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Combined hepatocyte-islet transplantation: an allograft model

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Abstract Experimental hepatocyte transplantation (Tx) has been shown to improve the survival rate of acute hepatic failure (AHF) in different models. Histological and biochemical data from some studies suggest more satisfactory function of hepatocytes after combined hepatocyte-islet Tx. The aim of the present study was to compare the survival rate between two different sites of Tx (kidney subcapsular and spleen) of hepatocytes alone or combined with islets of Langerhans in rats with surgically induced AHF (90% hepatectomy) accross a major histocompatibility barrier (WAG to Lewis). Rats were divided into five groups ($n = 6$ in each group). Group 1 consisted of AHF without treatment, group 2, AHF followed by hepatocyte Tx into the spleen, group 3, AHF followed by hepatocyte Tx subcapsular into the kidney, group 4, AHF followed by combined hepatocyte islet Tx into the spleen, and group 5, AHF followed by combined hepatocyte islet Tx subcapsular into the kidney. The number of hepatocytes was 10^7 and the number of islets was 400. All rats received cyclosporin A (CsA) i.v. (20 mg/kg on days 0–4 and 10 mg/kg on days 5–30). Hepatocytes were harvested using a modification of the portal vein collagenase perfusion (type V 1.3 mg/ml) and islets, with the collagenase digestive technique (type XI 1 mg/ml). All Tx took place 24 h after AHF. All rats in group 1 died within 48 h. In groups 2 and 3, the combined survival rate was 33% 1 month after Tx, while in groups 4 and 5, the combined survival rate was 50% at 1 month. All surviving animals were sacrificed and histological examination showed well-preserved hepatocellular aggregates in the spleen and beneath the renal capsule, as well as islets around the clusters of hepatocytes. SGOT and SGPT values were also measured. We concluded that the combined Tx in a rat experimental allo-Tx model in cases of AHF improves the survival rate in comparison with hepatocyte Tx alone. The survival rate at the two different sites for combined Tx was similar.

Key words Acute hepatic failure (AHF) · Combined allo-transplantation · Cyclosporin · Experimental transplantation · Hepatectomy Hepatocytes · Islets

Introduction

Acute hepatic failure (AHF) is associated with a high mortality. Liver transplantation is the current treatment, but the high rate of clinical complications, as well as difficulties in the management of organ sharing, makes hepatocyte transplantation (HTCx) an alternative and suitable future solution for the treatment of AHF. Matas et al. [1] have reported successful HCTx from heterozygous Gunn rats to homozygous recessive jaundiced Gunn rats, and many laboratories have reported successful iso- and allo-HCTx using a variety of models in order to induce AHF (hepatectomy and several hepatotoxins) as well as a variety of sites for the hepatocyte (HC) engraftment. Baumgardner et al. [2] have reported that long-term goals of such experimentation include development of the technique for HCTx associated with the highest rate of successful engraftment and the study of conditions that may promote HC implantation and regeneration.

Working on this idea, Ricordi et al. [3] and Xiangdong et al. [4] have reported success with combined HC islet Tx in rats. In the first report a model of allo-Tx was used and the cells were transplanted beneath the renal capsule. In the second report a model of iso-Tx was used with the spleen as the site of implantation. The aim of our study was to compare the survival rate between two different sites of Tx (kidney subcapsular and spleen) of HCs alone or combined with islets of Langerhans in an allograft model (WAG to Lewis).

Materials and methods

Animals

Adult WAG rats ($RT1^u$) weighing over 200 g were used as donors ($n = 30$) of HCs and islets. Thirty Lewis rats ($RT1^l$) weighing 250–300 g, were used as recipients.

Experimental protocol

The rats were divided into five groups ($n = 6$ in each group). AHF was induced in all groups by 90% hepatectomy prior to any treatment. The groups were treated as follows: group 1, AHF without treatment (controls); group 2, AHF followed by HCTx into the spleen; group 3, AHF followed by HCTx subcapsular into the kidney; group 4, AHF followed by combined HC-islet Tx into the spleen; group 5, AHF followed by combined HC-islet Tx subcapsular into the kidney. All the Txs took place 24 h after induction of AHF (day 0 = Tx and day 30 = end of experimental observation-euthanasia).

