Hiroshima J. Med. Sci.
Vol.45, No.3, 93~97, September, 1996
HIJM 45-14

Effect of Donor Fasting on Survival of Pancreas and Heart Grafts after Warm Ischemia

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ABSTRACT

Livers from fasted animals are believed to be more vulnerable to ischemic injury than those from fed donors. However, we have recently shown the opposite: livers from fasted rats were more tolerant to ischemic injury. Indeed, the survival rate of 60 min warm ischemic damaged livers increased from 0 to 90% if donor rats were fasted for three days. In this study, we examined how donor fasting affects the outcome of pancreas and heart preservation. BN rats were used as both donors and recipients, and recipients of pancreatic grafts were rendered diabetic prior to transplantation. Pancreatic or heart grafts were subjected to 90 min or 25 min of warm ischemia and were transplanted into the right side of the necks of recipient rats. The viability rate of hearts transplanted from fed donors into fed recipients was only about 11% (1/9) after transplantation. However, the viability rate with fasted donors was 75% (6/8). The rate of successful pancreatic grafting from fed donors into fed recipients was 28.6% (2/7), and that from fasted donors to fed recipients was 41.7% (5/12). These results confirm that the nutritional status of the donor is an important factor in the outcome of not only liver, but also pancreas and heart preservation during transplantation, although the effect of fasting on pancreatic graft is marginal.

Key words: *Heart transplantation, Pancreas transplantation, Warm ischemia, Fasting*

Fasting injures hepatocytes[1-4,6,11,12,18,26)]. On the other hand, however, long-term fasting improves viability of livers exposed to warm or cold ischemia[24)]. This has suggested to us, as well as other researchers[5,14,21)], that fasting the liver donor might be beneficial because of an effect on the sinusoidal lining cells, particularly Kupffer cells. Activation of Kupffer cells has been proposed to be the constraint on successful liver preservation[15)]. Thus, long-term fasting may suppress Kupffer cell activation, microvascular injury, platelet accumulation, and reduced reflow in the transplanted liver. Ischemic induced microvascular injury has also been suggested as the primary event in heart[16)], pancreas[20)], and kidney[19)] injury. If long-term fasting improves the integrity of the vascular system with regard to ischemia/reperfusion injury, then this may improve tolerance of these organs to ischemia as well as the liver. In this study, we have investigated how long-term fasting affects the viability of rat hearts and pancreases after injury by warm ischemia. The goal of this study was to determine whether the beneficial effects of long-term fasting exists in organs other than the liver. Documentation of such an observation could provide new insight into the mechanisms of ischemia/reperfusion injury and form the basis of more detailed studies to unravel the mechanistic effect of fasting on tolerance of organs to ischemia/reperfusion injury.

MATERIALS AND METHODS

Animals

Brown Norway rats were obtained from Seiwa Experimental Laboratoy, Japan. BN rats weighing 180 to 250 g were fed a standard laboratory diet (fed group) or fasted (access only to water) for up to 4 d (fasted group).

Heart transplantation model

Hearts were obtained by opening the abdominal cavity and injecting heparin (100U) into the infrahepatic vena cava. The heart was perfused with 5ml of chilled (4°C) cardioplegic solution (5w/v% glucose, 1.25w/v% mannitol, 30mEq/l KCl and 25mEq/l $NaHCO_3$) via a venotomy in the in-

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frahepatic vena cava. The thoracic cavity was then entered. The aorta and pulmonary artery were divided and the heart removed. The heart was perfused with 2ml of warm (37°C) cadioplegic solution by manual injection through the aortic root. The heart was stored at 37°C in saline for 25 min. After storage, the heart was flushed with 2ml of cold (4°C) saline and transplanted (ischemic time in the cold less than 2 min) into the right side of the neck of a recipient rat as described[13]. The vascular anastomosis was done using a cuff technique between the recipient carotid artey and donor aorta and the recipients jugular vein and donor pulmonary artery. Graft survival was judged by daily palpation of the heart and survival based upon a strong beat for at least 7 days, post-transplant.

