Egyptian Journal of Surgery Vol. (21), No. (1), Jan., 2002

THE VALUE OF HEPATOCYTE TRANSPLANTATION IN THE PROBLEM OF ACUTE LIVER FAILURE IN RATS

By
Tarek Badrawy, M.D., Solafa El Sharawy M.D and Khaled Zalata** M.D.*
Pediatric Surgery Unit, Clinical Pathology Department (Hematology unit)* and Pathology Department** Mansoura Faculty of Medicine, Egypt.

Acute liver failure after extensive hepatic resection constitutes a major cause of morbidity and mortality especially in patients with preexisting liver diseases. Although liver transplantation has been suggested as the most effective treatment of patients with acute liver failure, a variety of technical, functional and logistic obstecles limits its application. Hepatocyte transplantation is a new option for treatment of patients with acute liver failure. In the present study hepatocyte transplantation has been investigated by evaluation of the clinicopathological and laboratory effects in 56 rats with surgically induced acute liver failure by 90% hepatectomy. Rats were divided into two groups: group I transplanted rats including 30 rats in which intrasplenic hepatocyte transplantation has been performed 48 hours before 90% hepatectomy and group II including 26 rats in which no hepatocytes have been transplanted before hepatectomy. Comparison between both groups revealed a significant increase in the weight of the remaining liver mass in group I transplanted rats. There was also a significant improvement in the level of glucose, bilirubin and alanin transferase (ALT) while there was a tendency to improve in the level of albumin and prothrombin time in the same group. Moreover, group I transplanted rats showed a significant prolonged survival time and more long term servivors in relation to the non transplanted group. This clearly demonstrates that hepatocyte transplantation markedly improves the physiological function and regenerative capacity of the remaining liver mass after surgically induced acute liver failure by 90% hepatectomy. Thus hepatocyte transplantation can participate effectively to solve the problem of acute liver failure that may eliminate or even minimize the increasing demands for orthotopic liver transplantation.

Keywords: *Acute liver failure, hepatocyte transplantation.*

INTRODUCTION

Acute liver failure after extensive hepatic resection either due to the presence of underlying liver pathology or major liver trauma constitutes a major cause of morbidity and mortality especially in patients with preexisting liver diseases [1]. At present for practical purposes, some methods to improve liver functions during acute liver failure are not widely applicable. These methods include careful metabolic and nutritional supports, hemodialysis, hemoperfusion and plasmapharesis [2]. The bioartificial liver support devices represent a new horizon to bridge patients with acute hepatic failure until a suitable liver allograft is obtained for transplantation. However it is costy and does not represent a final solution [3,4,5]. Liver transplantation has been suggested as the most effective treatment to solve the problem of acute liver failure. However, a variety of technical, functional and logistic issues limits its application to a relatively small percentage of patients with liver diseases. These obstecles include shortage of donor organs, preservation of donated organs and the side effects of immunosuppression [6].

Recently, hepatocyte transplantation has emerged and increased the interest of many investigators as a simple and practical solution for cases with acute liver failure. Hepatocyte transplantation would be less invasive, less costy and technically less demanding than whole organ

transplantation [7]. The isolated liver cells can be cryopreserved for emergency use and potentially modified genetically before transplantation to abrogate rejection or enhance function [8,9]. However, the effect of hepatocyte transplantation on survival and metabolic support and also the effect regarding the regeneration of the remaining liver with acute liver failure are all still under evaluation. So, the aim of the present study is to demonstrate the value of hepatocyte transplantation in rats with acute liver failure induced by 90% hepatectomy.

MATERIAL AND METHODS

The study has been performed on fifty sex (56) seven weeks old male Lewis rats weighing 200-250 grams. Rats were housed in a climate controlled room (21°c) under a 12-hours light/dark cycle. Rats were given tap water and standard laboratory rat chew (Rodent chew 5001, Ralston Purina) ad libitum. All operation have been performed between 9am and afternoon under general ether anesthesia using clean surgical techniques. Animals were divided into two groups: group I (30 rats) for which hepatocyte transplantation has been performed two days before 90% hepatectomy and group II (26 rats) in which no hepatocytes has been transplanted before hepatectomy.

