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THE OUTCOMES OF BONE MARROW STROMAL CELL THERAPY IN SCHISTOSOMAL HEPATIC FIBROSIS: AN EXPERIMENTAL STUDY

By

DINA M. ABOU RAYIA[1*] SIRRIA M. ELMARHOUMY[1], HOWAIDA H. ISMAIL[1], FARHA A. ELCHENNAWY[2] AND KHALED R. ZALATA[3]

Department of Parasitology[1], Faculty of Medicine, Tanta University, Departments of Clinical Pathology[2], and Histopathology[3], Faculty of Medicine, Mansoura University, Egypt (*Correspondence:dina_aboraya@yahoo.com. Tel.: +2 (011) 44895025)

Abstract

Liver fibrosis is considered a serious health problem. Liver transplantation is almost the only curative treatment. The limited number of donors and the post-operative complications are major obstacles. Recent reports have shown that mesenchymal stem cells could improve chemically-induced hepatic fibrosis. The purpose of the present study was to evaluate the effect of bone marrow stromal cells (BMSCs) transplantation on hepatic fibrosis in *Schistosoma mansoni*-infected mice. Female mice infected with *S. mansoni* were divided into three test groups. One group received praziquantel drug orally. Another group received BMSCs, obtained from male mice, by intravenous injection. The last group received both. Two control groups were used, one was infected and untreated and the other was uninfected and untreated. Detection of BMSCs hepatic engraftment, morphometric analysis and histopathological evaluation of liver fragments, were done. Level of interleukin-10 (IL-10) mRNA expression was assessed. The results showed that BMSCs engrafted into the liver. There was significant improvement in morphometric and histopathological evaluation of liver fragments, and increase in IL-10 mRNA expression of all treated modalities. Promising result was achieved when BMSCs and PZQ used in treated mice.

Keywords: Stem cells; Bone marrow stromal cells; Mesenchymal stem cells; *Schistosoma mansoni*; Schistosomiasis; Liver fibrosis.

Introduction

Liver fibrosis is considered a serious worldwide public health problem (Friedman, 2003). Whatever its cause, there is to date no specific therapy for this disease and patients only receive treatment for its associated complications. Hence, the development of an effective therapy for hepatic fibrosis is a major aim of medical research (Saez-Lara *et al,* 2006). Schistosomiasis, among liver fibrosis different etiologies, is considered an important leading cause of hepatic fibrosis and portal hypertension especially in the developing countries, contributing to death of over half million people a year (Abdel Aziz *et al,* 2012). It also represents the second most important parasitic disease with a detrimental socioeconomic impact on more than 200 million people living in these countries (Farid *et al,* 2005).

Chemotherapy is an effective treatment that helped dramatically in decreasing the incidence of schistosomiasis. However, it doesn't prevent re-infection and has a little effect on the already developed hepatosplenic complications (Mbaye and Appleton, 2001). So, currently, the almost only curative treatment for patients who develop advanced hepatic fibrosis is liver transplantation. Unfortunately, most patients are unable to undergo transplantation due to limited availability of donors' livers. Many patients die while waiting for liver transpla-nts (Freeman *et al,* 2008).

New therapeutic strategies aiming to minimize damages caused by hepatic fibrogenesis in chronic liver diseases are of great interest (Oliveira *et al,* 2008). The new era of stem cell therapy has shown promising benefits in experimental models of hepatic diseases caused by drug administration (carbon tetrachloride-induced fibrosis) (Fang *et al,* 2004), surgical interventions (bile duct ligation-induced biliary cirrhosis) (George *et al,* 2005) or by genetic disorders as tyrosinemia (Wu *et al,* 2011).

Hepatic fibrosis due to experimental infection with *Schistosoma mansoni* is a model

that reflects the human form of the disease to a great extent. In the mouse model of schistosomiasis, the main immune-inflammatory response is directed against the parasite eggs which when swept to the portal circulation cause periportal hepatic fibrosis which resembles the pipe-stem fibrosis found in severe hepatosplenic form of the disease in human (Andrade and Cheever, 1993). In the present study, we used an experimental model of hepatic fibrogenesis caused by chronic infection with *S. mansoni* in order to evaluate the potential therapeutic effect of transplanted bone marrow-derived stromal cells (BMSCs).

