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THE USE OF MICROENCAPSULATED HEPATOCYTES TRANSPLANTATION REDUCES MORTALITY AND LIVER ALTERATIONS IN *SCHISTOSOMA MANSONI* INFECTED HAMSTERS

By

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Abstract

Hepatocyte transplantation is an attractive therapeutic modality for liver disease as an alternative for orthotropic liver transplantation. The goal of this work was to study the adequacy of intrasplenic hepatocyte transplantation (HCTx) in fresh and microencapsulated forms, in a hamster model of liver fibrosis by *Schistosoma mansoni* infected hamsters were divided into 6 groups; untreated for 11 weeks (GI) and for 15 weeks (GII), treated with praziquantel (PZQ) 7 weeks PI, and killed 4 weeks (GIII) and 8 weeks (GIV) post-treatment. Treated with PZQ 7 weeks PI, and then treated orally with immunosuppressive drug "cyclosporine (4 weeks post PZQ treatment), 24 hr. before interasplenic injection with fresh hepatocytes (V). Treated with PZQ 7 weeks PI, and then injected interasplenically (4 weeks post-treatment) with microencapsulated hepatocytes (GVI). GI & GIII were killed 11 weeks PI for assessment the anti-schistosomal efficacy of PZQ. The other four groups were killed 15 weeks PI for investigation of liver and spleen histology, serum liver enzymes and hepatic oxidative markers before and after HCTx. Freshly isolated hepatocytes with a mean viability 92.97±1.2% were used for microencapsulation and transplantation. Histological study showed the presence of transplanted hepatocytes in spleen of recipient. PZQ accelerated healing of hepatic granulomatous lesions as evidenced parasitologically by the increase in the percentage of dead eggs and histologically showing more granuloma circumscription with more ova degeneration and less inflammatory cells. The 25-day survival rates in GII, GIV, GV& GVI were 5/15 (33.3%), 8/15 (53.3%), 10/15 (66.7%) and 9/15 (60%) respectively. In addition, there were significantly better outcomes in serum biochemical indexes such as ALT, AST, γ-GT, ALP, and hepatic SOD and MDA in the fresh and microencapsulated groups than in PZQ-treated group, without great differences between the microencapsulated and the fresh transplanted groups. Liver pathological staining supported these findings.

Keywords: HCTx, *Schistosoma mansoni*, PZQ, microencapsulation, liver enzymes, oxidative stress, lipid peroxidation.

Introduction

Schistosomiasis is caused by several worm species of the genus *Schistosoma* and afflicts up to 600 million people in 74 tropical and sub-tropical countries in the developing world (El Ridi *et al*, 2014). Present disease control depends on treatment with the only available drug praziquantel. Chronic infection by *Schistosoma mansoni* (*S. mansoni*) is one of the experimental models of hepatic fibrosis used to elucidate the mechanisms involved in the fibrogenic processes. In schistosomiasis, the main immune inflammatory response is directed against the parasite eggs, which, when led to portal circulation, may become lodged into hepatic portal venules, eliciting a granulomatous response (Oliveira *et al*, 2008). In addition to schisto-

somiasis, hepatopathies due to alcohol, viral hepatitis, drugs, metabolic and autoimmune diseases, and congenital abnormalities are important causes of liver fibrosis (Bataller and Brenner, 2005).

Liver fibrosis occurs in the setting of chronic injury caused by different etiologies constituting a serious worldwide public health problem. Whereas in acute hepatic injury nonviable cells are replaced by normal tissue, during chronic injuries a persistent repair response may lead in fibrosis and scar formation as a result of an imbalance between proliferation and degradation of the extracellular matrix components (Bataller and Brenner, 2005). The increased deposition of extracellular matrix causes structural alterations in the liver and in its function, and portal hypertension due to the obstruction of vessels and focal ischemic lesions (Kumar and Sarin, 2007).

New therapeutic strategies aiming to minimize damages caused by hepatic fibrogenesis in chronic liver diseases are of great interest. Orthotropic liver transplantation "OLT" which include whole organ transplantation considers the only available effective treatment for patients with severe and end stage chronic liver diseases. However, the chronic shortage of donor livers finds parallel growth of transplant waiting list so substantial proportions of patients die while waiting for a donor graft (Akhter, 2007). This motivate the development of various alternative methods to conventional liver transplantation such as treatment to alleviate chronic or terminal liver illnesses at least as a temporary procedure until organ procurement is possible (Palmes, 2000; Yang, 2007). The most satisfactory results are obtained from hepatic cell transplantation by using a graft of already differentiated cells such as hepatocytes, which means transferring of normal hepatocytes into diseased liver to allow the retention of hepatic functions until procuring an organ and in some cases can achieve hepatic regeneration (Eugenia, 2010). Strom and Fisher (2003) reported that hepatocytes transplanted in the liver or ectopic sites such as spleen could support liver function in times of hepatic insufficiency.

Liver has been considered as an optimal site for hepatocyte transplantation; however even in this organ, the survival rate of transplanted hepatocytes is extremely low in comparison to spleen as hepatocytes are well engrafted when injected into splenic pulp and they are entrapped in the sinusoid and vascular spaces (Akhter, 2007). The spleen is considered as the most privileged anatomical site for hepatocyte transplantation. It is accessible by means of laparotomy. It can entrap a limited, but a sufficient number of hepatocytes within its sinusoids, providing conditions very similar to natural cell microenvironment. This process has been described by Mito in the early 1980's, by the term splenic hepatization. The main obstacle for wider usage of hepatocytes transplantation is the immune rejection or their rapid elimination by recipient macrophage (Gewartowska, 2007). The microencapsulating technique initiated by Lim and Sun (1980) brought new hopes for artificial liver and hepatocyte transplantation as result of immunoisolation of these cells and retaining in a semi permeable membrane that both protects them from immune system and maintain their survival and metabolic function (Akhter, 2007). In this study, a hamster model of liver fibrosis was induced by *S. mansoni* infection. The present work examines the parasitological criteria expressing *S. mansoni* infection and the resultant histopathology and biochemical changes in liver likely to be affected. The therapeutic efficacy of hepatocyte transplantation (HCTx) either being fresh or microencapsulated in splenic pulp, was investigated through biochemical and histological examinations.