Hepatectomy

Rats were anaesthetized with pentothal and stedon and 90% hepatectomy was performed by removing the left, median, right lower and right upper lobes of the liver, leaving only the caudate lobe. Coagulant (Spongostan) was used in order to stop bleeding on the cut surface. The resected liver was weighed to confirm the extent of resection.

Isolation of hepatocytes and islets

For HC isolation, a modification of the technique described by Xiangdong et al. [4] was used. The portal vein was cannulated. The hepatic artery and the inferior caval vein were tied. The liver was perfused slowly with 100 ml of lactated-ringers and total hepatectomy was carried out. The liver was placed in a sterile petri dish on ice and all the vessels were tied leaving only the cannula in the portal vein. A total of 50 ml of collagenase (Sigma, Type V, 1.3 mg/ml) was infused.

The liver was incubated at 37 °C for 45 min and was then cut into small parts and gently massaged through a metal sieve (pore size 400 μ) in order to take out the liver matrix and vessels. The cells were washed 3 times in Hanks' solution (Sigma-H 4385) with 5% newborn calf serum (Sigma-N 4637) and then isolated HCs were counted.

For islet isolation, the method described by Xiangdong et al. [4] was used with two modifications. The type of collagenase was (Sigma, Type XI, 1 mg/ml), in order to achieve higher purity. Also, instead of using a stereomicroscope to collect islets, the dispersed tissue was processed through a Dextran (Sigma-D 3759) gradient to separate the endocrine tissue from the exocrine (gradients 31%, 29%, 25% and 11%).

Viability of HC

The cellular viability was estimated using the trypan blue exclusion test.

Transplantation

Approximately 10^7 HCs were translanted in groups 2, 3, 4 and 5, as well as 400 islets in groups 4 and 5. The cell suspension was diluted in 0.5 ml Hanks and a 1- or 2.5-ml syringe was used. The cells were transplanted into the spleen as described in a previous study [4], and the cells were injected slowly into the renal capsule and leakage was avoided with microsurgical sutures.

Immunosuppression

Cyclosporin A (CsA) was injected i. v. as follows: 20 mg/kg on days 0–4 and 10 mg/kg on days 5–30.

Blood samples

Blood samples were collected every 2 days post-Tx (from the tail of the rat) in order to measure the levels of SGOT, SGPT (transaminases), alkaline phosphatase (ALP), albumin (ALB) and bilirubin (BIL).

Statistical analysis

The survival rates between groups were statistically analysed using the *t*-test ($\bar{x}$ = mean value, SD = standard deviation).

Results

Viability of isolated HCs

Viability of isolated HCs was over 90%. Microscopic observation in the chamber showed that the contamination rate of blood cells was less than 2%.

Survival rate

Results of the survival rate at 1 month (30 days) post-Tx are summarised in Table 1. In group 1, all rats died within 48 h. In group 2, approximately 33% of the animals transplanted intrasplenically survived for 1 month, two animals survived for 14 and 15 days, respectively, and two animals died early. In group 3, results were similar to group 2 (33% survival at 1 month). In group 4, 50% of the animals survived for 1 month post-Tx and two rats were alive for almost 20 days (19 and 21 days). One rat died on day 11. In group 5, results were similar to group 4, with 50% of the rats surviving for 1 month and, again, two rats were alive close to 20 days (no. 3) and more than 20 days (no. 5). One rat died on day 12.

Statistical analysis

The comparison of the mean values between groups 2 and 3 showed no significant difference in the survival rates. The same results were found between groups 4 and 5. Statistical comparison between the mean values of groups 4 and 5 and the mean values of groups 2 and 3 also showed no significant differences probably because the total period of observation (30 days) was not long enough to establish a difference. Also, the relatively small number of the animals did not permit sufficient statistical analysis. However, evaluation of the survival rate (30% and 50%) definitely proved an increased survival after combined Tx.