Materials and Methods

Animals: Male and female Swiss Albino mice (4-6wk old) were used as donors and recipients of BMSCs respectively. Male mice donors were used to be able to detect cell engraftment into the recipient female livers by PCR analysis of the Y male chromosome. Animals weighting (20-30g) were kept in the Medical Experimental Research Center of Mansoura University, under controlled conditions of temperature (22±2ºC), humidity (55%±10%) and air renovation. Animals were housed in a 12h light /12h dark cycle (6am-6pm) and allowed normal rodent diet and water *ad libitum*. Animals were dealt with the National Guidelines for Animal Experimentation.

Mice infection with *Schistosoma mansoni:* Infected *Biomphalaria alexandrina* were obtained from the Biological Unit Theodor-Bilharz Research Institute, Giza. Cercarial shedding was done and female mice were infected with 60 *S. mansoni* cercariae/mouse via subcutaneous injection in the thigh (Elkhafif *et al,* 2010). Infection was confirmed 6 weeks post injection by examination of feces for eggs, and those with viable eggs were used in the experiment.

Experimental design: Infected female mice were divided into two main groups; GI: control infected and non-treated and GII: infected treated and subdivided into three subgroups: group GIIa treated with PZQ drug orally that was available as Biltricide (Bayer) 600 mg tablets. It was given in a dose of 500mg/kg /dose in two doses; one was given six weeks post infection and second dose after nine days (Ismail *et al*, 2001). GIIb treated with 10^6 BMSCs eight weeks post infection via intravenous injection into the tail vein (Cho *et al*, 2009). GIIc given PZQ & BMSCs. GIII: Normal control was neither infected nor treated. Each mice group consisted of 20 mice and all mice groups were kept under the same laboratory conditions.

Preparation & transplantation of BMSCs: Femurs and tibiae of male mice were harvested and thoroughly cleaned of all muscle tissues. According to the method described by Dobson et al. (1999), the bone marrow was flushed from the medullary cavities using 25-gauge-needle with Dulbecco's Modified Eagle Medium (DMEM) into a centrifuge tube. The cell suspension was centrifuged at 1200 rpm for 5 minutes then the supernatant was removed and the pellet was suspended in 3ml media followed by filtration through a 70-µm nylon mesh filter. The cells were cultured in DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 2mM L-glutamine (all from Gibco). The culture flasks were maintained at 37ºC in a 5% CO2 incubator for about 24 hours (Fig. 1A). Then, the non-adherent cells were removed leaving only adherent cells to which fresh media was added. At approximately 80%- 90% confluence (Fig. 1B), cells were detached from the culture flasks using 0.25% trypsin-EDTA and re-plated. The cells were purified up to four passages, then detached, checked for viability using trypan blue and counted using the haemocytometer in the 4 squares used for WBCs count where the number of viable cells/ml = number of viable cells counted in the 4 squares × dilution factor × 10.000. Finally, 10^6 cells were injected I.V. into the tail vein of each mouse.

Male-specific Sry gene detection in female mice livers: To confirm the presence of male donor-derived cells in the livers of female

recipient mice, polymerase chain reaction (PCR) analysis of sex determination region on the Y chromosome (male-specific *Sry* gene) was carried out. Ten weeks post infection, mice were subjected to euthanasia with ketamine and xylazene, and their livers were taken. DNA extraction was done using DNA extraction kit (QIAamp® DNA Mini Kit). DNA amplification was done using Taq PCR Master Mix Kit all was supplied by QIAGEN, Germany. Mice-specific *Sry* gene primers according to Fang *et al.* (2004) were (5`CAGCTAACACTGATCTTTTC3`) and (5`TTACTGAGCCAGAATCATAG3`). Amplified products of PCR were detected by gel electrophoresis.