Material and Methods

Drugs and doses: Praziquantel tablets (Distocide®, EIPICO, El-Asher Men Ramadan, Egypt) was given orally as a suspension in 2% cremophore-El (Sigma-Aldrich Chemical Co., St. Louis, MO) in a dose of

1000 mg/kg (Gonnert and Andrews, 1977) each divided equally on 2 consecutive days. Neoral®, immunosuppressive drug "25mg cyclosporine" (Sandimmune, Novartis, Pharma AG, Basilea, Suiza) was given orally in single dose of 15 mg/kg b.w 24 hr before transplantation.

One hundred and five male Syrian golden hamsters, average weight 110 g ± 20, were bred and maintained at the Schistosome Biology Supply Center (SBSC) of Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Animals were housed in a controlled temperature and light environment, and were given water and commercial chow *ad libitum*. The animal experiments were conducted at the animal unit according to the international ethical guidelines for the care and use of animals for research purposes.

Cercariae of *S. mansoni* (Egyptian strain) were obtained from infected intermediate host snails maintained at SBSC. Hamsters were infected intraperitoneal with a single dose of 50±5 cercariae/hamster (Liang *et al*, 1987).

One hundred and five hamsters were divided into 3 main groups: donor group (10 hamsters), normal group (5 hamsters), and *Schistosoma* infected group (90 hamsters) which subdivided into six subgroups each containing fifteen hamsters at the start of the experiment as follow: untreated for 11 weeks (I) and for 15 weeks (II). Treated with PZQ 7 weeks PI, and killed 4 weeks (III) and 8 weeks (IV) post-treatment. Treated with PZQ 7 weeks PI, and then treated orally with immunosuppressive drug "cyclosporine (4 weeks post PZQ treatment), 24 hr. before interasplenic injection with fresh hepatocytes (V). Treated with PZQ 7 weeks PI, and then injected interasplenically (4 weeks post PZQ treatment) with microencapsulated hepatocytes (VI). Groups I and III were killed 11 weeks PI for assessment the antischistosomal efficacy of PZQ. The other four groups were killed 15 weeks PI for assessment of liver function and histology. After killing by decapitation, blood was collected and sera were separated by centrifugation at 1850 *g* for 10 min and stored frozen at –70°C pending assay.

Hepatocytes isolation: Hepatocytes were isolated using Seglen's *in situ* collagenase perfusion technique (Seglen, 1979), in which perfusion of the liver was done firstly with Krebs's Ringer Buffer "KRB" (137 mmol NaCl, 5.3 mmol KCl, 0.8 mmol Mg $SO_4.7H_2O$, 0.4 mmol Na_2HPO_4, 0.4 mmol KH_2PO_4, 5.5 mmol glucose, and 5 mmol HEPES), then liver digested by collagenase buffer (100 ml of KRB, 0.025 gm $CaCl_2$, and 0.05 gm collagenase) (Berry, 1991). After isolation of hepatocytes, count and test viability of yield cells was performed by using 0.4% trypan blue exclusion test.

Microencapsulation of hepatocytes: Isolated cells were microencapsulated according to Lim and Sun (1980) and Fritschy *et al*. (1991) methods with modifications. Hepatocytes were centrifuged at 1700 *g* for 2 minutes before resuspension in 4% sodium alginate (ALG) -normal saline solution (pH 7.2) at a concentration of $1x10^7$/ml. The suspension was stirred adequately into a homogeneous mixture before being injected into the syringe pump and dropped into 100 mmol/L calcium chloride solution (HEPES buffered, pH 7.2). Droplets were then washed in 4°C saline and reacted in sequence with 0.05% poly-L-lysine (PLL) for 8 minutes, Droplets were then rewashed in 4°C saline, and reacted with 0.2% (w/v) ALG for 4 minutes, then rewashed again in saline and finally it reacted with 30 mmol/l sodium citrate (SC) for 8 min.

Transplantation of fresh and microencapsulated hepatocytes: Fresh and microencapsulated hepatocytes were suspended in sterile normal saline in aseptic conditions. Transplantation of one million-cell suspension of fresh and microencapsulated hepatocytes was done by injection of cell suspension slowly in hamster's spleen. For intrasplenic transplantation of hepatocytes, hamsters were anesthetized and a small surgical incision was made in the flank and the

spleen was exposed. Freshly harvested hepatocytes suspended in normal saline were injected into the inferior pole of the spleen using a 25-gauge needle connected to a 1 ml syringe without constriction of the blood vessels. Homeostasis was secured with a ligature around the spleen proximal to the injection site. Before transplantation of fresh hepatocytes, hamsters were treated with cyclosporine as immunosuppressive drug.

