treatments on clinical parameters: The liver and spleen indices of mice were reduced in PZQ, PZQ/BM-MSCs and PZQ/AD-MSCs groups compared to the infected untreated control. The reduction was more obvious 4 wpt. The least indices were observed in PZQ/AD-MSCs group, after four wpt with a statistically significant difference as compared to either PZQ or PZQ/BM-MSCs groups at the same time period (Fig. 3).

The liver performance was monitored by estimating the serum levels of albumin, ALT and AST at two and four wpt. Generally, the infected untreated group had the lowest serum albumin levels. At two wpt, there was almost no difference in albumin levels in PZQ, PZQ/BM-MSCs or PZQ/AD-MSCs mice groups. However, at four wpt, both PZQ/BM-MSCs and PZQ/AD-MSCs groups exhibited significant elevation in serum albumin levels (2.98±0.02 and 3.2±0.07, respectively) as compared to PZQ group at the same time period (2.8±0.02). The best result was obtained from PZQ/AD-MSCs at four wpt which was very close to normal control and was significantly different from PZQ/BM-MSCs at the same time period. Likewise, there was an obvious improvement in the serum ALT and AST activities in both PZQ/BM-MSCs and PZQ/AD-MSCs groups at four wpt. The best outcomes were obtained in the PZQ/AD-MSCs group, which exhibited a statistically significant reduction in comparison to the PZQ/BM-MSCs group at four wpt (Table 1).

Histopathological and immunohistochemical examination of stained liver sections: Figure (4) displays H&E-stained liver sections; the normal control group showing intact hepatic lobules with hepatocytes arranged as plates or cords radiating from the central vein (Fig. 4a). Liver sections of

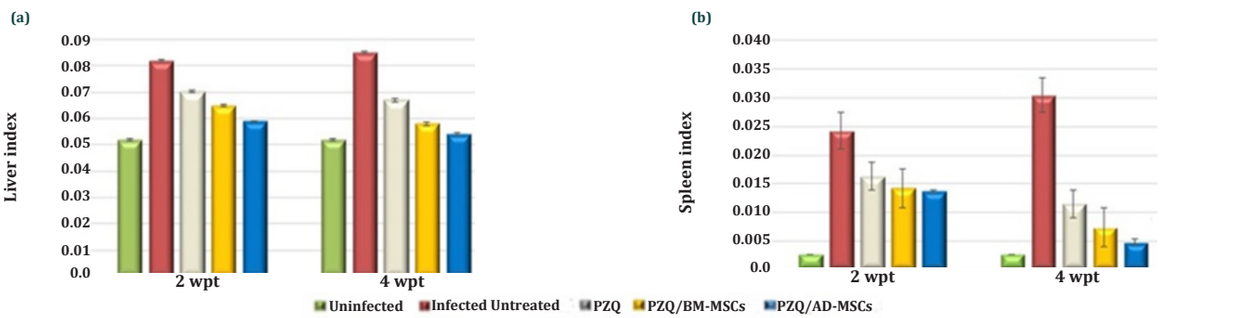

Fig. 3. Liver index **(a)** and spleen index **(b)** in different study groups at two and four wpt. **PZQ:** Praziquantel; **BM-MSCs:** Bone marrow-derived mesenchymal stem cells; **AD-MSCs:** Adipose-derived mesenchymal stem cells; **wpt:** Weeks post-MSCs treatment.

Table 1. Effect of MSCs treatment on liver function parameters in different study groups.

Wpt	Group	Albumin (g/dl) ± SD	ALT		AST	
			Level (IU/l) Mean ± SD	Reduction%	Level (IU/l) Mean ± SD	Reduction%
2	**Uninfected**	3.4 ± 0.06	44.5 ± 1.1	--	52.0 ± 1.3	--
	Infected untreated	2.8 ± 0.01	84.5 ± 3.5	--	134.9 ± 0.95	--
	PZQ	2.9 ± 0.03	73.2 ± 4.1	13.5	100.1 ± 3.7	25.8
	PZQ/BM-MSCs	2.9 ± 0.04	65.4 ± 1.3[a]	22.6	96.96 ± 2.3	28.1
	PZQ/AD-MSCs	2.9 ± 0.03	58.6 ± 1.2[a,b]	30.7	92.5 ± 2.2[a]	31.4
4	**Uninfected**	3.4 ± 0.06	44 ± 1.4	--	52.0 ± 1.3	--
	Infected untreated	2.7 ± 0.01	96.3 ± 3.3	--	141.9 ± 2.2	--
	PZQ	2.8 ± 0.02	72.5 ± 2.02	24.7	94.5 ± 5.3	33.4
	PZQ/BM-MSCs	2.98 ± 0.02[c]	60.8 ± 1.9[c]	36.9	73.9 ± 1.2[c]	47.9
	PZQ/AD-MSCs	3.2 ± 0.07[c,d,e]	48.2 ± 1.3[c,d,e]	49.9	64.2 ± 2.6[c,d,e]	54.8

