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Hepatic support by hepatocyte transplantation in congenitally metabolic diseased rats

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Abstract. Nagase analbuminemic rats (NAR), which lack albumin synthesis in the liver, underwent intrasplenic hepatocyte transplantation (HCTx), and the long-term effects were studied using functional and morphological examinations. Hepatocytes were isolated from congenic F344 rats with collagenase infusion, and 1×10^7 cells were injected into the spleen of 3-month-old NAR ($n = 10$). Serum albumin increased with time, reaching 53.9 mg/dl 14 months after HCTx, which was equivalent to 2.1% (maximum 4%) of serum albumin in normal rats. On the other hand, untreated NAR showed persistently low serum albumin levels (0.99 ± 0.23 mg/dl at 10 months). According to immunostaining with anti-rat albumin antibody at 16 months after HCTx, hepatocyte grafts occupied 27–41% of the spleen area and weighed 120–420 mg, which was equivalent to 0.8–2.9% of a whole liver. Our study demonstrated that grafted hepatocytes can grow in the spleen with the ability to synthesize albumin. HCTx in NAR is a new experimental system to monitor the function and survival of grafted heptocytes without sacrificing the animals by measuring serum albumin levels. Certain manipulations to facilitate the growth of grafted hepatocytes are necessary to achieve sufficient hepatic support in HCTx.

Key words: Intrasplenic hepatocyte transplantation – Nagase analbuminaemic rat

When isolated hepatocytes are grafted into a rat spleen, they can grow gradually until they ocuppy about 40% of the spleen area with preserved liver cell function as has been reported by Mito et al. [2]. The technique could be a useful treatment for metabolic diseases due to liver enzyme deficiencies and acute liver failure. However, it has been difficult to monitor the function of grafted hepatocytes in the spleen and to assess how much they assist the host liver function, because there are few suitable animal models for these studies. We performed intrasplenic hepatocellular transplantation (HCTx) in albumin-deficient rats, and monitored the function of grafted hepatocytes by measuring serum albumin levels in order to evaluate the effects of HCTx on congenital metabolic diseases.

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Materials and methods

Male NAR (RT1l) ($n = 10$, 200–250 g body weight, 3 months old, supplied by Sasaki Institute, Tokyo) underwent intrasplenic hepatocyte isotransplantation under ether anaesthesia. Hepatocytes were isolated from congenic F344 rats (RT1l) by collagenase infusion according to the method of Seglen [7], and approximately 1×10^7 hepatocytes in 0.2 ml of Hank's solution were injected into each recipient's spleen. The viability of the hepatocytes at the time of isolation, assesed by trypan blue staining, was 85%. Blood samples were taken from each rat at intervals for 20 months after HCTx, and serum albumin levels were measured by radioimmunoassay (RIA). The specific rabbit antiserum against rat albumin was prepared in the Hormone Assay Center, Institute of Endocrinology, Gunma University (Maebashi, Japan) and used for RIA [4]. Age-matched untreated NAR ($n = 9$) and adult F344 rats ($n = 5$) served as controls.

At 12 months after HCTx, spleens were removed from three HCTx rats, followed by serum albumin measurements at 2 month intervals. At 18 months after HCTx, another three rats were sacrificed for morphometric analysis of grafts in the spleen. The spleen was perfused through the portal vein for fixation with periodate-lysin-paraformaldehyde containing 0.1% glutal aldehyde. Then the spleen was sliced along the long axis and mixed with rabbit anti-rat albumin serum (Capple, USA) for immunostaining. The total area of the hepatocyte graft and graft fraction rate (%) were measured using a CB-Tasper system. The volume of the graft was also estimated from the fraction rate and the spleen weight.

The results were analysed using the Student's t-test or the Cochran-Cox test, and the values are shown as mean ± SE.

Results

All but two rats which underwent HCTx survived for at least 16 months after HCTx until some of the rats were sacrificed. Serum albumin levels of untreated NAR were very low throughout the observation period

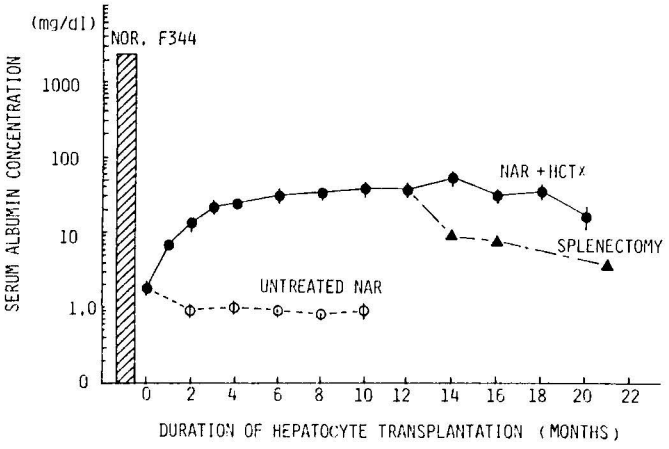

Fig. 1. Serum albumin levels in F344 normal rats ($n = 5$), untreated NAR ($n = 9$), and NAR with intrasplenic hepatocyte isotransplantation (HCTx) ($n = 10$). Some of the NAR with HCTx ($n = 2$) underwent splenectomy 12 months after HCTx

(0.99 ± 0.23 mg/dl at 10 months) compared with the normal F344 rats (2455 ± 62 mg/dl). Serum albumin levels of the transplanted group were 6.9 ± 1.1 mg/dl 1 month after HCTx, which was significantly higher than those of the untreated NAR group (0.92 ± 0.06 mg/dl at 2 months, $P < 0.01$). The levels increased until 14 months (53.9 ± 9.2 mg/dl, maximum 88.3 mg/dl) and declined from 16 to 18 months. Serum albumin dropped considerably after splenectomy in NAR with HCTx (8.8 ± 0.2 mg/dl 2 months after splenectomy), though it never decreased to the level of untreated NAR (Fig. 1).