Assessment of parasitological criteria: To recover mature and immature worms for subsequent counting and determination of sex, hepatic and mesenteric vessels of animals were perfused according to Duvall and DeWitt (1967), with no resort to general anesthesia. The number of ova per gram of liver or intestinal tissue (tissue egg load) was counted according to the method of Cheever (1968), in which a piece of small intestinal or hepatic tissue was weighed before digestion in 5% KOH. The percentage of different egg developmental stages (Oogram pattern) was studied (Pellegrino *et al*, 1962), in which eggs at different stages of maturity were identified (immature eggs) according to the size of the embryo and were counted. In addition, mature eggs containing fully developed miracidium and dead eggs (granular, dark, and semitransparent) were also counted in 3 fragments of small intestine and the mean number of each stage was calculated.

Histopathology and granuloma measurement: Murine livers and spleens recovered from hamsters were fixed in 10% buffered formalin and processed to paraffin blocks. Liver sections (4µm thick) were cut 250µm in apart from the proceeding sections to avoid measurement the same granuloma. Five paraffin liver sections were prepared from each animal and stained with hematoxylin and eosin (H&E). Measurements of the granulomas were conducted on non-contiguous granulomas, each containing a single egg (with intact or degenerated miracidia), using an ocular micrometer. The mean diameter of each granuloma was calculated by measuring two diameters of the lesion at right angles to each other (Von Lichtenberg, 1962). The percentages of viable and dead eggs were also calculated.

Serum liver enzymes: Concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT), and alkaline phosphatase in the collected sera were estimated by the methods of Reitman and Frankel (1957), Persijn and van der Slik (1976), and Kind and King (1954) respectively.

Assessment of oxidative stress and lipid peroxidation: Liver tissues were homogenized in four volumes (w/v) of ice-cold 0.1 mol potassium phosphate buffer (pH 7.4) containing 1 mmol EDTA, and centrifuged at 10,000 *g* for one hour at 4°C. The supernatant was collected and kept at -80°C for subsequent analysis for determination of liver content of glutathione (GSH) and superoxide dismutase (SOD) activity. The GSH level was determined in liver homogenate according to the method of Ellman (1959). Briefly: 0.5 ml homogenate was added to a tube with 0.5 ml of 10% trichloroacetic acid (TCA). The tubes were centrifuged at 3000 *g* for 10 min. A 0.2 ml aliquot of the resulting supernatant was added to a tube containing 5 ml of 0.1 mol potassium phosphate buffers and 0.1 ml of 5, 5'-dithio-bis-2-nitro benzoic acid solution (DTNB; Ellman's reagent) and the absorbance was measured at 412 nm. With the help of the standard curve drawn using gradual concentrations of a standard GSH, content of GSH in the liver homogenates of the experimental animals were calculated. SOD activity was assayed spectrophotometrically at 560 nm by the procedure of Winterbourn *et al*. (1975). The activity of SOD depends on its ability to inhibit phenazine methosulphate (PMT) mediated reaction of nitroblue tetrazolium (NBT) dye. One unit of enzyme activity is defined as the amount of enzyme that causes half-maximal inhibition of NBT reduction. Activity was expressed in µmole/min/g liver.

Assessment of hepatic lipid peroxidation products

Degree of lipid peroxidation in the liver tissue homogenate of mice was determined in terms of thiobarbituric acid reactive substances (TBARS) formation (Ohkawa *et al*, 1979). One milliliter of supernatant was mixed with one ml of TCA (10% w/v) in a centrifuge tube and centrifuged at 1850 *g* for 15 min at room temperature. One milliliter of TBA solution (0.67 % w/v) was added to one ml of supernatant and kept in a boiling water bath for 45 min. Absorbance was read after cooling at 530 nm against a blank containing all the reagents except the tissue homogenate. As 99% of the TBARS is malondialdehyde (MDA), TBARS concentrations of the samples were calculated using the extinction coefficient of MDA, which is $1.56x10^5$ $M^{-1}cm^{-1}$.

Statistical analysis: Data were analyzed with the use of SPSS software (Version 16.0). Values were expressed as mean± standard error of the mean. Means of groups were compared with the use of an unpaired *t*-test. For comparison of more than 2 groups, an ANOVA test was used. Data were considered significant at *P* value < 0.05.

Results

Number and viability of isolated hepatocytes: A mean of approximately 134±38.8 $x10^6$ of liver cells was harvested from each donor, with a mean viability about 92.97± 1.2%. Hepatocytes isolation from normal liver and their microencapsulation (Fig.1 A & B).

Survival rate: In this study, 66.7% (10/15) and 60% (9/15) of *S. mansoni* infected hamsters treated with PZQ, and transplanted with either freshly isolated or microencapsulated hepatocytes respectively, can stay alive for 25-days post transplantation. On the other hand, *S. mansoni*-untreated and PZQ–treated groups showed survival rate of 33.3% (5/15) and 53.3% (7/15) respectively (Figure 2).

Parasitological studies: Worm load, ova count and oogram pattern after *S. mansoni* infection and PZQ treatment, 11 weeks post infection were summarized in table (1). After *S. mansoni* infection with 50 cercariae for 11 weeks, the total worm burden was 15.2±1.02. Besides, the number of eggs per gram liver and intestine was $6.8x10^3$ and 11.2 $x10^3$ respectively. After treatment of hamsters with PZQ 7 weeks PI, and killed 4 weeks post the end of treatment, the total worm burden was 0.2±0.02 with a percentage reduction of 98.6% compared with the untreated group. Moreover, a significant decrease in both hepatic and intestinal egg count by 99.85% and 99.96% respectively, concomitant with complete absence of immature and mature eggs with complete degeneration of the remaining eggs were observed (Tab. 1).