Histopathological estimation of hepatic granulomas size: Paraffin blocks were made from the livers previously fixed in 10% formol-saline. Serial (5µm) sections were cut from each sample. The sections were stained with haematoxylin and eosin and with Masson's trichrome stain. The diameter of hepatic granulomas was measured using the fully automated Leica Image Processor with automated stage and the Leica Quin software 2004. Five randomly selected microscopic fields / liver section were examined at 40X magnification. Three liver sections for each mouse were examined. The hepatic granulomas mean diameter was determined (Ali and Hamed, 2006).

Determination of the inflammatory reactions density: The density of inflammatory reaction within the hepatic granulomas was determined (Botros *et al*, 2007), using a modified semi-quantitative histologic evaluation of inflammatory cellular infiltrate in five microscopic fields of highest inflammatory intensity at magnification 40x: Grade 0: no inflammation, Grade I: minimal (≤ 25 %), Grade II: mild (26-50%), Grade III: moderate (51%-75%) and Grade IV: marked (>75%).

Determination of hepatic parenchymal necrosis: Hepatic parenchymal necrosis was assessed (Ishak *et al*, 1995). The presence of visible small necrotic foci in the hepatic parenchyma away from the granuloma areas in five randomly selected fields at magnification 10x and expressed due to the severity as follows: Grade 0: no necrosis, Grade I: minimal (<2 necrotic foci/lobule), Grade II: mild (2-5 necrotic foci/lobule), Grade III: moderate (6-10 necrotic foci/lobule) & Grade IV: marked (>10 necrotic foci/lobule).

Morphometric assessment of liver fibrosis (Abdalla et al, 2009): the area of liver fibrosis was quantitated in percentage in Masson's trichrome stained liver sections using automated Leica image processor with automated stage and Leica Quin software 2004. Briefly, the liver section slide stained with Masson's trichrome was placed on the motorized stage of Leica microscope. At a magnification of 10X, automated sequential digitalized images were taken, converted into a binary image and stored. The area of liver parenchyma was considered as the reference area and the fractional surface occupied by fibrosis (the blue area) was measured in comparison to the reference area. A 20 random fields/group was analyzed independently and the percentage of the fibrotic areas in the livers was calculated by dividing the total blue-colored areas by the total areas of the livers.

Detection of IL-10 mRNA expression in liver tissue: Detection of IL-10 mRNA expression in liver tissue of all mice groups was done by reverse transcription polymerase chain reaction (RT-PCR). Briefly, RNA isolation and purification was done using TRIZOL Reagent supplied by (Invitrogen). Purified RNA was reversely transcribed into DNA (cDNA) followed by PCR amplification using RT-PCR One Step kit supplied by QIAGEN by the following primers (3`GCA GGACTTTAAGGGTTACT5`) & (5`TCAT GGGCCTTGTAGACACC3`) after Abdel Ghaffar *et al*. (2000). Detection of PCR amplified products was done by gel electrophoresis and all positive cases were analyzed by spectrophotometer where OD= 50µg/ ml & concentrations were expressed in ng/µl.

Statistical analyses: Data were computeri-

zed & tabulated using mean, standard deviation and chi-square test by SPSS V.16. Differences were significant if $P \leq 0.05$.

Results

Table 1: Percentage of stem cell engraftment into liver in groups.

Mice groups		GIIb	GIIc	Total
+VE	Number	15	16	31
	%	75	80	77.5
-VE	Number	5	4	9
	%	25	20	22.5
Total	Number	20	20	40
	%	100	100	100
Chi-Square	X^2	0.140		
	p-value	0.704*		

Table 2: Size of granulomas (µ) / liver section in groups

Mice	GI	GIIa	GIIb	GIIc	GIII
Mean (µ)± SD	216.1±26.1	133.8±22.8	116.1±13.8	108.5±7.82	-
Range(µ)	153-244	110-180	100-150	100-130	-
% of reduction	-	38.1%	46.3%	49.7%	-
F. test	19.632				
p. value	0.001**				
Scheffe test					

Mice	GI&IIa	GI&IIb	GI&IIc	GIIa&IIb	GIIa&IIc	GIIb&IIc
p. value	0.001**	0.001**	0.001**	0.006*	0.003*	0.048*