Pancreas transplantation model

Pancreatectomy and heterotopic transplantation into the right side of the neck of the recipient was performed as described previously[25]. The donor graft was removed after in situ flushing through the aorta with 2ml of warm (37°C) saline containing 20U heparin. The graft consisted of the pancreatic segment with a short length of the donor aorta connected to the celiac axis and splenic artery together with the splenic vein and portal vein to provide venous drainage. The pancreatic duct was ligated with a silk suture and the grafts stored at 37°C in saline for 90 min prior to transplantation into a recipient. Warm ischemia was terminated by flushing with cold saline (4°C, 1ml) containing heparin. Recipients were made diabetic prior to transplantation by injection of streptozotocin (55mg/kg) and animals were considered diabetic if blood glucose was greater than 300mg/dl. After transplantation, non-fasting blood glucose was measured at day 1, 3, 5, 7, 10, and 14, post-transplantation. The intravenous glucose tolerance test (IVGTT)[17] was performed in the rats in whose serum glucose was less than 400mg/dl on the 14th post-transplant day. A glucose bolus (0.5g/kg body weight) was given and changes in blood glucose measured over a 2 hour period. In this study (data not shown), the K values[17] in the untreated rats and the streptozotocin-treated rats that received pancreatic grafts with minimal preservation (fresh grafts) were 2.58 ± 0.46%/min and 2.35 ± 0.20%/min, respectively. The blood glucose concentrations in these rats were less than 200mg/dl throughout the 2-week post-transplant period. When the pancreatic grafts were subjected to 30 min or 60 min of warm ischemia under the same experimental conditions, the K values were decreased to 1.91 ± 0.16%/min and 1.45 ± 0.21%/min, respectively. The blood glucose concentrations were also less than 200mg/dl over the 2-week post-transplant period. Thus, transplantation of segmental pancreatic grafts in the neck can provide almost complete normalization of pancreatic function, which can be quantitatively assessed with the K value[17]. From these results, we defined graft success as a blood glucose concentration of less than 200mg/dl and a K value greater than 1.0%/min on the 14th post-transplant day.

For both heart and pancreas studies, the effluent flushed out by cold saline flushing of the organ was collected and used for the measurement of LDH and GOT.

Statistics

Statistical analysis was performed using unpaired t-test and Fisher's test. A probability value < 0.05 was considered statistically significant.

RESULTS

Fasting the donor improved the tolerance of the heart to warm ischemic injury. As shown in Table 1, hearts from fed donors exposed to 25 min of warm ischemia were only about 11% viable after transplantation (1 of 9 survivors for 7 days). However, those from fasted donors were 75% viable (six of eight survivors) for at least 7 d post-transplant. There was a significant difference between the two groups ($p=0.013$ by Fisher's Exact Test). Thus, when the heart is obtained from a rat fasted long term, there is improved tolerance to warm ischemia.

The results in Table 2 show the enzyme content of the effluent from hearts exposed to warm ischemia. There was a significant increase in LDH and GOT in the effluent from hearts in the fed group versus the fasted group. The increased LDH and GOT in the effluent of the fed group is thought to arise from plasma membrane injury occurring during ischemia. This suggest that the hearts from fasted donors have less membrane injury due to warm ischemia than hearts from fed donors.

Fasting also appeared to improve the tolerance of the pancreas to 90 min of warm ischemia, although the effect was marginal. There was no significant difference in pancreatic function (IVGTT) or survival between the fed and fasted groups (Table 3).

This study shows that donor fasting increases the tolerance of the rat heart and pancreas to warm ischemia/reperfusion injury, but the effect of fasting on pancreas graft is marginal. This beneficial effect of fasting is similar to what has been reported for the rat liver[24]. The mechanism for this effect is not well understood and may be different in each organ.

Table 1. Survival rate of hearts from fasted or fed donors after 25 min of warm ischemia.

Group	7-day survivors (%)	Stopped beating (day)
Fed (n=9)	11.1	0,0,0,1,1,1,1,3,>7
Fasted (n=8)	75.0	1,4,>7,>7,>7,>7,>7,>7

Rat hearts were obtained from fed rats or those fasted for 4 d as described in the text. The hearts were flushed out with warm (37°C) saline and stored at 37°C for 25 min to simulate warm ischemia. The hearts were transplanted in the right side of recipient rats (fed) and survival was based on continual strong heart beat for at least 7 d.

Table 2. Enzyme level in the effluent from warm ischemic rat hearts.

Group	GOT (U/L)	LDH (U/L)
Fed	387.7 ± 72.4 (n=9)	1968.6 ± 322.6 (n=7)
Fasted	173.3 ± 33.8 (n=8)	945.4 ± 235.1 (n=8)
p vs. fed	p=0.021	p=0.022

After 25 min warm ischemia rat hearts were flushed out with 2ml of saline and the activity of GOT and LDH measured as described in the text. Values are means with the standard error of mean for the number of hearts indicated (n=). Statistical comparison of the mean was done by the unpaired t-test.