Biochemical study: Infection of hamsters with *S. mansoni* produced significant elevation ($P<0.05$) in the serum concentrations of ALT, AST, γ-GT and ALP and a marked depletion in the hepatic content of GSH, with significant elevation in SOD activity and MDA level compared to their corresponding normal levels. Compared to the infected untreated group, these elevated serum enzymes were significantly lowered by treatment with PZQ. Moreover, the hepatic content of GSH, SOD activity and MDA level were partially but significantly restored (Tab. 2). After transplantation of hepatocytes either fresh or microencapsulated, these serum and hepatic markers related to liver damage were approximately restored to their normal levels in most test parameters. Moreover, the serum biochemical indexes of ALP, γ-GT and hepatic SOD and MDA in the fresh and microencapsulated groups differed significantly from those in the PZQ-treated group, but there were no great differences between the fresh and the microencapsulated groups in most test parameters (Tab. 3).

Histological study: Normal liver shows arrangement of hepatocytes in liver lobule and spaces between hepatic plates called sinusoid (Fig. 3A). Hepatic tissues of untreat-

ed-infected hamsters (Fig. 3B) showed cellular irregularly outlined granuloma encircling recently deposited intact or partially degenerated ova with a mean diameter of 190.30 ± 3.05 μm. Dosing of PZQ at 1000 mg/kg resulted in nearly complete degeneration of eggs and reduction of granulomatous reactions both in parenchymal or portal areas (Tabs. 1 & Fig. 3C), and in hepatocytes showed mild hydropic degeneration and mild steatotic changes. After intrasplenic injection of PZQ-treated hamsters with either fresh or microencapsulated hepatocytes, and killed 15 weeks PI, spleen showing rounded or hexagonal epithelial cells with rounded nucleus and esinophilic cytoplasm (hepatocytes like cells) (Figure 4 A-C).

Discussion

Schistosomiasis is the most common fibrotic disease to arise due to inflammation and the deposition of scar tissue around parasite eggs trapped in the liver (Burke *et al*., 2010). It is usually characterized by an unnoticed acute phase, followed by liver fibrosis at chronic and advanced stages (Cheever *et al*., 1998). Many investigators have evaluated hepatocyte transplantation (HCTx) as an alternative or supportive procedure to OLTx and have achieved significant progress in clinical therapy (Sokal *et al*, 2003). Some reports stated that isolated hepatocytes have been attacked by immune system of recipient so need to decrease its effect by either administration of immunosuppressive drug or immunoisolation of isolated hepatocytes by which known as microencapsulation of hepatocytes (Zhang *et al*, 2008). Cell transplantation by means of microencapsulation is a promising technique that can ameliorate immune reactions and thus help to induce tolerance as the ultimate goal of transplantation (Kobayashi *et al*, 2000). By reviewing published literatures, this is the first experimental study, investigating the effect of fresh and microencapsulated HCTx, in a model of *S. mansoni* induced hepatic fibrosis.

In this study, all hamsters received *S. mansoni* infection in addition to the presence of hepatic granulomas around deposited schistosome eggs affecting hepatocellular function. The observed elevation in ALT, AST, γ-GT & ALP reflected either acute active or chronic liver damage (Naik *et al*, 2007). This could be associated with malabsorption due to damaged intestinal mucosa resulting from the extrusion of large numbers of eggs, or could be due to decreased synthesis, which may result from parasitic injury to hepatic cells (Oliveira *et al*., 2009). In addition, a significant depletion of GSH content, with elevation of SOD activity and MDA level, which constitutes the first line of defence against free radicals and is a critical determinant of tissue susceptibility to oxidative damage, was observed. This agreed with Gharib *et al*. (1999); Seif el Din *et al*. (2006; 2007). Gharib *et al*. (1999) found that hepatic content of GSH were reduced in the livers of *S. mansoni*-infected mice. They added that the deposition of parasite eggs triggers the release of endogenous eosinophile peroxidase; an enzyme activity developed in the immediate vicinity of the eggs, which increases dramatically with time leading to a decrease in the antioxidant capacity of the liver. In the same context, Hanna *et al*. (2006) found decreased GSH content, increased MDA level and changes in the activities of GSH-related enzymes in patients with liver cirrhosis. Our results suggest that the increased SOD activity may be attributed to reactive oxygen products such as superoxide anions produced by *S. mansoni* worms or the deposited ova. In this context, Shaheen *et al.* (1994) reported increased SOD activities following *S. mansoni* infection and Par *et al.* (2000) recorded such increase in SOD accompanied by a decrease in GPX in patients with liver cirrhosis induced by alcohol or chronic hepatitis C.

After treatment of hamsters with PZQ 7 weeks PI, and killed 4 weeks post the end of treatment, the percentage worm reduction was 98.6% compared with the untreated

group. Moreover, administration of PZQ accelerated healing of hepatic granulomatous lesions as evidenced parasitologically by the increase in the percentage of dead ova in oogram pattern and histologically showing more granuloma circumscription with more ova degeneration and less inflammatory cells. This is in addition to improving serum markers of liver enzymes and decreasing of oxidative stress and lipid peroxidation. In previous studies, PZQ in full dose resulted in a significant reduction of granuloma diameter, number and cellularity. Zwingenberger *et al*. (1990) reported that elimination of adult worms by treatment with PZQ clearly leads to sustained diminution of egg-induced immunopathology. These findings are in accordance with those of Botros *et al*. (2000), El-Lakkany and Nosseir (2007). The antischistosomal properties of PZQ is already known where it prevents the possible formation of the toxic metabolites of *S. mansoni* worms (Schiller and Haese, 1973) and abolition of further insults to the liver through interruption of egg deposition after the death of adult worms, and to its direct effect on mature miracidia within egg granulomas (Hirose *et al*, 2003) and stopping emission of soluble egg antigens. These parasitological criteria-expressing cures indicate removal of the cause of disease (worms and eggs) before hepatocyte transplantation.

