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Original Study

Intrasplenic Autotransplantation of Hepatocytes in Dogs

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Abstract.

Objective- The liver has a unique location in the body for performing multiple functions such as. metabolism. detoxification and synthesis. There are limitation for liver transplantation due to shortage of available donors and requires for sophisticated technology and support surgical teams. The purpose of this study was to investigate the hepatocyte transplantation into the spleen of animal model such as dog to establish basic condition for further studies.

Design- In vivo experimental Study.

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Animals- Five Iranian indigenous mixed breed dogs of both sexes, 15/8+3 months old and 19.6±8.2 weighting were used in this sudy.

Procedures- Ventral midline celiotomy was performed under general aneasthesia and liver sampls were obtained using suction technique by a 50cc syring and 14 gauge needle. Then obtained samples were injected into the same dog's spleen directly and also via splenic artery, the celiotomy incision sutured and the animal left to recover. On the16th postoperative day, under general anaesthesia, the gross apearance of spleen were recorded and photographed and for histpathological evaluation, partial splenectomy was performed. The prepared sections were stained with Hemotoxylin Eosin [H& E] and Periodic Acid Schiff [PAS] and studied under light microscoy.

Results- The gross evaluations of the removed spleen showed a whitish area at the injection site in all of the 5 transplanted dogs, spleen vessels had been dilated and enlarged in diameter. Microscopically the groups of hepatocytes in the center of lymphoid follicles in the white pulp were noted.

Conclusion and Clinical Relevance- Hepatocyte transplantation could be useful for both supporting an acute liver failure and for surviving as a bridge to liver tansplantation in terminal liver failure.

Key words: intrasplenic, hepatocyte, transplantation, dog

Introduction

The liver has a unique location in the body for performing multiple functions such as, Metabolism, detoxification and synthesis. These complex processes occur (to a large extent) in paranchymal cells and impairment leads to high patient M(m)ortality [6, 7]. There are limitation for(in) liver transplantation due to shortage of available donors and requires for sophisticated technology and support surgical teams. During the past decade, interest has resurged in hepatocyte transplantation for metabolic support in liver failure and for the treatment of inherited metabolic disorders. [2, 3, 6, 7] The purpose of this study was to investigate the hepatocyte transplantation into the spleen of (animal models such as) dog to establish basic condition for further studies.

Materials and Methods

Five Iranian indigenous mix breed dogs (2males, 3 females) were used in this sudy. The study was approved by ethic committee of the Shiraz University. (They were handled on humane based study). All operations were performed under injectional general anasthesia using Ketamin Hcl (15 mg/kg, Rotexmedica GMBH, Germany) and Acepromazine (0.2mg/kg, Kla laboratoria, Belgian). Anaesthesia induced with intravenous injection of ketamin and Acepromazine then maintained with ketamin intramusculary during the surgery.Abdominal area from sternum to pubic was shaved and prepared aseptically for surgery. Ventral midline celiotomy was performed and liver sampls were obtained using suction technique by a 50cc syring and 14 gauge needle. Then obtained samples were injected into the same dog's spleen. Obtained samples injected directly into the spleen in different locations (posterior ,middle and anterior end), in all (the five) dogs. Linea alba, subcutaneous tissue and skin were closed routinely. Postoperatively all dogs were monitored clinicaly and received antibiotic (ampicilin, 22 mg/kg, daily injection for five days, Nasr Co) and anti-inflammatory (dexamethazone 2 mg/kg daily injection for five days, Nasr Co) daily for 5 days. (Also) blood sample were collected in ,0, 5, 10, 15, days for evaluation of CBC (compelet blood count) changes. On 16th postoperative day the dogs underwent ventral midline celitomy again under general anaesthesia, and gross apearance of spleen and reactions were recorded and photographed. For histpathological evaluation, partial splencetomy was performed. The spleen samples were fixed by 10% formalin and embeded in parafin blocks. The blocks were then cut in serial section of 4mm-5mm thickness and deparaffinized sections

were prepared with Hemotoxylin Eosin (H& E) and Periodic Acid schiff (PAS) stain and studied under light microscoy.

Results

The gross evaluations of the spleen showed a brownish white area at the injection site in all of the 5 transplanted dogs, spleen vessels had been dilated and enlarged in diameter (figure 1-A. Microscopically, red and white pulps were normal in appearance. The groups of hepatocytes in the center of lymphoid follicules in the white pulp were noted. These hepatocytes depicted typicall hepatocyte morphology. The hepatocyte nucleus was centerally located, round and contained one or two nucleoli. Its cytoplasm was eosinophilic, containing fine basophilic granules (figure 1-B). In the several area of capsule, there was fibroblastic activation, hemorrhage and infiltration of mononuclear inflamatory cells, this zone was thought to be the area where hepatocytes originally were injected into the spleen paranchyma, conveing reactive changes. The periodic Acid- Schiff proved PAS positive granules suggesting the presence of glycogon in the hepatocyte. The results of CBC evaluation and TPR (Temprature, Pulse, Respiration) were in expected normal range.