Table 3. Graft outcome and K values of pancreas transplants in fasted and fed donors (90 min of warm ischemia).

Group	K value (Mean ± SD)	Successful grafts
Fed	1.01 ± 0.31	2/7 (28.6%)
Fasted	1.37 ± 0.31	5/12 (41.7%)

Rat pancreas was obtained from fed or fasted 4 day donors. The grafts were flushed with warm saline and stored at 37°C for 90 min. Following storage, the pancreas was transplanted to the right side of the neck of the recipient. The intravenous glucose tolerance test (IVGTT) was performed 14 days following transplantation. In the fed group, transplantations were performed in 7 animals. The IVGTT was performed in 6 animals. The animal not subjected to the IVGTT had a blood glucose concentration of 460 mg/dl on day 14. In the fasted group, the IVGTT was performed in only 7 of the 12 transplanted rats. In the 5 animals that did not undergo IVGTT, the blood glucose concentration at 14 days was greater than 400mg/dl.

DISCUSSION

Nutritional factors have been shown to affect the tolerance of the liver to various injurious conditions[4,11,12,18]. Livers from fasted animals are more quickly injured by hypoxia, ischemia, or drugs than livers from fed animals, and the injury appears to be at the level of the hepatocyte. The cause of the increased injury has been suggested to be due to the lack of glycogen since providing another glycogenic substance (fructose), delayed the rate of onset of injury[1,6]. Liver glycogen depletion may occur in organ donors and the liver may be more sensitive to hypothermic preservation/reperfusion injury than nutiritionally replete livers. Thus, nutritional status of an organ donor could be a factor in primary nonfunction or initial delay in liver function after transplantation.

This conclusion was implied from the studies of Boudjema et al[2,3] which showed that livers from fasted rabbits were more quickly injured by cold storage than livers from fed rabbits. Also, Vreugdenhil et al[26] showed that key indices of preservation injury (protein synthesis and plasma membrane damage) were depressed more quickly in cold stored hepatocytes from fasted rats than fed rats. Thus, Vreugdenhil et al proposed that donor liver glycogen levels may be a critical determinant of the tolerance of the cadaveric liver to cold storage/reperfusion injury.

To test this hypothesis, we fasted donor rats for 1 to 3 days which depleted liver glycogen[24]. The livers were cold stored in University of Wisconsin solution (UW) for 30 hr. Survival after orthotopic transplantation was 50% (3/6), the same as in livers from fed rats. However, when the donor was fasted for 4 d, survival after 30 hr preservation increased to 100% (9/9) and after 44 hr preservation from 29% (2/7) to 83% (5/6). This remarkable protection of livers from cold ischemic injury by extensive donor fasting was also found in livers exposed to warm ischemia[24]. After 60 min of warm ischemia and orthotopic transplantation into a different rat, all rats died within 24 hr after the operations (0/8) while livers from 3 d fasted rats were 89% (8/9) viable (89% survival for 7 d).

In the liver, it appears that glycogen depletion

causes hepatocellular injury (increased plasma membrane leakage, decreased protein synthesis) but is not a requisite for survival after warm or cold ischemic injury[24]. In fact, long-term fasting improves survival. Because glycogen depletion causes hepatocellular injury, the beneficial effects of fasting on the liver might be due to protection of the microvascular system to ischemic injury. In the heart, however, fasting has been shown to increase glycogen[7,22] and elevated glycogen has been shown to increase tolerance to warm ischemia[9,10]. Thus, in the heart, fasting may increase glycogen and provide more substrate for anaerobic glycolysis and the synthesis of ATP in myocytes, thus suppressing the loss of ATP during cold or warm ischemia. Loss of ATP in the heart leads to ischemic contracture which is thought to be an irreversible event in warm[8] or cold[23] ischemia. A continual supply from glycogen metabolism may be critical to myocyte preservation. In addition, the beneficial effect of fasting in the heart may also involve an increase in the tolerance of the microvascular system to ischemia/reperfusion damage, as proposed for the liver.

Clearly understanding how long-term fasting protects the heart and liver from ischemic damage may be important to understanding how cold preservation causes injury to these organs. This information may allow development of better methods of donor management, improve organ preservation methods, or suppression of reperfusion injury. This could be very important in heart transplantation because current limits (about 4 hr) are not adequate for effective utilization of all cadaveric hearts at the national level.

(Received January 17, 1996)
(Accepted July 26, 1996)

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