Superscript letters represent significant difference of the group from [a]PZQ group 2 wpt, [b]PZQ/BM-MSCs group 2 wpt, [c]PZQ group 4 wpt, [d]PZQ/BM-MSCs group 4 wpt, and [e]PZQ/AD-MSCs group 2 wpt. **ALT:** Alanine aminotransferase; **AST:** Aspartate aminotransferase; **wpt:** Weeks post-MSCs treatment.

the infected untreated control revealed many large granulomas around trapped viable eggs, with marked inflammatory infiltrates and fibrosis, sparing only the narrow parts of the liver parenchyma in between (Fig. 4b). In the PZQ control group (two and four wpt), the granulomata number and size decreased moderately with a moderate amount of surrounding inflammatory infiltrates and fibrosis (Fig. 4c). Liver sections of groups two wpt with PZQ/BM-MSCs and PZQ/AD-MSCs showed granulomas with less cellular content and a greater reduction in both number and size, sparing larger areas of hepatic parenchyma in between. The demarcation between granuloma periphery and liver parenchyma was sharp. After four weeks of stem cell transplantation, the granulomas markedly decreased in size, with poor demarcation between them and liver parenchyma, which appeared normal. Additionally, small oval cells with deeply stained cytoplasm and oval nuclei were observed at the periphery of granulomas after four weeks of stem cell transplantation (Fig. 4 d-i). In PZQ/AD-MSCs group four wpt, the hepatic lobular organization was restored, and the majority of hepatocytes appeared normal, even near to the granulomas (Fig. 4h).

Masson's trichrome-stained liver sections (Fig. 5a) from the normal control group showed few collagen fibers around portal tracts and central veins. In sections of the infected untreated group (Fig. 5b), there was an abundance of collagen fibers concentrically arranged around the center of granulomas and dispersed in the nearby liver parenchyma. In the PZQ control group (two and four wpt), the collagen fibers content in granulomas was slightly decreased (Fig. 5c), while in the PZQ/BM-MSCs and PZQ/AD-MSCs groups, there was an obvious reduction in granulomas collagen content (Fig. 5d-i). The reduction was more noticeable at four wpt, especially in the PZQ/AD-MSCs group, where collagen fibers appeared very scanty and fragmented at the central areas of granulomas (Fig. 5h, i).

Immunohistochemically stained liver sections in the normal control group (Fig. 6a, b) showed α-SMA positive cells in tunica media of central veins, fusiform cells surrounding bile ducts and along blood sinusoids. The infected untreated group showed brownish regions of numerous immunostained cells within and in between granulomas, indicating marked active fibroblastic proliferation (Fig. 6c, d). In the PZQ control group (two and four wpt), liver sections showed a mild decrease in brownish immunostained regions (Fig. 6e, f). Both PZQ/BM-MSCs and PZQ/AD-MSCs groups at two wpt showed a moderate reduction of immunostained regions (Fig. 6g, i), while at four wpt, the reduction was more obvious in both groups, especially in the PZQ/AD-MSCs group (Fig. 6h, j).

Analysis of morphometric parameters: Both PZQ/BM-MSCs and PZQ/AD-MSCs groups at four wpt revealed a marked reduction in MGN and MGD (Table 2). The reduction was statistically significant in comparison to the same groups at two wpt and to the PZQ group at four wpt. However, these parameters were significantly lower in the PZQ/AD-MSCs group when compared to those in the PZQ/BM-MSCs group at four wpt. Similarly, the area percentage of collagen