**** Hepatocyte Preparation:***

Donor hepatocytes were prepared from the livers of allogenic normal Lewis rats. Hepatectomy was performed on normal Lewis rats anesthesized with ether. The liver was perfused with cold (4°c) saline through the portal vein until the effluent was clear. The liver was weighed, minced with a scissor into small pieces, pressed three times through a 100 mech wire screen (pore size 0.14 mm) and washed four times with cold saline using gentle centrifugation and lastly the cells were suspended in saline. The hepatocellular suspension consisted of individual hepatocytes or clumps of three to four cells. Hepatocyte validity was always higher than 95% as judged by trypan blue exclusion. Thus hepatocytes (2×10^7 cells) suspended in 0.3 ml of Dulbecco Modified Eagle's Medium (DMEM) were prepared for injection. Preliminary experiments established that a cell suspension prepared from 1.5 gm of liver was the maximum quantity feasible to be injected into the spleen without the development of intractable portal hypertension or portal vein thrombosis in the recipient rats [10]. Since the weight of normal Lewis rat liver used in the study ranged from 10-14 grams thus, hepatocytes prepared from about 10% of normal liver mass of adult Lewis rat will be ready for injection.

**** Hepatocyte Transplantation:***

The prepared hepatocytes were transplanted by intrasplenic injection 48 hours before 90% hepatectomy through a small left upper transverse incision under ether anesthesia. The spleen was exposed and hepatocytes suspended in 0.3 ml of DMEM were injected into the spleen using 25-gauze needle in group I rats while 0.3 ml of DMEM only was injected into the spleen in group II rats. During injection, the hilum of the spleen was occluded to avoid immediate passage of cells into the liver (Fig. 1). Splenic blood perfusion was reestablished immediately after splenic injection and the abdomen was closed in two layers using 4/0 vicryl sutures [11].

****Induction of acute liver failure:***

Acute liver failure was induced by 90% hepatectomy 48 hours after hepatocyte transplantation as follows: Laparotomy was performed again through the previous upper transverse incision but extended to involve the right side and the liver was exposed through the incision (Fig. II). The common pedicle to the right liver lobes (24% of liver mass) was ligated. The two anterior liver lobes (68% of liver mass) were removed using the standard technique [12]. The operation was performed with the aid of magnifying spectacles (Shin-Nippon loup × 2.5 magnification power) and special care was taken to fully mobilize the anterior liver lobes and to place ligature around their common pedicle high so that there was no interference with the arterial blood supply or venous drainage of the liver remnant. Thus, the two omental liver lobes (8% of liver mass) were left intact (Fig. III & IV). The suggested contributions of different liver lobes to the total liver mass were based on the data derived from Lewis rats subjected to selective portal branch ligation and hepatic resection [13,14]. The abdomen was closed again in two layers using 4/0 vicryl sutures. Animals were monitored till death and autopsied to confirm the presence of viable omental lobes and necrotic right lobes. Animals which died within 12 hours after hepatectomy were excluded from the study. (3 rats: one in group I and two in group II).

For proper identification of transplanted hepatocytes, several sections of hepatocyte bearing spleen were pathologically examined with hematoxylin and eosin stain. Because most of rats with acute liver failure induced by 90% hepatectomy died after 72 hours, we decided that the suitable time for identifying the growth of liver remnant and biochemical parameters was 48 hours after hepatectomy. Thus, eight rats in each group have been killed and evaluated for this purpose. The blood was collected by aortic cannulation and samples were analysed for glucose, albumin, total bilirubin, ALT in addition to prothrombin time. The residual omental lobes were removed and weighed. The growth of liver lobes was assessed using the ratio of the remnant lobes to the total body weight at 48 hours after acute liver failure. For survival analysis 21 rats in group I and 16 rats in group II

were monitored till death. Data were analysed statistically using Mann-Whitney test and student significance test.

