Does Fetal Hepatocyte Transplantation Provide Metabolic Support Immediately After Surgically Induced Acute Hepatic Failure in Rats?

SIÇANLARDA CERRAHİ OLARAK OLUŞTURULMUŞ AKUT KARACİĞER YETMEZLİĞİNDE FETAL HEPATOSİT TRANSPLANTASYONU HEMEN METABOLİK DESTEK SAĞLAR MI?

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Summary

Acute hepatic failure (AHF) is associated with a high mortality rate. The surgical approach for patients with AHF is orthotopic liver transplantation. However, this approach involves many risks and difficulties. If proper supportive therapy is given during the critical period of failure the liver may have the opportunity to regenerate and recover by itself.

In this study, intrasplenic fetal hepatocyte (FHc) transplantation was evaluated for providing metabolic support in Wistar Albino rats with AHF produced by 90% hepatectomy. We divided the rats into four groups as follow: Group 1: Fetal hepatocytes (0.1 ml of FHc suspension: $2x10^6$ cells) were transplanted into the spleen 4 months before AHF. Group II: Fetal hepatocytes were transplanted to the spleen at the same time of induced AHF. Group III: Hank's balanced buffer solution (HBBS) was injected into the spleen and 4 months later AHF was induced. Group IV: HBBS was injected into the spleen and AHF was induced at the same time. We evaluated mortality rates, blood glucose levels and histological appearance of the spleen. All animals in groups II, III and IV died in five days whereas two animals died and five animals survived in group I. In group I, blood glucose levels were significantly higher than the others.Macroscopic nodules of hepatocytes and microscopically organized hepatocytes were seen in spleen only in group I. The significant improvement of survival rates in group I compared to groups II- III-IV is due to the net support provided by functioning hepatocytes.

We therefore conclude that FHc transplantation provides metabolic support and improves survival rate if performed prior to AHF.

Key Words: Fetal hepatocyte transplantation, Acute hepatic failure

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Özet

Akut karaciğer yetmezliği yüksek mortalite oranı ile alakalıdır. Akut karaciğer yetmezliği (AKY) olan hastalarda cerrahi yaklaşım ortotopik karaciğer transplantıdır. Ancak bu yaklaşım birçok risk ve zorluk içerir. Eğer yetmezliğin kritik döneminde uygun destek tedavisi verilirse karaciğer rejenere olma ve kendiliğinden iyileşme fırsatını bulabilir.

Bu çalışmada %90 hepatektomi ile AKY yetmezliği oluşturulan Wistar Albino sıçanlarda intrasplenik fetal hepatosit transplantasyonunun metabolik destek sağlaması değerlendirildi. Sıçanlar aşağıdaki gibi dört gruba ayrıldı. Grup 1. AKY'den 4 ay önce fetal hepatositler (0.1 ml Fetal Hepatosit (FH) süspansiyonu, $2x10^6$ hücre) dalak içine transplante edildi. Grup 2. Fetal hepatositler AKY ile aynı zamanda transplante edildi. Grup 3. Hank'in dengeli tampon solüsyonu dalak içine enjekte edildi ve 4 ay sonra AKY oluşturuldu. Grup 4. Hank'ın dengeli tampon solüsyonunun enjeksiyonu ile AKY oluşturulması aynı zamanda yapıldı. Mortalite oranları, kan glukoz seviyeleri ve dalağın histopatolojik görünümü değerlendirildi. Grup 2, 3 ve 4'deki hayvanların hepsi 5 gün içinde öldü, grup 1'de 2 hayvan öldü, beşi ise yaşadı. Grup 1'de kan glukoz seviyeleri diğerlerine göre anlamlı olarak yüksekti. Hepatositlerin makroskopik nodülleri ve dalak içindeki mikroskopik organize hepatositler sadece grup 1'de gözlendi. Grup 2, 3 ve 4 ile kıyaslanınca grup 1'deki anlamlı yaşamda kalma oranları fonksiyon gören hepatositlerin sağladığı desteğe bağlıdır.

Sonuç olarak AKY'den önce yapılırsa fetal hepatosit transplantasyonu hayatta kalma oranını iyileştirmektedir.

