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Successful 48-h liver preservation by controlling nutritional status of donor and recipient

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Abstract The nutritional status of the donor has been shown to affect the outcome of liver transplantation in the rat. It has been proposed that this may be due to inhibition of Kupffer cell induced injury to the reperfused organ, which leads to an inflammatory type response. In this study we investigated how altering the nutritional status of the recipient affects the outcome of liver transplantation after preservation of the liver for 44 or 48 h in the University of Wisconsin (UW) solution. The nutritional status of the rats was altered by either fasting or by feeding an essential fatty acid free diet (EFAD) for 2 months. This type of diet has been shown to reduce significantly the inflammatory response in rats. Survival after 44-h preservation of livers from fed donors (fed a standard laboratory diet) transplanted to fed recipients was 29% (2/7) but increased to 80% (4/5) when the recipient was fed the EFAD diet. After 48-h preservation, there were no survivors under either of these two dietary combinations. However, survival was 100% after 48-h preservation if the donor had been fasted for 4 days and the recipient was fed the EFAD. These results showed that the nutritional status of the donor and recipient are important factors in the outcome of liver transplantation. How nutritional factors affect liver preservation and transplantation are not clear but may be related to the inflammatory response regulated by Kupffer cells and circulating neutrophils in the liver, both of which are influenced by the diet of the animal.

Key words Liver transplant
Nutritional status · Fasting
Essential fatty acid deficiency

Introduction

The outcome of liver transplantation is dependent upon the condition of the donor, the method and length of preservation, and the condition of the recipient. Our group [1] as well as others [2, 3] have shown that the nutritional status of the donor (i.e., fasting and reduction of liver glycogen) can sensitize the liver (hepatocytes) to preservation/reperfusion injury. However, we have recently shown [4] that although fasting may increase preservation injury in the hepatocytes, the whole liver from fasted rats is actually less sensitive to ischemia (warm or cold) when tested in the orthotopic transplant model. We have shown that livers from rats fasted for 4 days are 100% viable after 30 h preservation, whereas livers from fed donors or those fasted for 1–3 days are

only 50% viable [4]. Similar results were obtained with warm ischemia. There is currently no known answer to this dichotomy: fasting increases the sensitivity of hepatocytes to warm or cold ischemia but this is not translated into reduced survival on transplantation, in fact with long-term fasting survival increased.

One suggestion is that fasting alters other cells in the cold stored liver, such as Kupffer or endothelial cells, which have been suggested to be the cause of liver injury after preservation and reperfusion [5, 6]. Kupffer cell activation can lead to the production of a large number of potentially cytotoxic metabolites that could adversely affect the liver (hepatocytes) or induce an inflammatory response and infiltration of blood-borne cells to exacerbate preservation/reperfusion injury. A method of reducing the inflammatory response in rats was developed by Lefkowith [7] and involved feeding an essential fatty acid deficiency (EFAD) diet for a number of months. This altered the fatty acid composition of polymorphonuclear leukocytes (PMNs) and reduced the inflammatory response in rats treated with various inflammatory agents by reducing PMNs activation and reducing the production of cytotoxic metabolites. In this study, we tested the effect of feeding an EFAD to the recipients of liver transplantation on survival and liver injury after 44 and 48 h preservation.

Materials and methods

Brown Norway rats (4 weeks old) were fed either a standard laboratory diet (FED), fasted for up to 4 days (water ad libitum), or fed a diet deficient in fatty acids (essential fatty acid free diet, EFAD) for 2 months prior to the study. The EFAD was obtained from Harlan Teklad, Madison Wis., (TD 84224) and contained 19.2% casein hydrolysate, supplemented with 65.8% sucrose, 5% hydrogenated coconut oil, 5% dl-methionine cellulose, 3.5% minerals, 3.8% calcium carbonate, and 1% vitamins. This diet is estimated to provide only 0.01% linoleic acid to the animal from the 5% hydrogenated coconut oil. After 2 months on this diet or the standard diet, rats were used as donors or recipients for orthotopic liver transplantation. There were five groups in this study. Group 1: both donor and recipient were fed rats (FED-FED) and livers were preserved in University of Wisconsin (UW) solution for 44 or 48 h. Group 2: donors were fasted for 4 days and livers were preserved (44 or 48 h) and transplanted into fed recipients (FASTED-FED). Group 3: livers were from EFAD donors preserved as for other groups and transplanted into fed recipients (EFAD-FED). Group 4: donors were rats fed the standard diet and recipients were fed the EFAD diet (FED-EFAD). Group 5: donors were fasted for 4 days and livers transplanted into the EFAD fed rats (FASTED-EFAD).

The outcome of liver transplantation was judged by two criteria: survival for at least 7 days and liver enzymes measured 6 h after transplantation. Liver enzymes were measured by the method used in the clinics of University Hospital at University of Wisconsin. Results for only LDH are reported here.

The methods for liver harvesting, preservation, and transplantation have been well described previously and were identical to those procedures [8]. Portal cross clamping time and recipient total operation did not exceed 15 min or 45 min, respectively. Livers transplanted without any preservation time (controls) consistently gave 96% survifal for at least 1 week.

