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BRIEF REPORT

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# Quantitative measurements of collateral arterial blood flow in nonarterialized rat liver grafts

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**Abstract** The early development of arterial blood flow in the grafted liver after orthotopic liver transplantation in the rat without reconstruction of the hepatic artery was studied. Arterial liver blood flow was measured on day 21 after transplantation with NEN-TRAC microspheres (size 15.5 ± 0.1 μm) and labelled with $^{103}$Ru. The arterial liver blood flow in the grafted liver was 0.778 ± 0.247 ml/min per gram for transplanted rats after 21 days. One day after transplantation, the blood flow was only 0.006 ± 0.002 ml/min per gram. The results of this study demonstrate that there was no arterial blood flow on day 1 after transplantation, as expected, but that there was a high arterial blood flow in the transplanted liver by day 21. This was also supported by the angiographic findings. The early development of arterial blood flow via collaterals may account for the excellent results that we and others have attained in orthotopic liver transplantation without rearterialization in the rat.

**Key words** Liver transplantation, rat, nonarterialized · Rat, liver transplantation, nonarterialized Rearterialization, rat, liver transplantation

## Introduction

The technique of orthotopic liver transplantation in the rat was first described by Lee et al. in 1973. In this model, the vascular anastomosis was performed with a running suture technique without rearterialization (reanastomosis of the hepatic artery) [3, 4]. Later, several groups described a liver transplantation model with reconstruction of the hepatic artery [7, 9]. The question of whether arterialization is of significant value in rat liver transplantation has raised considerable controversy. Ulrichts et al. recommended rearterialization because they found improved survival and fewer bile duct complications [9]. However, others have had a high survival rate and have not experienced a high incidence of biliary tract complications in recipients of hepatic allografts receiving only portal venous blood [1, 2]. In one of our earlier studies, we found no significant differences in the biochemical markers and histopathological analyses between livergrafts with portal venous blood flow only and those with portal venous blood flow and re-established hepatic arterial blood flow on day 14 after liver transplantation [8]. The success of liver transplantation in the rat without hepatic artery reconstruction may be due to early development of arterial blood flow via collaterals. Late rearterialization of the grafted liver 102–245 days after transplantation has been angiographically demonstrated by Isai et al. [1], but quantitative studies have, to our knowledge, not been reported.

The objective of this study was to assess whether early rearterialization of the graft via collaterals occurs and to measure the collateral arterial blood flow.