Table 3: Percentage of hepatic fibrosis groups

Mice groups	GI	GIIa	GIIb	GIIc	GIII
Mean (%)± SD	6.45±0.99	2.55±0.18	1.74±0.24	1.27±0.10	-
Range (%)	4-7	2-2.7	1.2-2	1.2-1.5	-
% of reduction	-	60.5%	73.1%	80.3%	-
F. test	40.325				
p. value	0.001**				
Scheffe test					

Mice groups	GI&IIa	GI&IIb	GI&IIc	GIIa&IIb	GIIa&IIc	GIIb&IIc
p. value	0.001**	0.001**	0.001**	0.001**	0.001**	0.006*

Table 4: IL-10 mRNA expression (ng/µl) in groups

IL-10	GI	GIIa	GIIb	GIIc	GIII
Mean ng/µl± SD	32.5±3.5	41.5±2.8	46.8±0.86	49.7±0.55	20.6±1.2
Range (ng/µl)	30-37	37.5-47	45-48	48-50	20-24
Increased expression%	36.6%	50.4%	55.9%	58.5%	-
F. test	32.250				
p. value	0.001**				
Scheffe test					

Mice	GIII&IIa	GIII&IIb	GIII&IIc	GIIa&IIb	GIIa&IIc	GIIb&IIc
p value	0.001**	0.001**	0.001**	0.041*	0.009*	0.044*

Transplanted BMSCs were found in livers of *S. mansoni*-infected mice: Presence of donor male-specific *Sry* gene in mice groups that received BMSCs (GIIb) and GIc was confirmed, 10 wks post infection, by using PCR analysis in the livers of female recipient mice infected with *S. mansoni* (Fig. 2). In GIIb, the stem cell engraftment was (75%), and reached (80%) in GIIc with a total engraftment of (77.5%) in both groups ($p = 0.704$). These results suggested that peripherally intravenously injected BMSCs were capable of liver engraftment in mice infected with *S. mansoni* (Tab. 1).

BMSCs therapy improved morphological the hepatic changes in *S. mansoni*-infected mice: Liver sections of *S. mansoni* infected mice were examined 10wks post infection, the GI showed a granulomatous inflammatory reaction with multiple, large sized-granulomas of cellular and fibrocellular types. The cellular granulomas showed marked collection of inflammatory cells (Fig. 3A). Some sections showed the adult worm in the

portal tract (Fig. 3B). The inflammatory cellular infiltrate within the granulomas was of grade (IV). After the treatment with PZQ, BMSCs or both, a great positive impact was observed on schistosomal hepatic pathology, where all detected granulomas were mainly of fibrotic type with the cellular elements markedly decreased and replaced by fibrous tissue (Fig. 3C, 3D, 3E). The inflammatory cellular infiltrate within the granulomas was of GIII in mice GIIa, grade (II) in mice GIIb and grade (I) in mice GIIc (Fig. 4). Mean diameter of hepatic granulomas was significantly reduced after all types of treatment in comparison to the GI. Also, mean diameter of granulomas in GI was 216.1µ ± 26.1, and reduced to 133.8µ±22.8 in GIIa, 116.1µ± 13.8 in GIIIb and 108.5µ±7.82 in GIIc (p=0.001). Highest percentage of reduction in the mean diameter of granulomas was in both PZQ and BMSCs treated GIIc (Tab. 2). As to the hepatic parenchymal necrosis in mice groups; demonstrated in GI there was moderate hepatic parenchymal necrosis of grade (III), while in GIIa there was minimal hepatic parenchymal necrosis of grade (I) and in GIIb and GIIc no hepatic parenchymal necrosis was detected (Fig. 5).

Therapy with BMSCs reduced liver fibrosis in *S. mansoni* chronically infected mice: Liver sections of *S. mansoni* were analyzed. In GI, the mean percentage of hepatic fibrosis was 6.45% ± 0.99, but with significant decreased in other groups. In GIIa, mean percentage of hepatic fibrosis was s significantly decreased to 2.55%±0.18 (p = 0.001). In GIIb), mean percentage of hepatic fibrosis was significantly decreased to 1.74%± 0.24 (p=0.001). In GIIc, mean percentage of hepatic fibrosis was significantly decreased to 1.27% ± 0.10 (p value = 0.001) (Tab. 3).