Anahtar Kelimeler: Fetal hepatosit transplantasyonu, Akut karaciğer yetmezliği

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Acute hepatic failure (AHF) causes high mortality. Orthotopic liver transplantation has become a standard procedure for end stage liver disease including fulminant hepatic failure. Due to the diffi-

culties of this procedure, alternative therapeutic approaches are being investigated. One of them is hepatocyte transplantation.Although adult hepatocyte transplantation has been most widely accepted and extensively studied (1-7), the fetal hepatocytes have a higher proliferation capacity and lower immunogenicity in comparision with adult hepatocytes (8-10). The transplantation of FHc has been recommended for the treatment of diseases involving hematopoietic stem cell and inborn errors of metabolism rather than AHF (11-14). FHc transplantation for AHF has been considered only in few experiments (15-18).

In this study, the efficiency of FHc transplantation prior to or simultaneously with surgically induced AHF to provide metabolic support and improve survival rate were evaluated in rats.

Materials and Methods

Wistar albino, 5 month-old, male, syngeneic rats (250-300 g) were used as recipients. All animals were kept in standard laboratory chow and tap water, at 25°C. We obtained fetal hepatocytes from 14-15 day old fetuses. To obtain fetal hepatocytes,modified Hata's method was used (16). Fetuses at 14-15 days of gestation were obtained from pregnant rats under sterile conditions. Pregnant rats and their fetuses were decapitated and fetal livers were collected immediately. We pooled the livers, incised with fine scissors into small pieces and pressed through a mesh wire screen (104 µ pore size, Sigma Cot.No: CD 1). They were washed several times with cold HBBS for 10 minutes under 2000 rpm centrifugation, until the supernatant was clear. The viability of fetal liver cells was determined by trypan blue dye exclusion and we found that the percentage of viable cells was 80-85. The donor cell number was adjusted to $2x10^7$/ml in HBBS. FHc suspension was stored at 4°C till transplantation (15-22 minutes).

Hepatectomy was performed under light ether anaesthesia. Recipient rats underwent a median upper abdominal incision, the right and left hepatic ligaments were separated. The median, left and two right lobes were elevated and ligated at their bases. The lobes were removed taking care to prevent injury to the portal vein and hepatic artery. Two caudate lobes were salvaged and 90% hepatectomy was performed as described in the Emond and colleagues study (19). After the splenic vein was clamped, 0.1 ml ($2x10^6$ cells) cell suspension was injected deeply and slowly into the spleen from the lower pole. Leakage of the cell suspension and blood was minimized by tamponade of injection site with a cotton tip.All experimented rats were given electrolyte solution (IsolyteM®, Eczacıbaşı-Baxter, İstanbul) subcutaneously for 24 hours, after then oral feeding was started.

The animals were divided into four experimental groups as follow: Group I (n=7); 0.1 ml of FHc suspension ($2x10^6$ cells) was transplanted intrasplenically, and AHF was induced by 90% hepatectomy 4 months later. Group II (n=6); 0.1 ml of FHc suspension ($2x10^6$ cells) was transplanted intrasplenically and 90% hepatectomy was performed at the same time. Group III (n=6); only HBBS solution (0.1 ml) was injected into spleen and AHF was induced by 90% hepatectomy 4 months later. Group IV(n=6); HBBS solution was injected into the spleen and 90% hepatectomy was performed at the same time. The effect of transplanted fetal hepatocytes was evaluated by survival rates. Blood was drawn through the tail vein to determine glucose levels by the glucose oxidase method, before hepatectomy and then at 12 hours intervals for 3 days. The tissues were fixed in 10% buffered neutral formaline and embedded in paraffin. The histological examination was carried out using hematoxylin and eosin (H&E) staining.

The statistical analysis was performed using the Mann Whitney - U Test.

Results

All animals in groups II, III and IV died in five days after AHF induced by 90% hepatectomy. In group I, two animals died and five animals survived. The survival rate in group I was significantly higher than the other groups ($p<0.05$) (Figure 1).

Twelve hours after the resection, blood glucose levels were significantly lower in all groups compared with preoperative levels ($p<0.05$). The blood glucose levels decreased from 100 mg/dL to 30 mg/dL. Hypoglycemia persisted below 40 mg/dL for four days after resections in groups II, III and IV. But in group I, hypoglycemia was significantly