RESULTS

In this study the effect of transplanted hepatocytes on the function of liver remnants and the survival of transplanted rats has greatly investigated.

In several sections of the spleens of transplanted rats (gp. I.) there were numerous clusters and cords of hepatocytes that were proved pathologically by hematoxylin and eosin staining (Fig. 5 & 6). In the same group there was a significant increase in the ratio of weight of remnant liver lobes to the total body weight in comparison to group II rats (1.5 in group IVs 0.98 in group II (Table 1). Group I transplanted rats also showed a significant improvement in the level of glucose, total bilirubin and ALT while levels of albumin and prothrombin time showed a non significant improvement in comparison to the group II rats (Fig. 7 & 8).

Regarding survival group I transplanted rats showed significant prolonged survival time and had more long term servivors when compared to group II rats (mean survival time: 128.6 hours Vs 79.2 hours) (long term survivors [5 days or more]: 15 rats Vs 2 rats).

Table (1): Percentage ratio of liver remnant in relation to the total body weight and survival time in transplanted group compared to the non transplanted one.

	Transplanted group	*Non transplanted group*	
Percentage ratio of liver remnant			
range	1.39-1.51	0.93-1.02	Mann-Whitney test
median	1.5	0.98	Z= 3.4 P < 0.001
number	8	8	
Survival time (hours)			
X ± SD	128.6 ± 20.5	79.2 ± 18.03	Sign test
number	21	16	t= 7.5 P < 0.001

Fig. (1): Intrasplenic hepatocyte injection with temporary occlusion of the splenic pedicle

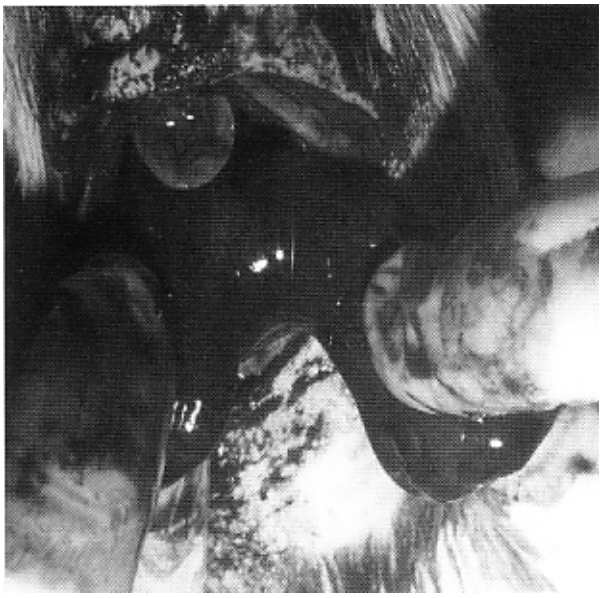

Fig. (2): Exposure of the liver.