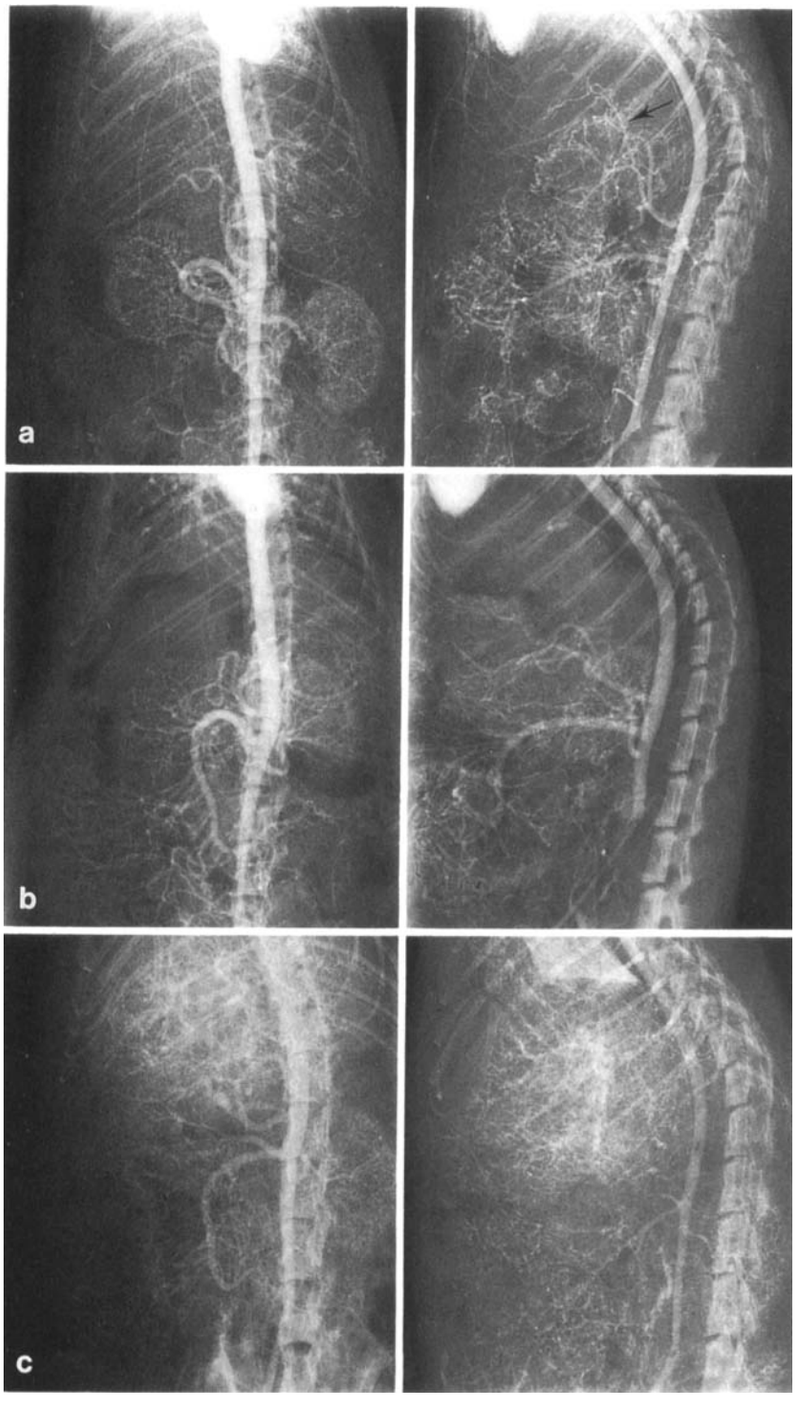


**Fig. 1 a–c** Aortogram of:
**a** nontransplanted rat;
**b** transplanted rat on day 1 after transplantation;
**c** transplanted rat on day 21 after transplantation (Kodak Mini-R 100). In the nontransplanted rat the normal arterial tree with the hepatic artery *(arrow)* is visualized. On day 1 after transplantation, the angiogram shows that there is no arterial liver blood flow. On day 21, the rearterialization of the grafted liver is clearly shown

## Materials and methods

### Transplant operation

Orthotopic liver transplantation in the rat without rearterialization was performed in this study using a technique described in detail in a previous paper [8]. Female Wistar-Furth rats weighing around 200 g were used as donors and recipients. Ether was used for induction and maintenance of anesthesia in donor and recipient animals. Briefly, in this transplantation model, the vascular anastomosis was performed with a running suture technique and without using any cuffs. The bile duct anastomosis was performed with a polyethylene tube as a splint in the bile duct. The donor liver was perfused in situ under low pressure through the portal vein with 5 ml University of Wisconsin (UW) solution at 4°C. The preservation time was less than 45 min, the anhepatic time less than 20 min, and the recipient operation time less than 45 min in all cases.

### Arterial blood flow measurements

NEN-TRAC microspheres (E. I. duPont de Nemours, Mass., USA), size 15.5 ± 0.1 μm and labelled with $^{103}Ru$, were used. In each experiment approximately $0.4 \times 10^6$ spheres were injected with 0.1 ml NaCl solution (a suspension of 9 mg/ml) and 0.01 percent Tween 80 was added.

The microsphere method has been described in detail and validated for arterial liver blood flow measurements [6]. A catheter (PE25) was advanced through the right carotid artery into the left ventricle. For withdrawal of a reference sample, a catheter (PE25) was introduced in to the left femoral artery with the tip close to the bifurcation of the abdominal aorta. A reference sample was withdrawn at constant speed (0.55 ± 0.01 ml/min) with a pump (Sage Instruments).

The microsphere suspencion was aspirated into PK50 catheters and the activity of each catheter was measured with a NaI (T1) detector (75 × 75 mm thick at a distance of 450 mm). When the microspheres were to be injected into the animal, the catheter with the microspheres was placed on a Vortex shaker and connected at one end to the injection catheter. The other end was connected to a pump. The microspheres were injected for 5 s. The specimen and the reference sample were weighed and placed in tubes, filling up no more than 15 mm of the height. The samples were assayed in a well counter (Picker) equipped with a 75 × 75 mm NaI (T1) well crystal and a single-channel pulse height spectrometer.

Cardiac output and arterial liver blood flows were calculated with the formula: $Q_o = \frac{N_o \times Q_r}{N_r}$ where $Q_o$ refers to cardiac output or arterial liver blood flow in ml/min, $Q_r$ to reference sample in ml/min, $N_o$ to radioactivity of microspheres in the injection catheter or liver samples, and $N_r$ to radioactivity of microspheres in the reference sample.

After the microspheres were injected, the animals were sacrificed. Each animal's liver was removed and great care taken to avoid excising the surrounding tissue.

### Experimental design

Seventeen isogenic rat liver transplantations were included in this study. The donor livers were assigned to three groups with five animals in the first group, eight animals in the second group, and four animals in the third group. In the first group, the liver blood flow measurements were performed on day 1 post-transplantation. In the second group, the measurements were performed on day 21. In the third group, the portal vein blood was collected during the blood flow measurements on day 21. A fourth group with eight sham-operated, non-transplanted rats served as a control group. To visualize the rearterialization, aortography was performed in one non-transplanted rat, in one rat on day 1 post-transplantation, and in one transplanted rat on day 21. Four milliliters of barium was used as contrast medium and the angiography was performed via a catheter in the aorta.