Results

The results are shown in Table 1. In the 44-h preservation group, survival for 7 days was only 29% in the FED-FED group. However, if the donor was fasted for 4 days (FASTED-FED) survival increased to 83%. If the donor was fed the EFAD diet and the recipient fed the standard diet (EFAD-FED) survival was 0%. However, if the donor was fed the standard diet and the recipient fed an EFAD diet (FED-EFAD) survival increased to 80%. Post-transplant LDH was measured in three groups and the results were quite variable within the individual groups so that no statistical significance was reached between the differences in the mean values. However, the means were quite different and showed less damage to the livers in the groups that produced the best survival. After 48 h preservation of the liver, no animal survived in the FED-FED, FASTED-FED, or FED-EFAD groups. However, 1-week survival was 100% in the group

Table 1 Effect of nutritional status on survival and LDH release from livers preserved for 44 or 48 h. Survival includes number of animals that survived/total number transplanted for each group in parentheses. LDH values (means ± standard error of the mean) are given for total number of animals in each group (ND not done)

Group	Preservation time (h)	Group description	Survival	LDH (U/l, g) 6 h post-tx
1.	44	FED-FED	29% (2/7)	24973 ± 11844
2.	44	FASTED-FED	83% (5/6)	6677 ± 1738
3.	44	EFAD-FED	0% (0/4)	ND
4.	44	FED-EFAD	80% (4/5)	8709 ± 1915
5.	44	FASTED-EFAD	ND	ND
1.	48	FED-FED	0% (0/4)	ND
2.	48	FASTED-FED	0% (0/4)	ND
3.	48	EFAD-FED	ND	ND
4.	48	FED-EFAD	0% (0/4)	ND
5.	48	FASTED-EFAD	100% (7/7)	3113 ± 186

(FASTED-EFAD) in which the donor was fasted for 4 days and the recipient fed an EFAD diet. In this group, LDH release was lowest among all the groups tested but the difference between the mean LDH in this group and the FED-FED group was not significant using Student's *t*-test.

Discussion

The results of this study showed that the nutritional status of the recipient can have a profound impact on the outcome of liver preservation/transplantation. When the recipient was a rat that was maintained on an EFAD diet for 2 months, survival was 80% when the donor was a fed rat (fed the standard laboratory diet) and the liver preserved for 44 h. If the donor was a fed rat and the recipient was a fed rat, survival, in contrast, was only 29%.

Also, 100% survival was obtained after preservation of the liver for 48 h if the donor was a fasted rat and recipient fed the EFAD diet. In contrast, there were no survivors if the donor was fed and the recipient fed the EFAD diet. Therefore, recipients fed an EFAD diet appeared to be capable of maintaining the viability of livers preserved for relatively long periods of time (44–48 h) and they either reverse preservation injury or do not contribute to reperfusion injury.

The origin of these experiments were the results of the studies by Lemasters et al [6] who have suggested that Kupffer cell activation in the preserved liver is a cause of preservation/reperfusion injury, and the studies of Lefkowith [7] who has shown that an EFAD diet inhibits the inflammatory response in rats and in livers. Thus, we thought that an EFAD might alter the inflammatory response caused by transplantation of a liver injured by long-term hypothermic preservation and improve survival. This appeared to be true in our set of experiments and suggests that reperfusion injury in liver transplantation may be due to neutrophil infiltration into the injured tissue after reperfusion followed by activation. What causes this inflammatory response is not known but could be due to Kupffer cell activation, production of cytokines, and attraction of PMNs, etc, followed by the generation of oxygen free radicals, protease, and arachidonic acid metabolites that could lead to injury to the microvascular structures of the liver and cause irreversible injury. Such mechanisms have been described for livers injured by warm ischemia [9, 10].

Reperfusion injury may be a two-stage event and involve both Kupffer cells and circulating neutrophils. Thus, the first stage takes place within the preserved liver and Kupffer cell activation due to poor or long-term preservation may lead to liver injury directly by the production of cytotoxic end products of metabolism. Under normal conditions the production of these agents is either strictly controlled by the Kupffer cells or the amounts produced can be metabolized by healthy hepatocytes. However, when the hepatocytes are injured by hypothermic storage they may be unable to efficiently metabolize these end products of Kupffer cell activation. Fasting the donor, therefore, may alter the capacity of the Kupffer cells to cause injury to the liver as suggested by Sankary et al. [11], and this may be why survival is increased when the donor is fasted for a long period of time. The second stage of inflammatory injury to the reperfused liver may be related to circulating neutrophils that become activated when exposed to an injured liver. Thus, recipients that have been maintained on an EFAD diet have a less active inflammatory response due to changes in the circulating neutrophils. The combination of a fasted donor and an EFAD-fed recipient act synergistically to inhibit the inflammatory reaction and results in increased survival of rats receiving livers preserved for 48 h.

The clinical utility of these results is unclear at this time. What is clear, however, is that both donor and recipient nutritional status affects the outcome of liver transplantation, even though we do not know fully why this is true. It may be possible to alter the donor nutritional status or to improve the tolerance of the liver to preservation/reperfusion injury by donor treatment, but how to do this requires further study. It is certainly possible, however, to initiate recipient treatments that may decrease the inflammatory response and suppress reperfusion injury. A limitation to liver preservation by simple cold storge, therefore, may have more to do with the quality of the donor or recipient than the quality of the preservation solution.

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