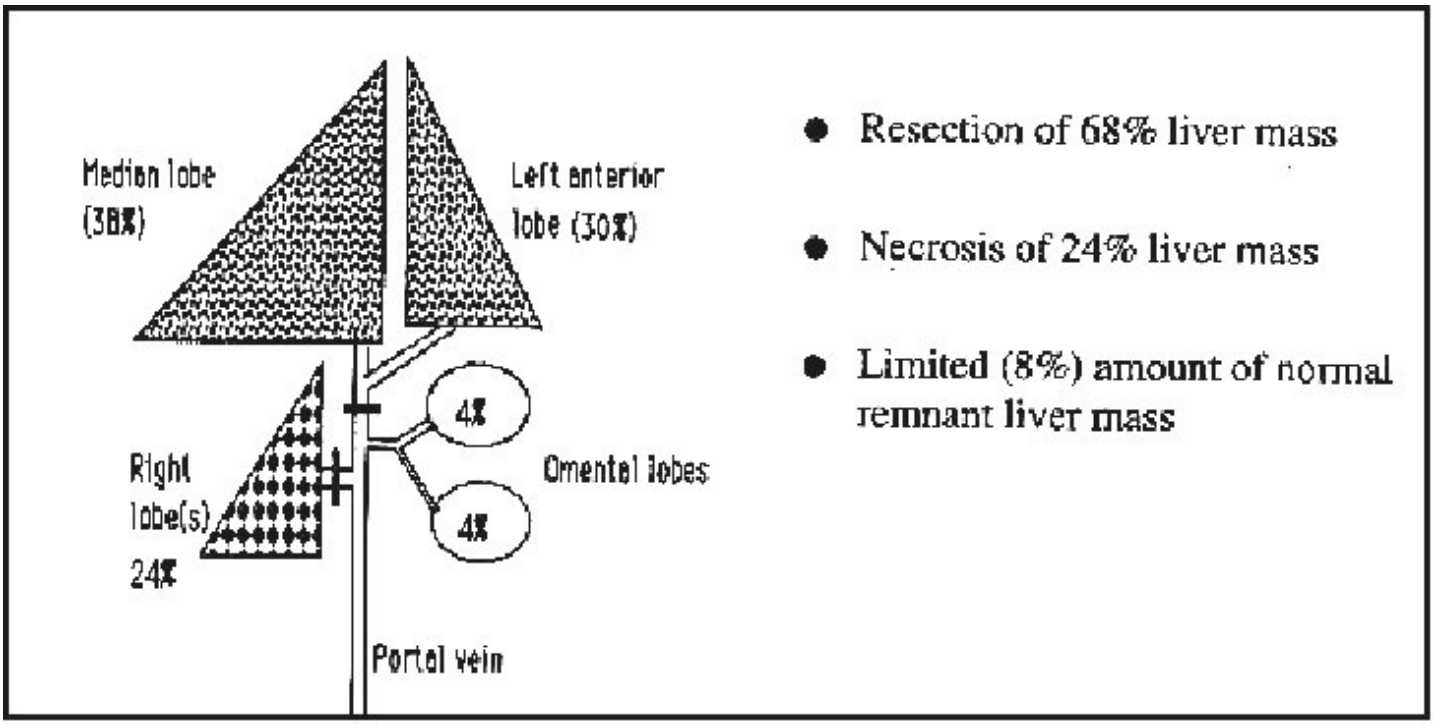

Fig. (3): Selective hepatic resection preserving only the omental lobes.

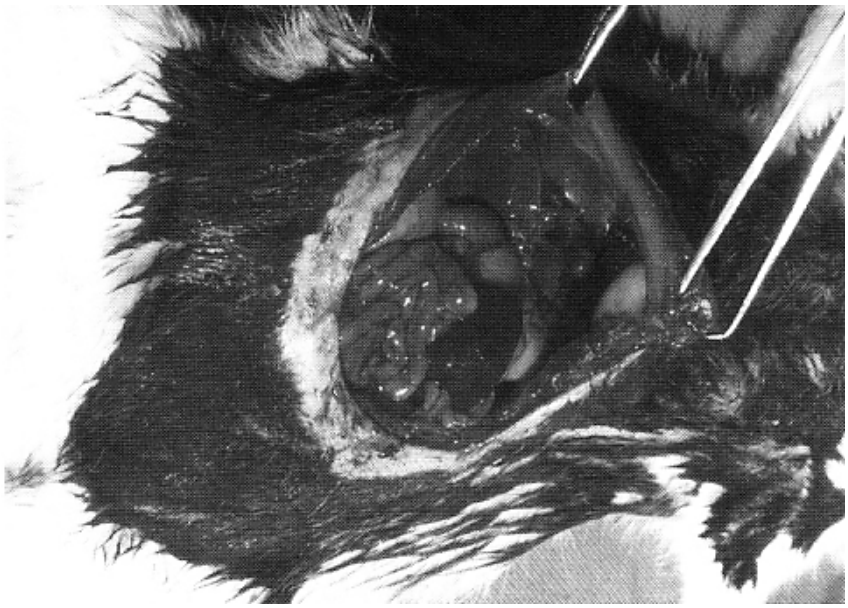

Fig. (4): 90% hepatectomy has been completed.