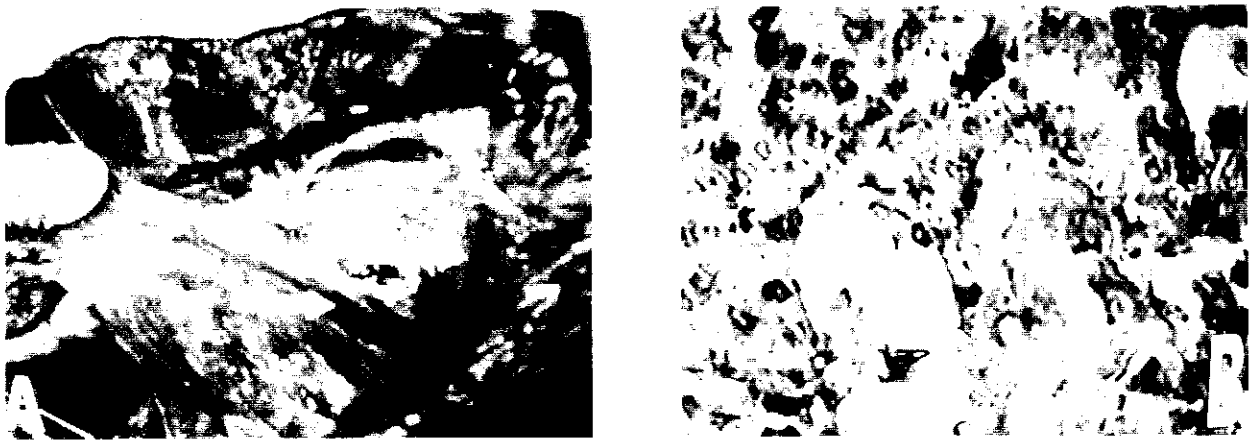

Figure 1. Intrasplenic hepatocyte transplantation. A: Note the splenic artery and vein engorgment after 6 post operative day (green arrow). B: Microscopically the hepatocyte in the center of lymphoid follicles in the white pulp were noted showing central nucleoles (H&E X 400 (yellow arrow).

Discussion

In animal models of acute and chronic hepatic failure the beneficial effects of hepatocyte transplantation in prolonging the survival of the animal have been reported . The majority of the studies have been performed in rats using allograft transplantation of hepatocytes. The successful engraftment of these studies have been dependent on the immunosuppressive drugs use . However in our study autograft transplantation of the hepatocytes have been used and there was no need for immunosupressive drugs postoperatively. Hepatocyte transplantation could be useful for both supporting an acute liver failure and for surviving as a bridge to liver tansplantation in terminal liver failure . The study by Fox, et al demonstrated the beneficial effects of hepatocyte transplantation in treatment of Crigler-Najjar syndrome, reflecting its possible use in other metabolically based liver disease . There are also some studies on the development of liver directed gene therapy . These studies designed to isolate primary hepatocytes, transducing them in vitro

with a therapeutic gene transplantation of the genetically modified cells into recipients. Based on animal works, liver cells can be transplanted into multiple body sites including the spleen, liver, adipose tissue, renal capsul, pancreas and peritoneal cavity[5,6]. Rosenthal et al evaluated five methods of spleen hepatocyte transplantation, includes: 1-Retrograde injection of cells via the splenic vein. 2- Intra arterial injection of cells. 3- Direct intrasplenic injection of cells after laparatomy. 4- Precutaneous intrasplenic injection of cells under laparascopic control. 5- Laparascopic intrasplenic injection of cells. Then he suggested direct intrasplenic injection of cells that could be compatible with survival [13]. Gurden, et al reported that spleen is favorable site for engraftment and survival of transplanted hepatocytes [7]. In another study it was demonstrated that intrasplenically transplanted cells can prolifrate tremendously within several weeks resulting in hepatized spleen [1]. According to these reasons we selected spleen for hepatocyte transplantation and results were succesful.

Conclusion

This study stablishes the feasibility of hepatocyte transplantation in the dog spleen. This modified method of "liver sampling" instead of "pure hepatocyte isolation" was used and successful engraftment was observed grossly and histopathologically. It is clear that this exciting area of hepatology still faces many challenges and that further and more extensive studies are needed to open the way for broad clinical application of hepatocyte transplantation.

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چکیده:

پیوند خودی سلولهای کبدی در طحال سگ

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هدف: بررسی امکان پیوند سلولهای کبدی درون طحال در مدل حیوانی.
طرح: مطالعه تجربی.
حیوانات: پنج قلاده سگ مخلوط بومی از هر دو جنس.
روش کار: بوسیله بیهوشی عمومی خط میانی شکم باز شده و از کبد نمونه هایی به طریق مکش بوسیله یک سرنگ ۵۰ میلی لیتری و سوزن ۱۴ اخذ گردید. نمونه های تهیه شده درون طحال به روش مستقیم درون بافت طحال و همچنین از طریق شریان طحالی تزریق گردید. شکاف خط میانی بخیه گردید و حیوانات به هوش آمدند. در روز ۱۶ بعد از عمل مجدداً حیوانات بیهوش شدند وضعیت ظاهری طحال ثبت گردید و تصاویری تهیه شد. برای مطالعه هیستوپاتولوژیکی قسمتی از طحال قطع گردید. لامهای تهیه شده بوسیله هماتوکسیلین ائوزین (H & E) و (PAS) پریودیک اسید شیفت رنگ آمیزی شد و با میکروسکوپ نوری مطالعه گردید.
نتایج: بررسی وضعیت ظاهری طحال نقاط سفید رنگی در محلهای تزریق نشان داد و قطر عروق طحال بزرگ شده و پر به نظر می رسد. مطالعه میکروسکوپی گروهی از سلولهای کبدی در مرکز یک فولیکول لنفی در لایه سفید رنگی را نشان داد.
نتیجه گیری: پیوند سلولهای کبدی درون طحال هم برای تقویت کبدهای ضعیف و هم در موارد نارسایی حاد کبدی می تواند مفید باشد. نیز برای ایجاد پل ارتباطی تقویتی تا زمان پیوند کبد مناسب در موقعیت های نارسایی نهایی حاد کبدی می تواند مؤثر باشد.

کلید واژه ها: طحال، سلولهای کبدی، پیوند، سگ.