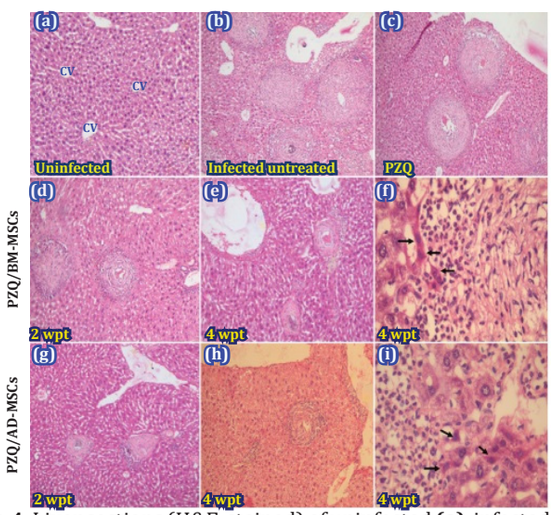

Fig. 4. Liver sections (H&E-stained) of uninfected **(a)**, infected untreated **(b)**, PZQ **(c)**, PZQ/BM-MSCs 2 and 4 wpt **(d-f)**, PZQ/AD-MSCs 2 and 4 wpt **(g-i)** groups showing marked reduction in granuloma count and diameter in PZQ/AD-MSCs group 4 wpt with poor demarcation between granulomas and liver parenchyma (**a-e, g and h:** x40; **f and i:** x200). Arrows represent oval cells with deeply stained cytoplasm and central nucleus. **CV:** Central vein; **wpt:** Weeks post-MSCs treatment.

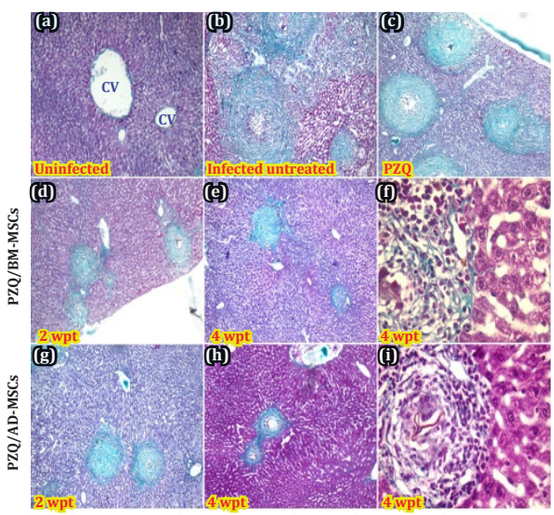

Fig. 5. Masson's trichrome-stained liver sections of the uninfected group **(a)** showing few collagen fibers around CV, infected untreated group **(b)** showing an abundant amount of collagen fibers within and nearby granulomatous lesions, PZQ group **(c)**, PZQ/BM-MSCs 2 and 4 wpt **(d-f)**, PZQ/AD-MSCs 2 and 4 wpt **(g-i)** showing the least amount of collagen fibers that appear scanty and fragmented in PZQ/AD-MSCs 4 wpt (**a-e, g and h:** x40; **f and i:** x200). **CV:** Central vein; **wpt:** Weeks post-MSCs treatment.

and expression of α-SMA were significantly reduced in the PZQ/BM-MSCs and PZQ/AD-MSCs groups at four wpt with a significantly lower percentage reported in the PZQ/AD-MSCs group as compared to the PZQ/BM-MSCs group (Table 3).

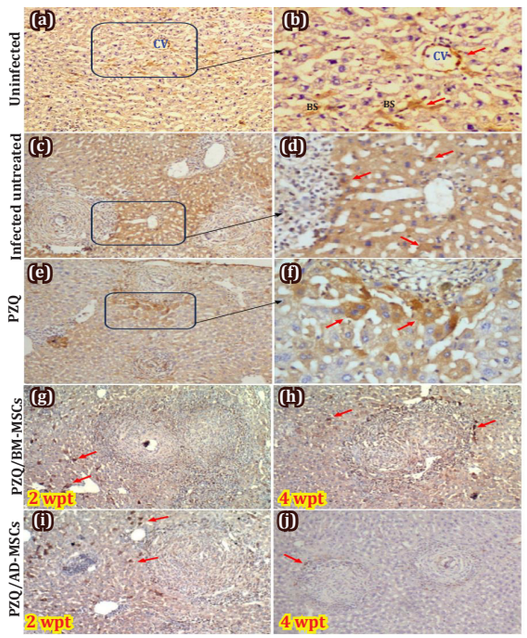

Fig. 6. Liver sections (immunohistochemically stained for α-SMA) of the uninfected group **(a, b)** showing brownish positive cells around CV and BS, infected untreated group **(c, d)** with numerous brownish areas of positive cells, PZQ group **(e, f)** showing positive cells concentrated mainly around granulomas, PZQ/BM-MSCs group 2 and 4 wpt **(g, h)**, PZQ/AD-MSCs group 2 and 4 wpt **(i, j)** showing marked decrease in immunostained regions in PZQ/AD-MSCs group 4 wpt with few scattered positive cells around granulomas (x40 and magnified areas at x200). Arrows indicate positive immunostained cells. **α-SMA:** Alpha-smooth muscle actin; **CV:** Central vein; **BS:** Blood sinusoids; **wpt:** Weeks post-MSCs treatment.