The graft fraction rates in three rats which were sacrificed 16 months after HCTx were 27.1, 36.1 and 40.9% (Fig. 2, Table 1). There was a clear correlation between serum albumin levels and estimated hepatocyte graft volumes in the spleen (Table 1).

Discussion

Liver transplantation has been performed successfully in humans for the treatment of congenital metabolic diseases [8]. However, many transplant candidates, especially children, do not get the benefits of liver transplantation because of a serious donor shortage. Thus, various alternative methods have been sought clinically as well as experimentally. We have reported short-term and long-term effects of fetal liver transplantation in congenital metabolically diseased rat models [5, 6]. In the present study we transplanted adult hepatocytes in NAR and observed the long-term growth and function of the grafts.

NAR is a rat mutant in which serum albumin levels are very low (0.05% of normal rats) due to an albumin mRNA defect in the liver [3]. This trait is transmitted in an autosomal recessive fashion. Following HCTx, serum albumin increased considerably with time. It reached 88.3 mg/dl in one rat 14 months after HCTx, which was equivalent to 4% of normal rat serum albumin. Morphological study showed that grafted hepatocytes occupied 40.9% of the spleen in the same rat 16 months after HCTx, which was equivalent to 2.9% of a whole liver weight. The volumes of grafted hepatocytes in the spleen pararelled the serum albumin levels in NAR with HCTx. Moreover, serum albumin levels dropped significantly following splenectomy in HCTx rats. All these results suggest that grafted hepatocytes in the spleen grow with time and give the main contribution to albumin production in albumin-deficient rats.

Gupta et al. reported that when hepatocytes from the HBsAg-producing transgenic mouse were isografted into the spleen of normal mice, serum HBsAg was present from 3 days after HCTx, and increased from 20 to 168 ng/ml over a period of 20 weeks [1]. Thus, measuring serum albumin levels in NAR following HCTx or measuring serum HBsAg levels in mice following HCTx of

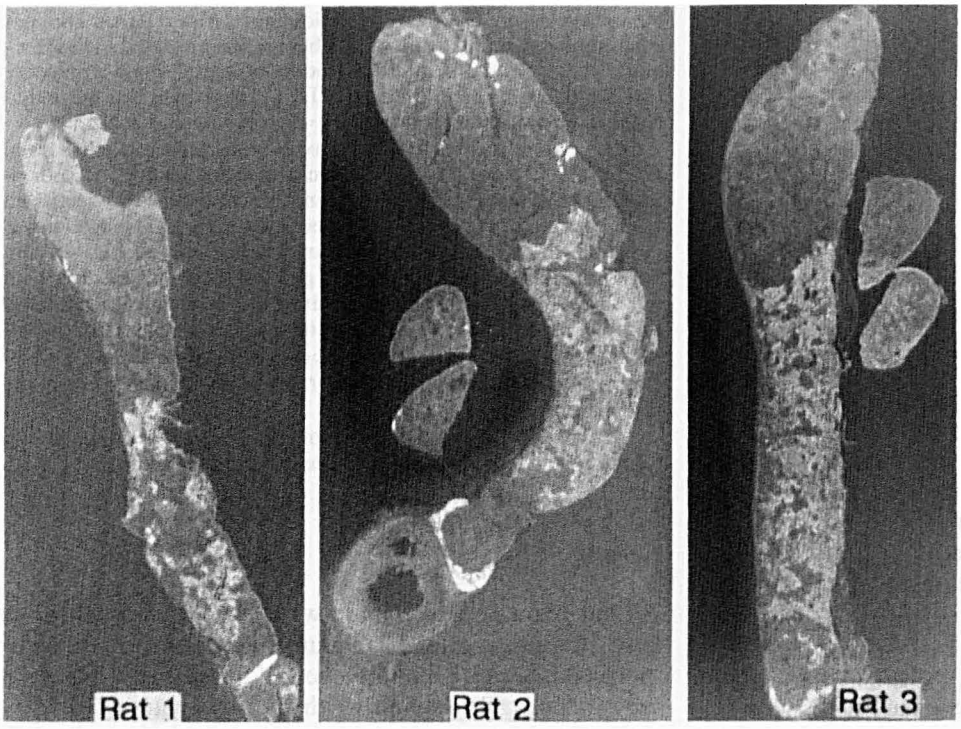

Fig. 2. Rat albumin immunostainings of hepatocyte grafts in the spleen 16 months after HCTx

Table 1. Morphometric analysis of intrasplenic hepatocyte grafts in the rats shown in Fig. 2

Rat no	Serum albumin (mg/dl)	Total area of HC in the spleen (mm^2)	Total volume of HC in the spleen (mg)
1	12.3	16.5 (27.1%)	120
2	31.5	29.7 (36.1%)	350
3	42.3	32.7 (40.9%)	420

HC, hepatocytes

HBsAg-producing hepatocytes enabled us to monitor the function, survival and fate of grafted hepatocytes without sacrificing the animals.

We have reported the effects of orthotopic whole liver transplantation (OLTx) in NAR and found that serum albumin increases rapidly to normal range immediately after OLTx [4]. Since the function of grafted hepatocytes is equivalent to only 2% that of OLTx, certain manipulations to facilitate hepatocyte growth are necessary to achieve sufficient function. However, HCTx may be useful as a method for liver enzyme replacement in such conditions as congenital enzyme-deficient disease.

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