Several reports have demonstrated the feasibility and efficacy of cell transplantation in providing specific function in various experimental animal models of human disease. However, without adequate immunosuppression, complications due to tissue rejection remain a significant problem. Microencapsulation of cells within a synthetic semipermeable membrane, prior to transplantation, has been proposed for circumventing immunological complications following transplantation. The microcapsule's semipermeable membrane allows permeant molecules to freely diffuse across while preventing the microencapsulated cells from escaping. This membrane also keeps unwanted substances, such as cells and antibodies, from entering the microcapsule. Thus, microencapsulation provides an innovative and unique technique for the transplantation of foreign tissue and cells without the need for immunosuppression drugs (Dixit and Gitnick, 1995).

In the current study, $1x10^6$ isolated hepatocytes with viability mean 92.97±1.2% were used for transplantation. This is in consistent with that of Badrawy *et al*. (2002) who found successful hepatocyte engraftment in rats when cell viability was higher than 85%. Our study examined the hepatocytes derived from donor liver and capsulated by the APA microencapsulation technique. The obtained microcapsules exhibited a good smooth surface and integrated appearance. Furthermore, living cells inside the microcapsules were > 90% as determined by trypan blue staining. The mortality rate of *S. mansoni*-infected and PZQ treated hamsters transplanted with fresh or microencapsulated hepatocytes were nearly the same and ranging from 33- 40% respectively. Although the mortality rate between the two transplanted groups is comparable to each other, the transplantation of microencapsulated the hepatocytes is more beneficial to avoid the use of immunosuppressive agents with the known undesirable effects. A better survival rate of intrasplenically transplanted hamsters has been demonstrated, suggesting the superiority of the spleen as a site for cell implantation in this experimental model. Pilichos *et al*. (2004) stated that, the rats intrasplenicaly transplanted with hepatocytes succeeding to survive until day 6 post-transplantation. In addition, there were significantly better outcomes in serum biochemical indexes such as ALT, AST, γ-GT, ALP, and hepatic SOD and MDA in the micrencapsulated group than in the PZQ-treated group, but no great differences were observed between the fresh and the microencapsulated groups. These findings agreed with Bin *et al*. (2012) who stated that two weeks after hepatocytes were transplanted into the rats; serum levels of

transaminases were significantly reduced. The rate of reduction in γ-GT level in the group administered cyclosporin with hepatocyte transplantation was lower versus their level in microencapsulated group and this may be due to postoperative cholestasis, which in accordance with Fusai *et al*. (2006) who stated that administration of either cyclosporin or azathioprine after liver transplantation was associated with severe cholestasis. In this study, SOD activity was elevated after hepatocyte transplantation than normal, meanwhile, GSH was reduced. These data were in accordance with Hassan *et al*. (2005) who stated that reactive oxygen species (ROS) play a central role in ischemia reperfusion injury after organ transplantation.

In this study, liver and spleen pathological staining supported the present findings where hepatocyte like cells was observed in spleen sections. The reason why the latter two groups showed no difference requires further exploration, although it is possibly related to the lower number of encapsulated cells. Some studies supported the notion that microcapsules provide the encapsulated cells with a good living space, and can significantly increase their survival time, thus theoretically reduce the number of transplanted cells (Wang *et al*, 2005).

The intrasplenic transplantation of hepatocytes is an effective strategy for the treatment of acute or chronic liver failure and several congenital metabolic defects in animals (Kobayashi *et al*, 2000a; b). Furthermore, intrasplenic transplantation of hepatocytes has been demonstrated to be the most effective way to transplant hepatocytes because only by this method can exogenous gene-modified hepatocytes survive, express the target gene effectively and release the target products into the peripheral blood other than those transplanted intraperitoneally or subcutaneously (Raper, 1995; Fox *et al*, 1998). Zhang *et al*. (1997) showed that NeoR gene modified hepatocytes transplanted intrasplenically into mice could migrate to the liver within 24 h after transplantation and express the exogenous gene for 11 weeks and intrasplenic transplantation of IL-2 gene-modified hepatocytes could effectively activate liver immune function and exert potent therapeutic effects on liver carcinoma in mice (Cao *et al*, 1996).

Conclusion

The intrasplenic microencapsulated HCTx could provide a temporary liver support and reducing mortality in *S. mansoni*-infected animals, because its mechanism is not only related to the immunosuppressive and substitution effects of the transplanted cells, but also is associated with liver repair promoted by the transplanted cells. Although there are still many unanswered questions regarding the mechanisms of action of transplanted cells in hepatic lesions, our results reinforce the use microencapsulated hepatocytes for severe liver diseases.

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Table 1: Effect of praziquantel treatment on worm burden, ova count, egg developmental stages % and histo-pathological changes in *S. mansoni* infected hamsters; 4 weeks post treatment (11 weeks post infection).

Animals	Parasitological parameters						Histopathological changes		
		Ova count x 10^3		Egg development stages %					
	Total worms	Hepatic	Intestinal	Total immature	Mature	Dead	Granuloma diameter	Degenera-ted eggs %	Living eggs%
Infected untreated	15.2 ± 1.02	6.8 ± 0.8	11.2 ± 2.23	52.8 ± 2.6	40.4 ± 2.46	6.8± 0.86	190.30 ± 3.05	29.4± 0.73	70.6± 3.95
PZQ-treated	0.2±0.2*** (98.60%)	0.96± 0.19 *** (99.95%)	0.38 ± 0.07 *** (99.96%)	0.00***	0.00***	100***	91.00± 1.28***	84.5± 4.65***	15.5± 0.86***

Values = mean ± SE, each group= 10 hamsters, PZQ at 7th wk post infection (PI) for 2 days and killed 11 wk PI (4 wk post-treatment). Numbers between parentheses = percentage of reduction from infected untreated.
*** Significantly different from infected untreated group at $P < 0.001$.