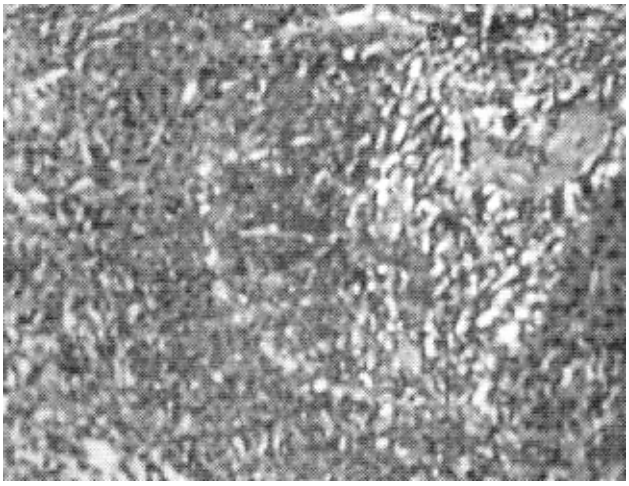

Fig. (5): Section of the spleen revealing liver Paryncymal tissues found involving and replacing the white pulp by low power magnification.

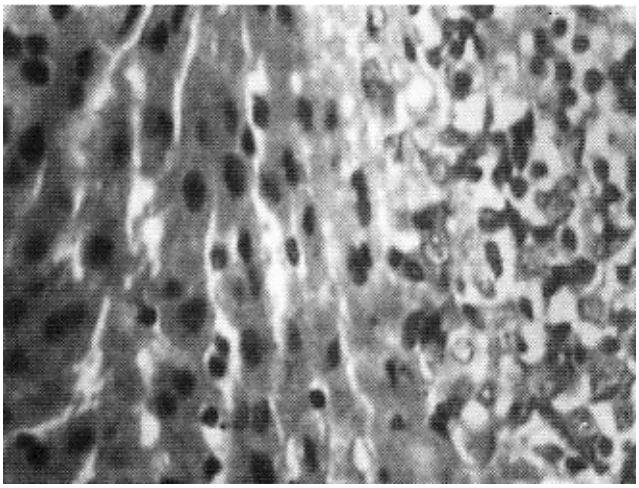

Fig. (6): Hepatocytes arranged in solid cords among the white pulp with evident Kupffer cells by high power magnification.