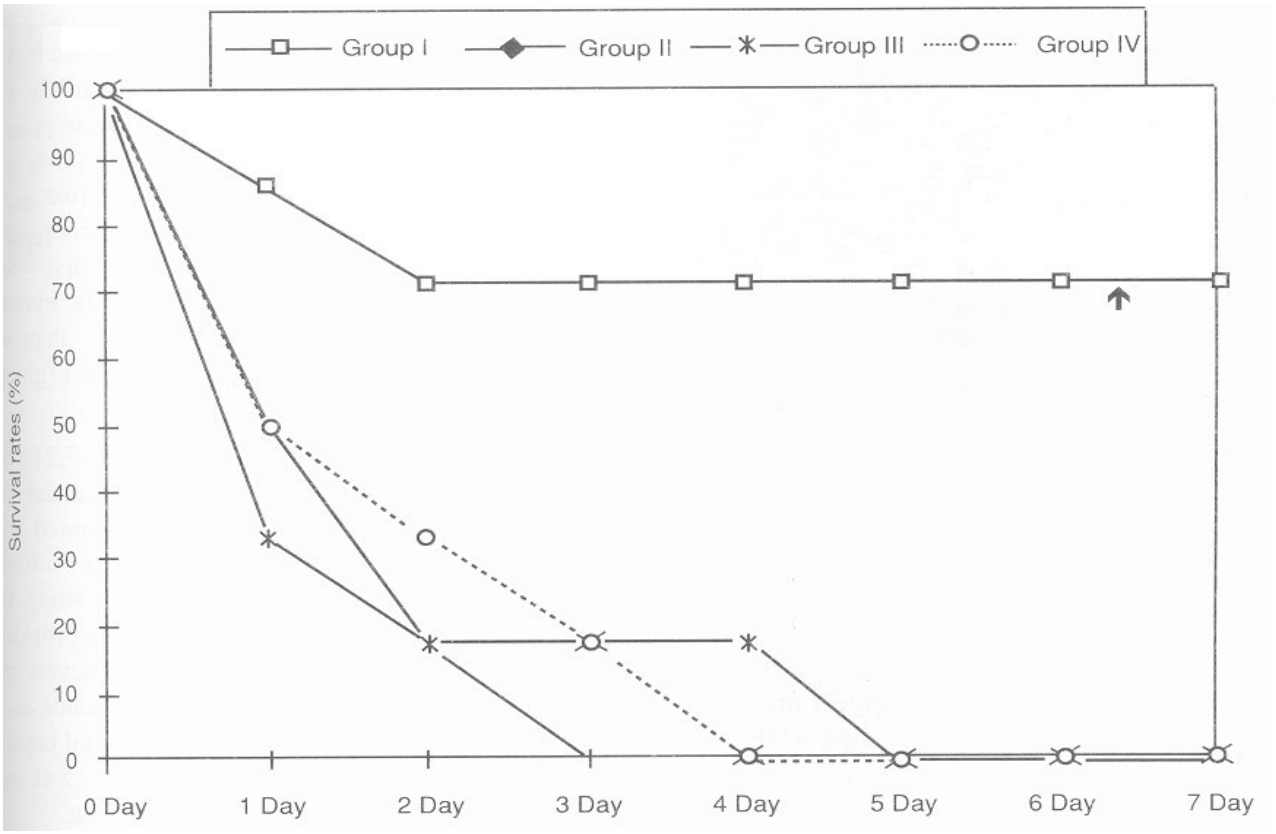

Figure 1. Survival rates of the 4 groups after AHF induced by 90% hepatectomy (↑ statistically significant, $p<0.05$).

less than that in groups II, III, IV and blood glucose returned to normal levels within 72 hours ($p<0.05$) (Figure 2).

In group I, macroscopic nodules of hepatocytes (in 3-5 mm diameters) were seen 4 months after transplantation.The histological examination showed that the transplanted hepatocytes were organized, forming a cord like structure (Figure 3). Histological examination of the spleen, in group II, revealed that transplanted hepatocytes were alive in the splenic red pulp without necrosis, but appeared disorganized. In groups III and IV, the histological appearance of the spleen was normal.

Discussion

Fetal liver has both fetal hepatocytes and hematopoietic stem cells. Thus fetal liver cell transplantation has been applied in the treatment of diseases involving hematopoietic stem cells and inborn errors of metabolism (14,20). Experimentally or clinically, few study have been carried out on FHc transplantation for AHF (16,17,21).