Table 2: Effect of praziquantel treatment on serum liver enzymes, antioxidants activity and lipid peroxidation in *S. mansoni* infected hamsters; 4 weeks post treatment (11 weeks post infection).

Animals	ALT (g/dl)	AST (mg/dl)	ALP (U/l)	γ-GT (U/l)	GSH (mmol/g)	SOD (U/g)	MDA (nmol/g)
Uninfected	15.04±1.06	18.3±0.9	27.9 ± 1.2	575.9±1.9	1.68±0.18	180.13±47.3	16.3±0.54
Infected untreated	47.9±3.0‡‡‡	120.8 ± 4.3‡‡‡	48.7 ±1.8‡‡‡	779.3 ± 26.2‡‡‡	0.64± 0.14‡‡‡	986.00± 41.0‡‡‡	49.3±2.9‡‡‡
PZQ-treated	31.2±2.6 ‡‡‡*	22.6 ± 2.1*	44.4 ± 0.3 ‡‡‡	522.3 ± 30 ‡‡‡*	1.00 ± 0.2‡‡‡	501.7 ±59.4‡‡‡*	32.8± 6.4*‡

Values = mean ± SE. In each group= 10 hamsters, ‡‡‡ Significantly different from uninfected group at $P < 0.001$.
* Significantly different from infected untreated group at $P < 0.05$.

Table 3: Effect of usage of fresh and microencapsulated hepatocytes (HCTx) with praziquantel treatment on serum liver enzymes, antioxidants activity and lipid peroxidation in *S. mansoni* infected hamsters; 8 weeks post treatment (15 weeks post infection).

Animal groups	ALT (g/dl)	AST (mg/dl)	ALP (U/l)	γ-GT (U/l)	GSH (mmol/g)	SOD (U/g)	MDA (nmol/g)
Uninfected (n=5)	15.04±1.06	18.3±0.9	27.9 ± 1.2	575.9±1.9	1.68±0.18	180.13±47.3	16.3±0.54
Infected untreated (n= 5)	72.4 ± 6.0‡‡‡	133.8 ± 3.6‡‡‡	81.3 ± 5.5‡‡‡	1011.7 ± 4.3‡‡‡	0.61± 0.18‡‡‡	2038.5 ± 135‡‡	42.1± 4.5‡‡‡
PZQ-treated (n= 6)	30.04 ±2.3‡‡‡***	24.06 ± 1.8***	50.6 ±2.4‡‡‡***	647.4 ± 12.5‡***	1.12 ± 0.2‡‡‡***	604.5 ± 41‡‡‡***	35.7±5.9‡***
PZQ + fresh HCTx (n= 10)	25.08 ± 2.8‡***	18.97 ± 0.6***	37.28 ± 0.3‡***	600.06 ± 2.8***$$	0.53 ± 0.02‡‡‡***$	270 ± 31.5***$$$	20.6±1.14***$
PZQ + microencapsulated HCTx (n=9)	27.1 ± 2.7‡‡***	17.8 ± 0.39***	35.01 ± 0.5***$	563.5 ±2.7***$$$	0.17 ±0.0 2 ‡‡‡***$$$	244.8 ± 36.8***$$$	13.8±0.66***$$

Values = mean ± SE. Hamsters were administered PZQ at 7th wk post infection (PI) for 2 days and then injected interasplenically (11 weeks PI) with either fresh or microencapsulated hepatocytes and killed 15 wk PI (8 wk post the end of PZQ treatment and 25 days post HCTx transplantation). ‡ Significantly different from normal uninfected group at $P < 0.05$, ‡‡ at $P < 0.01$ and ‡‡‡ at $P < 0.001$. *Significantly different from infected untreated group at $P < 0.05$, ** at $P < 0.01$ and *** at $P < 0.001$. $ Significantly different from PZQ-treated group at $P < 0.05$, $$ at $P < 0.01$ and $$$ at $P < 0.001$.