Table 2. Effect of MSCs treatment on granulama number and diameter in different study groups.

Wpt	Groups	Granuloma No. Mean ± SD	Reduction%	Granuloma diameter (µm) Mean ± SD	Reduction%
2	**Uninfected**	--	--	--	--
	Infected untreated	22.4 ± 1.1	--	302.3 ± 5.9	--
	PZQ	18.2 ± 1.3	18.8	267.6 ± 6.4	11.5
	PZQ/BM-MSCs	14.6 ± 1.8[a]	34.8	261.7 ± 2.6	13.4
	PZQ/AD-MSCs	13.2 ± 2.05[a]	41.1	216.4 ± 4.3[a,b]	28.4
4	**Uninfected**	--	--	--	--
	Infected untreated	21.6 ± 1.1	--	282.1 ± 5.7	--
	PZQ	17.6 ± 1.5	18.5	228.7 ± 1.9	18.9
	PZQ/BM-MSCs	9.8 ± 0.8[b,c]	54.6	170.7 ± 9.1[b,c]	39.5
	PZQ/AD-MSCs	6.8 ± 0.8[c,d,e]	68.5	103.6 ± 3.6[c,d,e]	63.3

Superscript letters represent significant difference of the group from [a]PZQ group 2 wpt, [b]PZQ/BM-MSCs group 2 wpt, [c]PZQ group 4 wpt, [d]PZQ/BM-MSCs group 4 wpt and [e]PZQ/AD-MSCs group 2 wpt. **wpt:** Weeks post-MSCs treatment.

Table 3. Effect of MSCs treatment on the hepatic area percentage of collagen and α-SMA expression in different study groups.

Wpt	Groups	Collagen % ± SD	Reduction%	α-SMA expression % ± SD	Reduction%
2	**Uninfected**	1.7 ± 0.05	--	1.5 ± 0.2	--
	Infected untreated	19.3 ± 0.69	--	21.2 ± 1.1	--
	PZQ	14.3 ± 0.73	25.9	17.4 ± 0.4	17.9
	PZQ/BM-MSCs	11.2 ± 0.76[a]	42	14.6 ± 0.6[a]	31.1
	PZQ/AD-MSCs	9.6 ± 0.26[a,b]	50.3	13.8 ± 0.4[a]	34.9
4	**Uninfected**	1.7 ± 0.05	--	1.5 ± 0.2	--
	Infected untreated	29.7 ± 1.3	--	22.6 ± 2	--
	PZQ	11.9 ± 0.98	60	12.4 ± 0.6	45.1
	PZQ/BM-MSCs	9.5 ± 0.38[b,c]	68	10.4 ± 0.7[b,c]	54
	PZQ/AD-MSCs	7.2 ± 0.78[c,d,e]	75.8	6.2 ± 0.7[c,d,e]	72.6

Superscript letters represent significant difference of the group from [a]PZQ group 2 wpt, [b]PZQ/BM-MSCs group 2 wpt, [c]PZQ group 4 wpt, [d]PZQ/BM-MSCs group 4 wpt, and [e]PZQ/AD-MSCs group 2 wpt. **α-SMA:** Alpha-smooth muscle actin; **wpt:** Weeks post-MSCs treatment.

DISCUSSION

Schistosomiasis is a prevalent helminthic chronic infection in tropics, contributing to the death of more than 11792 people yearly[29]. Although chemotherapy using PZQ confers great success in infection elimination, the advanced hepatic fibrosis resulting from granulomatous liver affection in chronic schistosomiasis is irreversible[30]. Our current study aimed to compare the effect of BM- and AD-MSCs in ameliorating liver fibrosis in *S. mansoni* chronically infected mice treated by PZQ.

In the current study, both BM- and AD-MSCs displayed a uniform population of fibroblast-like spindle-shaped cells, with AD-MSCs having a higher proliferation potential and smaller cell size than BM-MSCs. Similarly, Li *et al.*[31] demonstrated no obvious significant differences regarding the morphology of these MSCs; however, differences could be observed in colony frequency, which was higher in AD- than BM-MSCs[31]. Previously, a significant abundance of colony-forming unit-fibroblast was reported in AD- than in BM-MSCs[32].