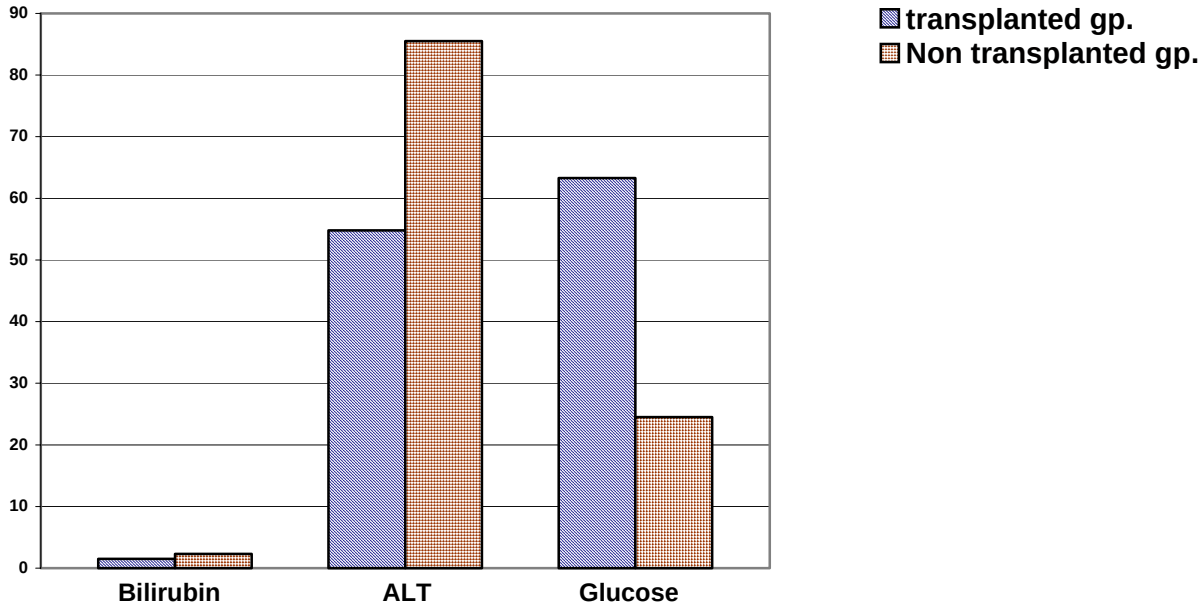

Fig. (7): Significant improvment of bilirubin, ALT and glucose in the transplanted group compared to the non transplanted one (P< 0.001)

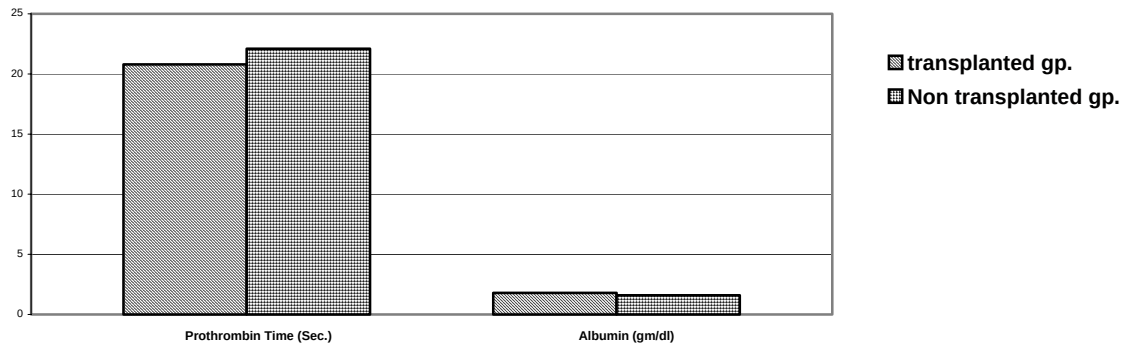

Fig. (8): Non significant improvment of prothrombin time and albumin in transplanted group compared to the non transplanted one (P>0.05)

DISCUSSION

Hepatocyte transplantation represents a new option and has attracted the attention of many investigators for treatment of patients with acute liver failure. So, hepatocyte transplantation has been investigated in this study by evaluating the clinicopathological and laboratory aspects in group I transplanted rats in comparison to group II non transplanted rats and both were exposed to surgically induced acute hepatic failure by 90% hepatectomy. In this study, surgically induced acute hepatic failure by 90% hepatectomy is much preferred than the drug induced acute liver failure because in the drug induced failure it is difficult to predict accurately the extent of hepatic necrosis and functional impairement while the surgically induced failure is simple, highly standaradized and enables the investigator to detect clearly the pathophysiologic actions during acute liver failure. The suggested contributions of different liver lobes to the total liver mass were based on the data derived from Lewis rats subjected to selective portal branch ligation and hepatic resection [13,14]. The spleen has been considered the most suitable place for hepatocyte transplantation as most of the injected hepatocytes migrates to the liver via the portal venous system [15,16,17].