Transplantation of BMSCs increased Il-10 mRNA expression: Expression of IL-10 m RNA (ng/µl) in all mice. In GIII, mean value of IL-10 mRNA expression was 20.6ng/ µl±1.2, but was significantly increased in all other groups (p=0.001). In GI, IL-10 mRNA expression increased to 32.5ng/µl±3.5, while it reached 41.5ng/ µl±2.8 in GIIa, 46.8ng/ µl±0.86 in GIIb, 49.7ng/µl±0.55 in GIIc. Highest increased IL-10 mRNA expression was 58.5% in GIIc followed by 55.9% in GIIb, 50.4% in GIIa and least was in GI and reached 36.6% (Tab. 4; Fig. 6).

Discussion

Liver fibrosis and its end stage cirrhosis, represent a serious worldwide public health problem. Most patients with cirrhosis die from one or more clinical complications as ascites, hepatic encephalopathy and variceal bleeding (Bataller and Brenner, 2005). Among 1.4 million liver disease-related deaths each year worldwide, over 55% of them are directly attributed to cirrhosis (Dai *et al*, 2009). Many reports have demonstrated that transplantation of bone marrow-derived stem cells in experimental models of hepatic fibrosis could improve liver function, decreases fibrosis index and contributes to parenchymal regeneration (Fang *et al*, 2004; Zhao *et al*, 2005; Piryaei *et al*, 2011). In the present study, the effects of BMSCs transplantation in a model of chronic liver disease caused by *S. mansoni* infection were given. The recruitment of BMSCs to liver lesions was recorded in several models of liver diseases (Oliveira *et al*, 2008, Elkhafif *et al*, 2010; Piryaei *et al*, 2011). In the present study, liver injury caused by *S. mansoni* infection elicited the migration of peripherally injected BMSCs into the livers of female recipient mice. However, in the current study, BMSCs were not detected in all liver samples. This could be attributed to the engraftment of some cells in organs other than the liver e.g. colon. Schistosomiasis is considered a systemic disease affecting organs other than the liver producing widespread tissue injuries which may have favored the migration of BMSCs to these injured-tissues. Elkhafif *et al.* (2010) reported that organ injury was the key factor directing the migration of systemically-injected stem cells and that no engraftment occurred when stem cells were injected into normal non-injured-mice, supported our explanation.

Fibrosis is a common feature of the chronic liver diseases. It is characterized by increasing deposition of extracellular matrix proteins, portal hypertension due to obstruction of the blood vessels and focal ischaemic lesions. All of these features contribute to alterations in liver structure and function. Although spontaneous regression of fibrosis occurs when the stimulus for liver damage is removed, in some liver diseases e.g. viral hepatitis, the stimulus can't be completely removed in addition to being a slow process (Kumar and Sarin, 2007). Thus, schistosomiasis proved an interesting model of chronic fibrotic liver diseases even when worms die, the presence of eggs in tissues releasing soluble egg antigens contributes to continuous occurrence of many fibrotic lesions.

In the present study, there was a significant improvement of hepatic pathology after all treatment modalities (PZQ, BMSCs or both), with the best results obtained when PZQ and BMSCs were simultaneously administered. As demonstrated by reduction in granuloma size, density of inflammatory reaction and hepatic parenchymal necrosis. Also, the morphometric assessment of total liver fibrosis revealed marked reduction. Besides, as the BMSCs were administered intravenously, this reveals that fibrosis doesn't hamper the influx of cells to liver of *S. mansoni*-infected mice.