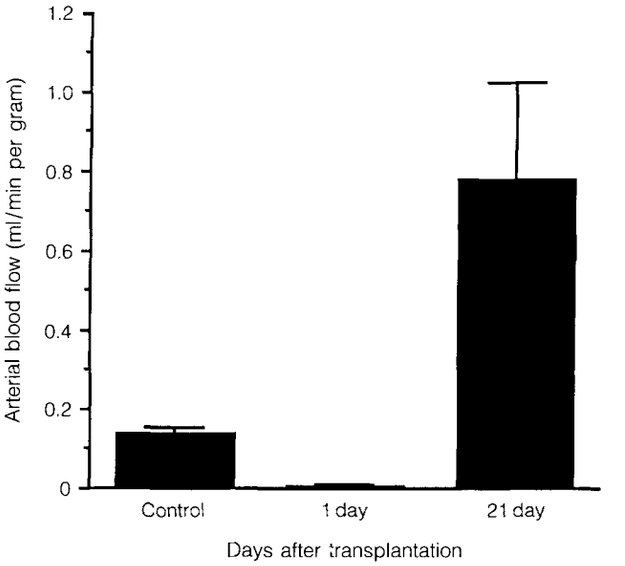


**Fig. 2** Arterial liver blood flow in ml/min per gram liver tissue (wet weight) measured with 16-μm microspheres for control group (sham-operated, nontransplanted rats), transplanted rats 1 day after transplantation, and transplanted rats 21 days after transplantation

## Results

The development of arterial collaterals on day 21 is visualized in an angiogram (Fig. 1 c). There was no arterialization of the grafted liver in transplanted rats on day 1 after transplantation (Fig. 1 b). By comparison, an aortogram with the hepatic artery in a non-transplanted rat is shown (Fig. 1 a).

Figure 2 shows the results of the microsphere measurements as arterial liver blood flow. The arterial liver blood flow in the grafted liver was 0.778 ± 0.247 ml/min per gram for transplanted rats after 21 days. One day post-transplantation, the blood flow was only 0.006 ± 0.002 ml/min per gram. The arterial liver blood flow in sham-operated rats was 0.141 ± 0.012 ml/min per gram and comparable to what has previously been reported as measured with 16-μm spheres [6]. The rat weight was 203 ± 2 g and the liver weight was 6.6 ± 0.2 g for all rats and there were no significant differences between the

groups. The mean arterial pressure was 110 ± 4 mmHg and there were no differences between the groups. As a control, in four transplanted rats on day 21 after transplantation, the portal vein was cut when the microsphere injection started and the portal blood was collected during the blood flow experiment. The measurements of microspheres in portal venous blood showed very low concentrations – 122 ± 36 spheres – compared to 37891 ± 10529 trapped in the liver.

## Discussion

The microsphere method has been widely used for blood flow measurements and was used in this study for arterial liver blood flow measurements [6]. A prerequisite is that the microspheres are trapped in the capillary bed and distributed evenly with the blood flow. The capillary bed is between 7 and 9 μm in diameter. Sixteen-micrometer microspheres should, therefore, not bypass the splanchnic capillary bed and, indeed, we found practically no spheres in portal blood. Therefore, microspheres trapped in the liver reflect the arterial blood supply to the liver.

The results of the microsphere measurements demonstrate, as expected, that there was no arterial blood flow on day 1 after transplantation. This was also in accordance with the angiographic observation that there was no arterialization of the grafted liver on day 1 after transplantation. In contrast, there was a high arterial blood flow measured with microspheres in the transplanted liver on day 21. The high arterial blood flow on day 21 was also confirmed by the angiogram, where a well-developed collateral rearterialization was visualized.

The results thus demonstrate that the arterial blood flow was not only restored but also significantly increased compared to that in nontransplanted control rats. On day 21, when the animals were sacrificed, there were thick adhesions from the stomach, duodenum, diaphragm, retroperitoneum, and greater omentum to the liver. In a morphological study in which the arterial vasculature was filled with heated gelatin and minium, Lie et al. found that vessels proceed from the stomach, duodenum, and retroperitoneum to the portal hilum in orthotopic hepatic grafts without hepatic artery anastomosis [5].

The early rearterialization demonstrated in the present study may account for the excellent results that we and others have attained in orthotopic liver transplantation without rearterialization in the rat. The cause of the overcompensation in arterial liver blood flow remains totally obscure, but it is of sufficient interest to report on separately in order to allow a better understanding of the results obtained in experimental rat liver transplantation.

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