The timing for hepatocyte transplantation (48 hours before hepatectomy) was chosen so as to clearly demonstrate the function of transplanted hepatocytes. The chosen time was not longer than 48 hours so as to avoid the effect of rejection episodes thus eliminating the need for immunosuppression because several reports stated that most of the transplanted hepatocytes died within 5 days without immuno suppression due to the rejection episodes [15]. The time of transplantation was not shorter than 48 hours as the isolated hepatocytes injured by cellular isolation and purification might need some kind of recovery from injury. This problem can be solved either by cultivation with hepatocellular trophic factor as insulin or transplant few days before hepatectomy [18,19].

In this study the transplanted hepatocytes showed a great effect regarding the function and regenerative capacity of the remnant liver lebes. There was a significant increase in the ratio of the weight of remnant liver lobes to the total body weight in group I transplanted rats in comparison to group II rats. Really this may be due to two factors; the first one was migration of intrasplenic transplanted hepatocytes to the liver remnant via the portal venous system and the second one was the more active regenerative response of liver remnant induced by transplanted hepatocytes.

Regarding the function of transplanted hepatocytes group I transplanted rats showed a significant improvement in the level of glucose, total bilirubin and ALT in comparison to group II rats. However, there was a non significant improvement in the level of albumin and prothrombin time. This possibly may be because the transplanted hepatocytes need a more longer time to improve the synthetic capacity of albumin and prothrombin. Thus, these results indicate that hepatocyte transplantation significantly improves the function of liver remnant and increases the regenerative capacity of the remaining liver cells. This can be clearly observed in prolonged survival times and more long term servivors in group I transplanted rats in comparison to group II rats. The results of this study agree with the results of some investigators [7] who stated

that liver repopulation following acute liver failure could potentially eliminate the requirement for orthotopic liver transplantation. Moreover the ability to repopulate the liver with disease resistant hepatocytes offers new opportunities for treating patients with chronic liver diseases. The results of this study also come in agreement with the results of other investigators [1,9] in addition to the results of certain investigators [20] who utilized Xenograft of immortalized human hepatocytes for acute liver failure in rats. Moreover, the results of other investigators [21] were encouraging considering hepatocyte transplantation as a bridge assigned to provide liver function and enable the native liver to recover in patients with acute liver failure that may eliminate the need for liver transplantation. In a new jump for successful human hepatocyte transplantation, certain investigator [22] has reported case of successful transplantation in a 10 years old girl having Crigler- Najjar syndrome with significant improvement of hyperbilirubinemia as well as other clinical and laboratory parameters. Thus we can conclude that hepatocyte transplantation represents a new option with encouraging results for treating patients with acute liver failure. It is simple, easy and less costy than other topics and on the long run, it may eliminate or at least minimize the increasing demands for orthotopic liver transplantation.

REFERENCES

1. Nyberg SL. (2000): Improved survival in acute liver failure with hepatocyte therapies. Liver transpl, 6 (4): 508-511.

2. Bilir BM, Guinette D, Karrer F., Kumpe DA, Krysl J, Stephens J, Mc Gavran L, Ostrowska A and Durham J. (2000): Hepatocyte transplantation in acute liver failure. Liver transpl. Jan 6 (1): 41-43.

3. Riordan SM and Williams R. (2000): Acute liver failure: targeted artificial and hepatocyte- based support of liver regeneration and reversal of multiorgan failure. J. Hepatol. 32 (Suppl): 63-76.

4. Hui T, Rozga J and Demetriou AA (2001): Bioartificial liver support. J. Hepatobiliary Pancreat. Surg. 8 (1): 1-15.

5. Sechser A, Osorio J, Freise C and Osorio RW. (2001): Artificial liver support devices for fulminant liver failure. Clin Liver Dis, 5 (2): 415-430.