The improvement of hepatic fibrosis after BMSCs transplantation was also recorded by other studies (Fang *et al,* 2004; Zhao *et al,* 2005; Almeida-Porada *et al,* 2010). The mechanisms underlying the improvement in liver fibrosis and liver functions observed BMSCs transplantation in various experimental models of chronic hepatic injuries are still a matter of researches. Many reports have suggested that MSCs may act via paracrine secretion of factors which help in organ repair. Ozaki *et al.* (2002) demonstrated the ability of BMSCs to express hepatocyte growth factor (HGF) which increases the expression and activity of proteases that are involved in the breakdown of extracellular matrix. Besides, Higashiyama *et al.* (2007) and Chang *et al*. (2009) reported that the stem cell paracrine expression of the matrix metalloproteinases (MMPs) facilitated recovery from chemically-induced liver damage. So, stem cells in the fibrosis resolution. BMSCs might also have an inhibitory effect on quiescent hepatic stellate cells (main fibrogenic cells concerned with the production of extracellular matrix proteins) preventing their activation into myofibroblasts (Zhao *et al,* 2005). They reported also the ability of stem cells to induce apoptosis of the activated hepatic stellate cells. Thus, they prevented the fibrosis occurrence. Also, Oliveira *et al*. (2008) reported that TGF-β was significantly lowered after bone marrow transplantation to mice with hepatic fibrosis. They stated that this cytokine was considered as fibrosis-promoting cytokine and its reduction denotes resolving of fibrosis.

Almeida-Porada *et al*. (2010) reported that a potent anti-inflammatory effect of bone marrow derived the mesenchymal stem cells when transplanted into rats with chemical fibrosis. This may explains the reduction in the density of inflammatory reaction and size of hepatic granulomas observed in the current study. Parekkadan *et al*. (2007) recorded that mesenchymal stem cells (MSCs) have an anti-apoptotic activity for hepatocytes and verified presence of some anti-apoptotic molecules such as the vascular endothelial growth factor and hepatocyte growth factor (HGF) in MSCs culture medium. This explained the absence of parenchymal necrosis, in mice treated with BMSCs, reported in the present study.

Improvement of liver regeneration and functions after stem cell therapy was clarified by Jung *et al*. (2009) who reported the differentiation of infused stem cells into hepatocyte-like cells. These hepatocyte-like cells expressed albumin and α fetoprotein. Also, Tsai *et al*. (2009) found that stem cell engraftment in liver was associated with an increase in prolactin hormone which was linked to liver regeneration. Also, Fang *et al*.

(2004) noticed an increase in the number of oval cells (hepatic progenitor cells), they attributed this increase in the number of oval cells to the ability of stem cells to stimulate resident oval cells to proliferate and replace areas where fibrosis degradation occurs. So, stem cells may promote liver repair via prevention of hepatocyte apoptosis together with replacement of damaged hepatocytes by normal functioning cells.

In the present study, using PZQ drugs caused significant improvement in hepatic pathology. But, the best improvement was obtained when both PZQ and BMSCs were given together. The mechanism of action of PZQ in schistosomiasis was studied by many authors who stated that PZQ treatment mainly affects the adult schistosomes causing their paralysis and destruction. Hence, egg deposition and subsequently associated fibrosis is dramatically decreased. These authors added that PZQ had a potent anti-fibrotic and anti-inflammatory effect (Caffrey, 2007; El-Lakkany *et al,* 2012). This may explain the improvement of hepatic fibrosis with PZQ treatment, observed in the current study. And the best results were obtained when BMSCs were given with PZQ, this could be referred to the elimination of adult worms by PZQ and reduction of fibrosis by BMSCs.

Cytokines, mainly those produced by Th2 cells, have a very important role in schistosomiasis. They can help in controlling the pathology of schistosomal granulomas by the production of a state of the immune-modulation decreasing both inflammation and fibrosis, thus contributing to less adjacent tissue injury (Von Boehmer, 2005). One of the chief soluble mediators with known immune-regulatory functions that induced early after exposure to schistosome cercariae, was IL-10 (Hogg *et al,* 2003).

Interleukin-10 is a cytokine which has wide range regulatory effects upon antigen presentation, co-stimulation and development of acquired T cell responses. This immune-regulatory effect is highly beneficial to patients suffering from schistosomiasis concerning regulation of the inflammatory response against egg deposition that may cause more aggressive pathology, with reduction of granuloma size and influx of inflammatory cells (Moore *et al,* 2001). It was also found that the transgenic mice over-expressing Il-10 from hepatocytes shown to be protected from fibrosis. This proved the anti-fibrogenic properties of this cytokine (Muddu *et al,* 2007).