Table 1 Survival rate at 1 month (30 days)

No. of experiment	Groups				
	1	2	3	4	5
1	1	30	8	21	12
2	1	3	30	19	30
3	2	14	10	30	18
4	1	2	5	30	30
5	2	15	30	11	24
6	2	30	2	30	30
% Survival at 1 month	0	33	33	50	50
$\bar{x}$	1.5	15.66	14.16	23.5	24.00
SD	0.5	11.26	11.46	7.1	6.92

Table 2 Higher biochemical values pre-Tx and lower values post-Tx (*BIL bilirubin, ALP* alkaline phophatase, *ALB* albumin; Normal values: BIL = 0.09, SGOT = 114, SGPT = 40, ALP = 120, ALP = 3)

Biochemical data		Groups				
		1	2	3	4	5
BIL	Pre-Tx	2.67	0.15	0.09	0.09	0.04
(mg/dl)	Post-Tx	–	0.03	0.04	0.05	0.02
SGOT	Pre-Tx	3600	1143	1033	> 3000	> 3000
(IU/L)	Post-Tx	–	164	192	147	126
SGPT	Pre-Tx	> 3000	1050	927	> 3000	898
(IU/L)	Post-Tx	–	66	46	22	89
ALP	Pre-Tx	1391	1382	1100	1314	1010
(IU/L)	Post-Tx	–	430	106	145	384
ALB	Pre-Tx	3.6	3.4	4.6	3.3	4.4
(g/dl)	Post-Tx	–	3.0	3.9	3.1	4.1

Biochemical data

A summary of biochemical values can be seen in Table 2, with lower values post-Tx. After 90% hepatectomy, rats responded with a large increase in transaminases, but not always the same way. Values greater than 3000 IU/l were observed as well as lower values. There was also a large increase in ALP. Hepatic function was recovered (HTx alone or combined with islets) between days 3 and 5 post-Tx. Animals that were sacrificed on day 30 still had functioning grafts in contrast to the animals that died.

Autopsy

Visible aggregates of cells were observed at autopsy in the injection site. Also, adhesions were observed in the spleen in several cases. No evidence of intraperitoneal sepsis was found.

Histological examination

Single layers of clusters of HCs were detected in groups 3 and 5 close to the renal capsule or renal parenchyma. Islets were observed alone in the renal parenchyma or as aggregates with HCs. In the spleen, sections from animals that died early showed cells mainly in the injection site in contrast to the long-term survivors in which cells were found in the splenic parenchyma.

Discussion

As an experimental technique, HCTx has important advantages such as: (1) isolation of high numbers of HCs, with enough for multiple donations, (2) cryopreservation until needed and (3) recovery of hepatic function using only small numbers of HCs. In our experimental data, we injected a small number of HCs (10^7) and we supported the graft surival by CsA, decreasing the dose on day 5.

We found no evidence of technical complications at autopsy, so we concluded the experimental animals that died between days 2–5 had primary non-function of the injected cells. Furthermore, the biochemical data and the histological findings from the animals that survived longer (but less than 30 days) showed that the injected cells functioned early but the histology confirmed difficulties of implantation and probably rejection. In contrast, biochemical and histological findings from the animals that were alive on day 30 showed well-preserved hepatocellular aggregates and islets in the parenchyma of the organs and this was evidence of implantation.

The significant improvement in survival after combined Tx is related to trophic factors from pancreatic islets [2, 3]. Insulin and glucagon are important regulators of liver regeneration, so the improvement in survival was probably related to implantation of the HCs because of the injected islets. In addition, the remaining host liver demonstrated signs of regeneration after day 14.

We concluded that the successful allo-Tx of HCs depends on factors that help the implantation mechanisms. Also, for the conditions used in this experiment and for the specific rat model (WAG to Lewis), the long-term survival of the cells was not related to the Tx site (spleen or renal subcapsular).

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