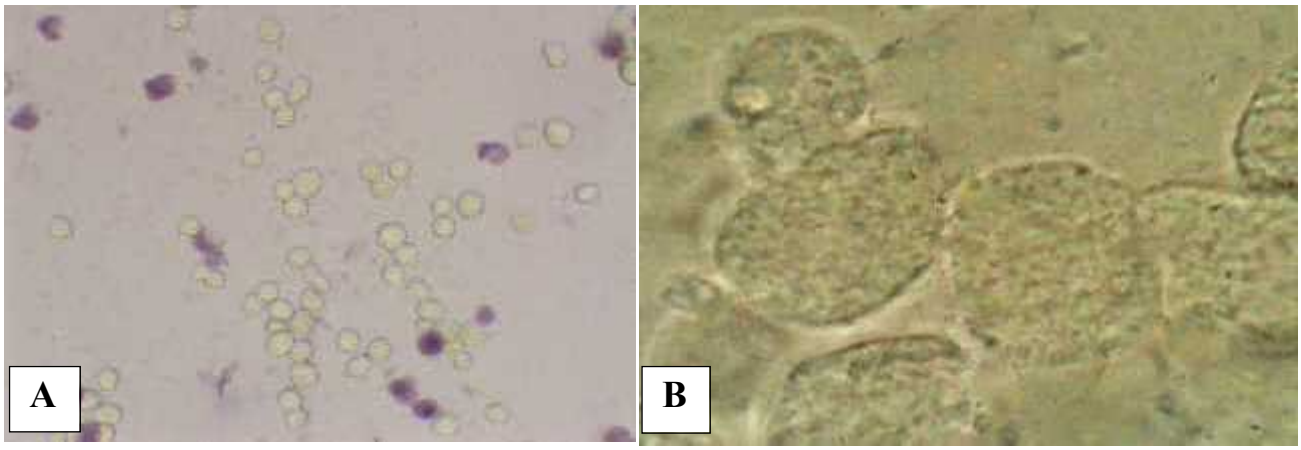

Fig. 1A & B: Hepatocytes isolation from normal liver and microencapsulation: (A) A high power magnification of normal hamster liver isolated hepatocytes (A) showing numerous viable cells that exclude-trypan blue dye and few darkly stained (dead) cells failed to exclude dye (x40). (B) A high power magnification of sodium alginate capsule contains hepatocytes (x100 oil lens).

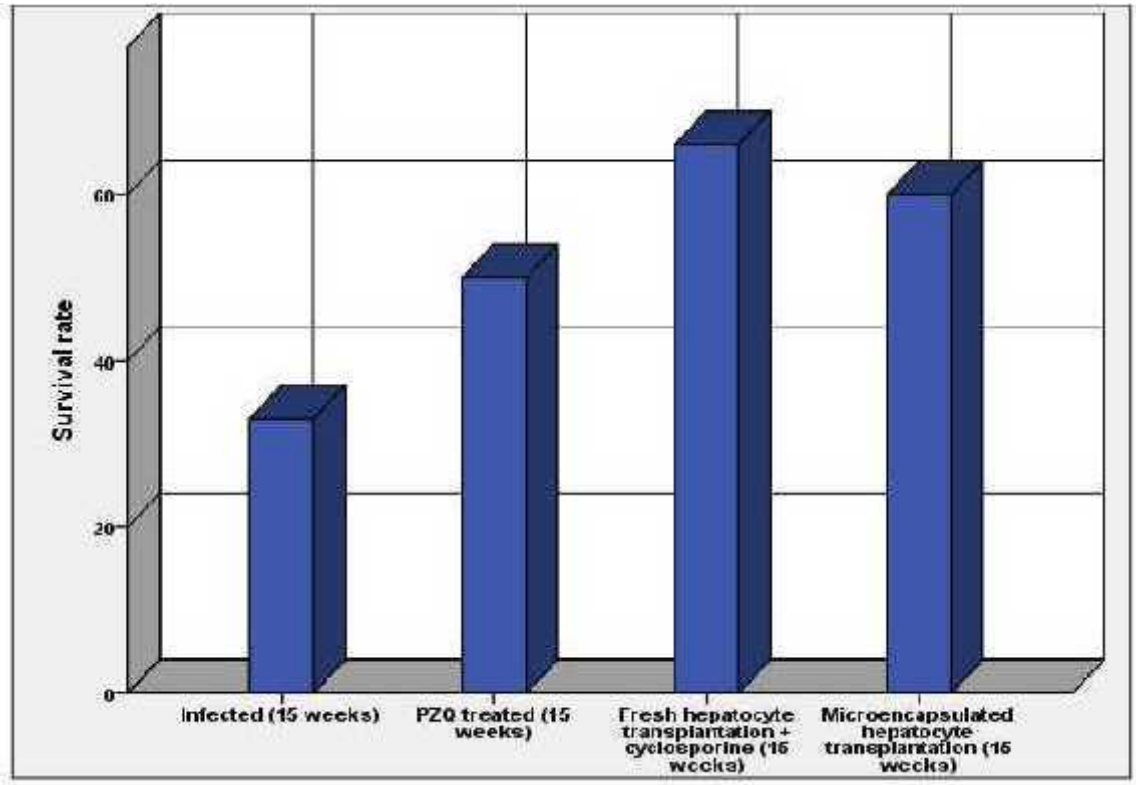

Fig. 2: Survival rate of *S. mansoni* infected hamsters, treated with PZQ, and transplanted with either freshly isolated or microencapsulated hepatocytes, and killed 15 weeks post infection.