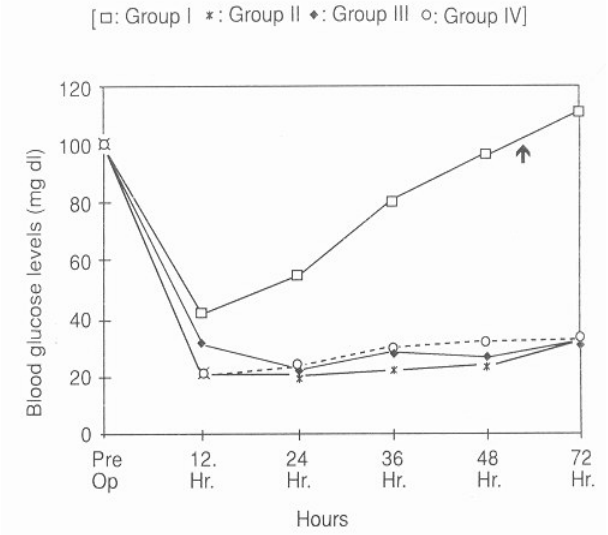

Figure 2. Mean blood glucose levels of the 4 groups after AHF induced by 90% hepatectomy [statistically significant, ($p<0.05$)].

In one of these studies, Hata and colleagues (16) suggested that intraperitoneal hepatocyte

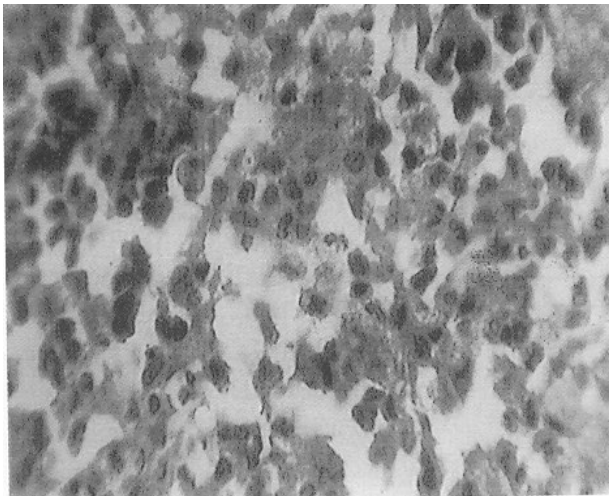

Figure 3. Organization of the transplanted hepatocytes in the spleen forming cord like structures 4 months after transplantation.

transplantation provided metabolic support and increased the survival rates of rats which had AHF by hepatectomy, their study also demonstrated that the intraperitoneal nodules consisted of organized hepatocytes. Shimura and colleagues (17) transplanted fetal liver fragments into the omentum of recipient rats a day after AHF which was induced with D-galactosamine, and their results confirmed that metabolic supporting of these animals were established. Habibullah and colleagues (21) concluded that intraperitoneal human FHc infusion, about 12 days after the onset of the disease improved the survival of patients with fulminant hepatic failure.

Although the benefits of hepatocyte transplantation into different body locations were reported, the spleen has been choosen as the site of transplantation, because hepatocytes can efficently localize and organize in it (22,23). It is shown that syngeneic hepatocytes may survive in the spleen for up to 18 months in rats (24,25). Ebata and colleagues (26) showed macroscopic nodules of hepatocytes inside the spleen 6 months after intrasplenic infusion of fetal liver fragments. They also showed that inoculated fetal liver fragments formed cord structures and bile ducts with marked proliferation of hepatocytes up, 4 weeks following transplantation (22,26). In our study, macroscopic nodules and microscopic organization of fetal hepatocytes were seen 4 months after intrasplenic transplantation.

In our study, the course of blood glucose levels after 90% hepatectomy showed a decrease within the first 12 hours in all groups.It means all hepatectomised rats produced AHF. The return to prehepatectomic values was determined only in group I. In group II, low glucose levels did not increase until the rats died. Five animals survived in group I, but all animals died in group II within the first week. FHc transplantation with simultaneously induced AHF had no more beneficial effects than intrasplenic injection of HBBS in our model (groups III-IV).

In the studies mentioned above (16,17,21), hepatocyte infusions were performed immediately after AHF and they reported that transplanted fetal hepatocytes provided metabolic support with improved survival rate. However, in our study, the supportive data to their reports was not provided. The significant differences in glucose levels and survival rates between group I and II leads us to suggest that the ability of transplanted fetal hepatocytes to provide metabolic support was not achieved immediately after transplantation.

In conclusion, histological and biological results and the survival rates of rats suggested that transplanted fetal hepatocytes were functional and provided metabolic support only after their organization. Because they need organization time, transplanted fetal hepatocytes do not provide metabolic support if AHF was induced during transplantation.

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