The efficient MSCs' homing to the injury site is essential for initiating tissue repair and regeneration[31]. This was confirmed in our study where ferumoxide-labelled MSCs were detected in liver histopathological sections of MSCs-treated mice using Prussian blue stain ensuring the presence of transplanted MSCs in treated mice's livers. A similar approach was previously employed to highlight the presence of transplanted MSCs in liver tissue[33]. The homing capacity is apparently attributed to MSCs' expression of a cytokine (stem cell-derived factor-1) from the chemokine family that could promote MSCs mobilization, recruitment, and entrapment[31].

Additionally, we demonstrated that MSCs treatment promoted the recovery of the clinical parameters in schistosomiasis *mansoni* chronically infected mice, with significant improvement in PZQ/AD-MSCs treated group close to normal levels four wpt. This was evident by the reduction of liver and spleen indices as well as improvement of liver performance monitored by Albumin, ALT and AST levels. Marked reduction of liver and spleen indices was previously reported following BM-MSCs administration in *S. japonicum*-infected mice with liver fibrosis[21]. Moreover, transplantation of BM-MSCs restored serum albumin and suppressed transaminases activity in an experimental model of liver fibrosis[34]. On the other hand, improvement in liver weight and normalization of ALT was reported in *S. mansoni*-infected mice treated with AD-MSCs following PZQ treatment[35]. El-Mahdi *et al.*[3] suggested that the generation of new hepatocytes following MSCs treatment could improve overall hepatic functions. Besides, the reversal of dysregulated functions of fibrotic liver after MSCs treatment has been attributed to the prevention of parenchymal cells' apoptosis and/or suppression of inflammatory cells infiltration and proliferation[3].

Our examination of mice's liver sections stained with H&E in MSCs-treated groups revealed significant improvement in the hepatic histopathological changes with detection of newly formed oval cells within liver parenchyma, especially along the periphery of the granulomatous lesions after four weeks of MSCs transplantation. Meanwhile, the most profound reduction in MGN and MGD was among PZQ/AD-MSCs mice group at four wpt. In accordance, marked improvement in the hepatic histopathological changes was reported in *S. mansoni*-infected mice treated with BM-MSCs by several studies[3,36]. Additionally, significantly reduced hepatic granuloma volume in *S. mansoni*-infected mice treated by PZQ followed by AD-MSCs was demonstrated[35]. Indeed, no comparative studies were performed between BM- and AD-MSCs concerning their corresponding histopathological findings in schistosomiasis liver injury. However, compared to BM-MSCs, AD-MSCs implantation induced better histopathological improvement in hepatic inflammatory activity and fibrosis in rats with CCl_4-induced liver injury, despite the difference being insignificant[37].

Previously, it was shown that oval cells, progenitors of hepatocytes and biliary epithelium, could be induced to differentiate into hepatocytes when hepatocyte proliferation is hampered by severe liver injury[38]. Another previous report demonstrated that liver regeneration following MSCs treatment could occur through stem cells' ability to stimulate the proliferation of resident oval cells to substitute fibrosis-affected areas[4]. A prior study found that the number of these oval cells increased three to fourfold after BM-MSCs administration to *S. mansoni* chronically infected mice[39]. In the current study, these cells were demonstrated only at four wpt in mice groups treated with BM-MSCs or AD-MSCs and did not occur before that time. This finding indicates that at least four weeks were essential for MSCs to stimulate oval cells' proliferation. This agreed with El-Mahdi *et al.*[3] who detected oval cells one-month post-treatment with BM-MSCs and for three months afterwards.

Hepatic fibrosis in schistosomiasis is mainly attributed to excessive extracellular matrix (ECM) deposition over degradation[8]. Collagens are the most prevalent proteins in hepatic ECM. In the fibrotic liver, collagen is mostly produced by activated HSCs after their differentiation into myofibroblast-like cells[7,40]. In our study, a significant reduction in the percentage of collagen fibers was observed two and four wpt with both types of stem cells compared to the PZQ-treated control. Furthermore, the PZQ/AD-MSCs group had the least percentage of collagen fibers four wpt with a significant statistical difference as compared to

the PZQ/BM-MSCs group at the same time period. Several prior studies elucidated the antifibrotic effect of MSCs as reflected by collagen content reduction in hepatic fibrosis[3,26,41]. A significant reduction in hepatic collagen percentage was reported four weeks after BM-MSCs infusion in *S. mansoni* chronically infected mice using either Sirius Red or Masson's trichrome staining[3,26]. This reduction was attributed to an increase in collagen disintegration rather than a decrease in its synthesis, probably through MSCs amending the relative gene expression of MMP and TIMP[3,41].