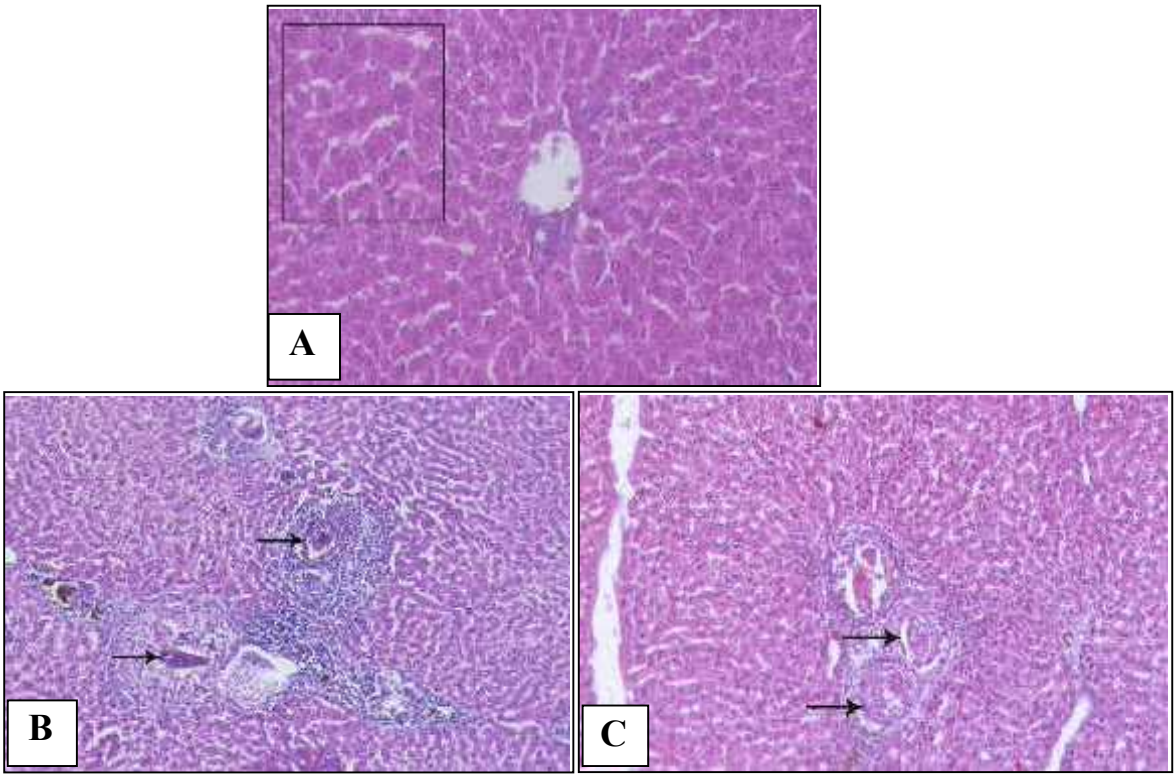

Fig. 3 A-C: H & E stained liver sections: (A) Normal liver section showing liver sinusoid. (B) *S. mansoni* infected hamsters for 11 weeks showing larger size fibrocellular granuloma formed of central egg (with living miracidia), surrounded by lymphocytes, esino-phils & collagen fibers (arrow). (C) Infected & treated with PZQ 7 weeks PI; killed 11 weeks PI showing small (regressive) fibro-cellular granuloma formed of central dead egg & miracidia, surrounded by lymphocytes, esinophils & collagen fibers (arrows, x100).

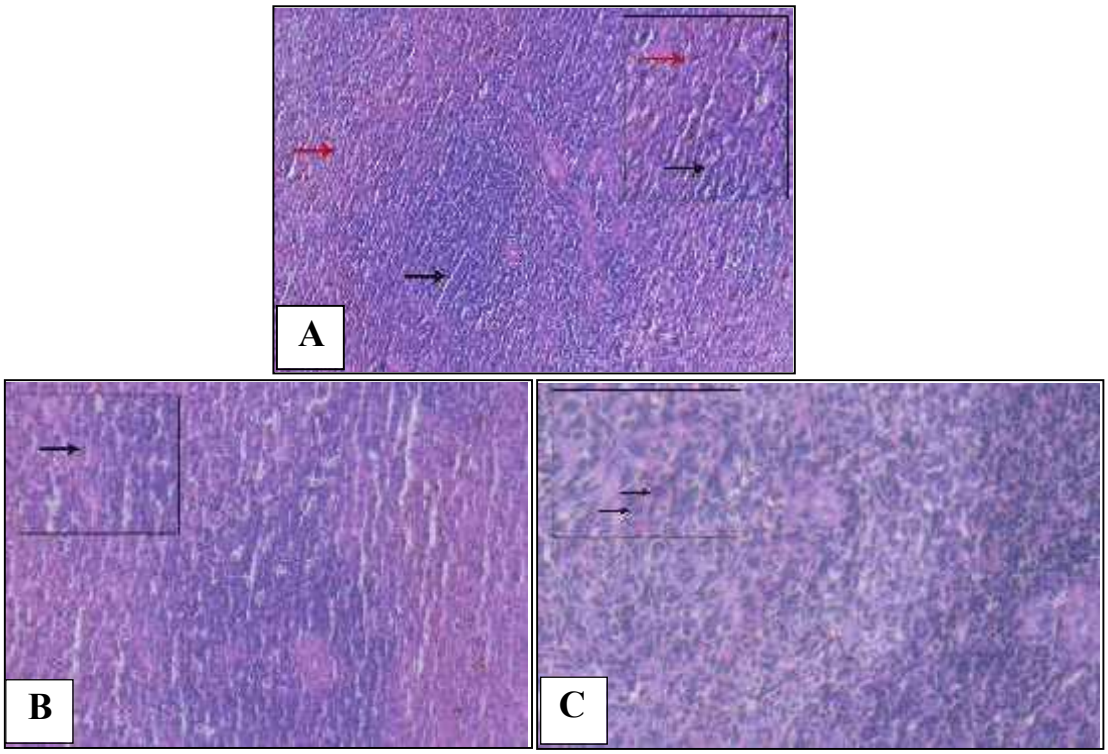

Fig. 4 A-C: H & E stained spleen sections of: (A) Normal spleen of hamster showing red pulp (red arrow), and white pulp (black arrow). (B) *S. mansoni* infected and PZQ treated hamsters transplanted with fresh hepatocytes after cyclosporine administration, and sacrificed 15 weeks PI showing rounded or hexagonal epithelial cells with rounded nucleus and esinophilic cytoplasm (hepatocytes like cells) (arrows). (C) *S. mansoni* infected and PZQ treated hamsters transplanted with microencapsulated hepatocytes in alginate capsule and sacrificed 15 weeks PI showing rounded or hexagonal epithelial cells with rounded nucleus and esinophilic cytoplasm (hepatocytes like cells) (arrows) (x100).