In the present study, IL-10 mRNA expression was measured as an anti-fibrotic related marker and to determine if the used therapy performed its anti-fibrotic action via IL-10. According to the current study, all treatment modalities caused significant increase in IL-10 mRNA expression with the highest increase in mice received both PZQ and BMSCs. The present findings coincide with those of Wilson et al. (2011) who recorded increase in IL-10 producing cells following PZQ therapy using IL-10 green fluorescent protein reporter mice. Also, Parekkadan *et al*. (2007) found that co-culture of mesenchymal stem cells (MSCs) and hepatic stellate cells (HSCs) caused apoptosis of HSCs and marked reduction of collagen production by these cells. When MSCs were devoid of their IL-10, they lost their effect on HSCs. This highlights that stem cells may exhibit reactions via paracrine mechanisms involving IL-10.

In the present study, BMSCs were engrafted into injured livers, improved fibrosis percentage, and decreased hepatic granulomas size with mild inflammatory reactions and non- parenchymal necrosis and provoked the production of immune-regulatory IL-10 cytokine. When combined with the PZQ gave good results. It was hypothesized that these cells acted via paracrine secretion of factors that degraded the extracellular matrix proteins. Moreover, they mostly produced an immune-modulatory environment that decreased the tissue inflammatory reaction together with protection of the residual hepatocytes from apoptosis as observed by the

absence of hepatic parenchymal necrosis. But, many aspects offering the grounds for optimism in this concern were discovered recently, the most important is that British scientists not only proved that stem cells could produce hepatocytes, and have grown the world's first artificial liver from umbilical cord blood stem cells (Jurga *et al*, 2010).

Conclusion

The outcome results showed that BMSCs improved schistosomal hepatic fibrosis specially when combined with PZQ in a mice model raising the possibility for their clinical use after assurance of their safety. Stem cell research, in spite of the great progress in the last few years, is still in its infancy and more in-depth studies are needed. Thus, there is a great hope that stem cell therapy may be the golden key for all patients suffering from end stage liver diseases after the assurance of their complete safety.

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Explanations to figures

Fig. 1A: Rounded glistening BMSCs on second day of culture.
Fig. 1B: BMSCs one week after cultivation. Majority acquired fibroblastic spindle shape morphology.
Fig. 2: PCR analysis of *Sry* gene in liver of experimental mice 10 weeks post infection, 800bp band in male normal liver. No signal was detected in control (GI), a distinct band in GIIb & GIIc.
Fig. 3A: Liver section of GI (control infected, non-treated) showing multiple schistosomal granulomas. Some of cellular types while others fibrocellular. Some showed *S. mansoni* ova (H&E 100X).
Fig. 3B: Liver section of an infected female mouse GI showed adult *S. mansoni* in portal tract (H&E 200X).
Fig. 3C: Liver section of GIIa showed one small well defined granuloma surrounding *S. mansoni* ovum (MT 100X).
Fig. 3D: Liver section of GIIb (BM MSCs-treated) showing one small well defined granuloma (MT 100X).
Fig. 3E: Liver section of GIIc (PZQ+BM MSCs-treated) showing minute bluish bands of fibrous tissue with lack of significant fibrosis (MT 100X).
Fig. 4: Relation between grades of inflammatory reaction within hepatic granulomas in groups. Grade I: ≤ 25% Grade II: 25%-50% Grade III: 51%-75% Grade IV: >75%
Fig. 5: Relation between the grades of hepatic parenchymal necrosis in groups. Grade I: < 2 necrotic foci / lobule Grade II: 2-5 necrotic foci / lobule Grade III: 6-10 necrotic foci / lobule Grade IV: > 10 necrotic foci / lobule.
Fig. 6: Gel electrophoresis of IL-10 mRNA expressions in groups showing positive bands at 242 bp.