Likewise, AD-MSC transplantation to CCl_4-damaged rats' livers ameliorated hepatic fibrosis, as evidenced by reduced collagen content using Sirius Red staining[16]. Moreover, AD-MSCs were superior to BM-MSCs in producing fibrinolytic MMPs known to degrade collagen types I and III that constitute the major compartment of fibrotic liver[16]. Another study demonstrated comparable efficacy of both BM- and AD-MSC in attenuating induced hepatic fibrosis using CCl_4 in rats through promoting HSCs' apoptosis and inhibiting their activation and proliferation[37]. On the other hand, no differences in hepatic granuloma collagen deposition were observed after AD-MSC treatment given to acutely-infected *S. mansoni* mice previously treated by PZQ compared to PZQ-only-treated ones[35]. Indeed, the maximum collagen production was found to coincide with the peak of granulomatous reaction, and both are affected by the duration of infection[41].

Expression of α-SMA represents a trustworthy biomarker that reflects HSCs activation and differentiation into a myofibroblastic phenotype and has been directly related to the degree of hepatic fibrosis[17]. Our immunohistochemical study revealed a significant reduction in α-SMA positive cells two and four wpt with both types of MSCs compared to PZQ control group at the same time period. The least percentage of immunoreactive cells was observed in the PZQ/AD-MSCs group four wpt. These findings indicate successful alleviation of fibrosis following treatment with both types of MSCs as a result of reduction of α-SMA expressing myofibroblasts that are presumed to be the primary source of pathologic ECM, specifically with better results following AD-MSCs treatment.

Previous studies demonstrated a significant reduction in α-SMA positive cells in schistosomiasis mice livers following BM-MSCs treatment[17,36]. Unfortunately, few researches investigated AD-MSCs' effect on animal schistosomiasis models[35]. However, AD-MSCs significantly reduced hepatic α-SMA expression in a liver fibrosis rat model induced by CCl_4[42]. Moreover, on comparing the impact of BM- and AD-MSCs on HSCs α-SMA expression *in vitro* using western blot analysis, the AD-MSCs showed significantly lower α-SMA expression[37]. Suppression of HSCs' activation, and induction of their apoptosis were postulated as potential mechanisms for MSCs antifibrotic effect[36]. Analysis of *in vitro* BM and AD-MSCs cytokine secretion demonstrated significantly higher levels of transforming growth factor β1, and nerve growth factor in AD-MSCs culture medium compared to BM-MSCs culture medium. These cytokines were widely implicated in regulating HSCs' activation, proliferation, and apoptosis[37].

Collectively, results of the present study suggest the ability of both BM- and AD-MSCs to significantly attenuate *S. mansoni*-induced liver damage and enhance hepatic regeneration four wpt, with better results achieved following AD-MSCs treatment. Both kinds of stem cells have shown comparable hepatogenic differentiation potential as represented by similar levels of liver-enriched transcription factors expression[19]. Nevertheless, in several clinical and biological aspects, AD-MSCs are known to be superior to BM-MSCs. Clinically, they have the advantages of source abundance and easy collection manipulation with minimal invasive procedure[14]. Biologically, AD-MSCs possess higher proliferative capacity, longer life span in culture and more potent immunomodulatory effects compared to BM-MSCs[19]. They demonstrated higher expression of interferon-γ, an essential cytokine that controls MSCs' immunomodulatory functions which could further enhance engraftment following allogeneic stem cell transplantation[31].

In conclusion, AD-MSCs are a potentially more potent therapeutic candidate than BM-MSCs in combating hepatic fibrosis in chronic murine schistosomiasis *mansoni*, with best results obtained after four weeks of treatment. The superiority of AD-MSCs was obvious both *in vitro* and *in vivo*. This was represented in higher proliferative potential in culture and better therapeutic impact *in vivo*, with restoration of hepatic functions, marked improvement of the hepatic histopathological changes and significant reduction of collagen and percentage of α-SMA expressing cells. However, the appropriate dosage, route, and time of administration of transplanted AD-MSCs, as well as the possibly involved anti-fibrotic mechanism(s) are important issues that still need to be further investigated.

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