6. Laconi S, pillai S, Porcu PP, Shafritz DA, Pani P and Laconi E (2001): Massive liver replacement by transplanted hepatocytes in the absence of exogenous growth stimuli in rats treated with retrosine. Am. J pathol. Feb; 158 (2): 771-777.

7. Malhi H and Gupta S (2001): Hepatocyte transplantation: new horizons and challenges. J. Hepatobiliary Pancreat Surg. 8 (1): 40-50.

8. Kobayashi N., Ito M, Nakamura J, Cai J, Gao C., Hammel J.M. and Fax I J. (2000): Hepatocyte transplantation in rats with decompensated Cirrhosis. Hepatology 31 (4): 851-857.

9. Ostrowska A. (2000): Strategies for hepatocyte transplantation. Postepy Hig Med Dosw. 54 (2): 149-81.

10. Sutherland DER, Numata M., Matas A.J., Simmans RL and Najarian J.S. (1977): Hepatocellular transplantation in acute liver failure. Surgery 82 (1): 124-132.

11. Nieto JA, Escandon J, Betancer C, Ramos J Canton T and Cuervas-Mons V. (1989): Evidence that temporary complete occlusion of the splenic vessels prevents massive embolization and sudden death associated with intrasplenic hepatocellular transplantation. Transplantation 47: 449-450.

12. Higgins GM and Anderson RM (1931): Restoration of the liver of the white rats following partial surgical removal: Arch Path. 12; 186-202. Coated from. Eur. J. Surg. 1995; 161: 475-481.

13. Emond J, capron-laudereau M, Meriggi.F Bernuau J, Reynes M and Houssin D. (1989): Extent of hepatectomy in the rat: evaluation of basal conditions and effect of therapy. Eur. Surg-Res. 21:251-259.

14. Eguchi S, Kamlot A, Ljubimova J, Hewitt WR, Lebow LT, Demetriou AA and Rozga J. (1996): Fulminant hepatic failure in rats: survival and effect on blood chemistry and liver regeneration. Hepatology 24, (6): 1452-1459.

15. Kim W H, Lee JH, JinYM, Han SU, Kim BH, Wang HJ and Kim MW (1998): Biodistribution of intrasplenically transplanted hepatocytes in the rat. Kor. J. Surg. Soc. 54: 772-779.

16. Gupta S, Gorla GR and Irani AN (1999): Hepatocyte transplantation: emerging insights into mechanisms of liver repopulation and their relevance to potential therapies. J. Hepatol. 30: 162-171.

17. Gupta S, Rajvanshi P, Sokhi RP et al (1999): Entery and integration of transplanted hepatocytes in liver plates occur by disruption of hepatic sinusoidal endotheluim. Hepatology 29: 509-519.

18. Wang X, Zhao X, and Anderson R. (1995): The effect of transplantation of hepatocytes cultured with insulin on acute liver failure induced by 90% hepatectomy in the rat. Eur J Surg. 161: 475-481.

19. Jiang B, sawa M., Yamamoto T and kasai S. (1997): Enhancement of proliferation of intrasplenically transplanted hepatocytes in cirrhotic rats by hepatic stimulatory substance. Transplantation 63 (1): 131-135.

20. Kobayashi N., Miyazaki M., Fukaya K, Inoue Y, sakaguchi M, Noguchi H, Matsumura T, watanabe T, Totsugawa T, Tanaka N. and Namba M. (2000): Treatment of surgically induced acute liver failure with transplantation of highly differentiated immortalized human hepatocytes. Cell transplant Sep-Oct.; 9 (5): 733-735.

21. Palmes D., Qayumi AK, and Spiegel Hu (2000): Liver bridging techniques in the treatment of acute liver failure. J. Invest. Surg. Nov- Dec.; 13 (6): 299-311.

22. Emerson R.W. (1998): Human hepatocyte transplantation: Gene therapy and more? This time is a very good one if we but know what to do with it. Pediatrics 102, (3): 647-648.