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Prediction of graft dysfunction by analysis of liver biopsies after cold storage

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Abstract Failure of the hepatic allograft continues to be a serious life-threatening risk for the recipient. Because no effective method of extracorporeal support is available for these patients, early retransplantation is the only alternative that offers the potential for survival. The aim of this prospective analysis was to search for a predictor of primary non-function of hepatic allografts before reperfusion. From March to June 1993 we investigated 19 liver biopsies which were obtained during the preparation of the donor liver in the back table bath immediately before the implantation of the organ. All organs were preserved by UW solution. Biopsies were stored at −80 °C, the working-up process was started by dividing the biopsy into several portions for the determination of fat (petrol-ether extraction), water (weighing before thawing and after drying) and free amino acids (OPA-HPLC method). Graft function was categorized into three groups: (1) good function; (2) fair function; (3) primary non-function (PNF). In addition to known risk factors for delayed graft function such as a long stay of the donor in intensive care and a prolonged anhepatic period of the recipient, we were able to demonstrate that organs with malfunction had a higher fat and water content. Donor livers developing PNF showed a trend towards higher total and subdivided amino acids, which could be explained by the incapacity of the liver to utilize available substrates for gluconeogenesis.

Key words Liver transplantation · Liver biopsy · Primary non-function

Introduction

Although orthotopic liver transplantation has become an established therapy for patients with end-stage liver disease, several problems remain to be solved. Despite improvements in organ retrieval, preservation and controlled reperfusion, primary non-function still occurs in 2–20 % of the patients [5, 11]. Multiple factors are involved in graft dysfunction and some of them have been identified as predictors for delayed graft function [7, 9], but none of these so-called predictors is a potent indicator for the development of primary non-function. One important factor in the suitability of the liver graft seems to be the nutritional status of the donor [2, 3]. This factor should be considered because many donors have a period of hyponutrition before organ harvesting, correlating with the length of stay in an intensive care unit (ICU).

The aim of our prospective study was to address the period after cold storage immediately before organ replacement to determine whether the organ had sustained any grave injury.

Materials and methods

From March to June 1993 during a series of 19 consecutive orthotopic liver transplantations we obtained liver biopsies from the fourth segment. Biopsies were taken at the time of unwrapping the liver from the storage pack and were immediately stored at

−80 °C. All organs were preserved with the University of Wisconsin (UW) solution after the standard procurement of organ retrieval and in situ liver perfusion with up to 5000 ml of UW solution during the donor operation. The working-up process was started by dividing the biopsies into several portions for the determination of fat, water, and free amino acids. Fat was measured by the method of petrol-ether extraction, water content was measured by weighing the liver piece before thawing and after drying, and the free amino acids were measured by the OPA-HPLC method. Results of the measurement of the free amino acids were divided into those for total amino acids, essential amino acids, aromatic amino acids, and branched-chain amino acids to observe any subgroup differences. The results of the preparation of the liver biopsies were compared with the postoperative function of the transplanted livers. Two further risk factors for delayed postoperative graft function were taken into consideration, the length of donor stay at the ICU before organ procurement and the length of the intraoperative anhepatic period during the liver transplantation. Postoperative graft function was categorized as described before [7] and divided into three groups: (1) good function (GOT under 1000 U/l, spontaneous PT higher than 50 %, bile production above 100 ml/day); (2) fair function (highest GOT above 1000 U/l, clotting factor support, bile production under 100 ml/day); and (3) primary non-function (death without retransplantation within 7 days because of complete liver failure.

Results

Distribution of the postoperative organ function showed 13 livers (68 %) having good function 4 livers (21 %) with fair function, and 2 livers (11 %) with primary non-function (PNF). Concerning the water content of the biopsies, we observed a mean of 313 g/100 g fat-free substance (FFS) with a standard deviation of ± 89 g/100 g FFS in the group of organs with good postoperative liver function. The biopsies of the livers showing fair postoperative graft function had a water content of 289 ± 37 g/100 g FFS. The liver biopsies of the two livers with postoperative non-function had a water content of 335 ± 8 g/100 g FFS. Examination of the fat content within the liver biopsies of the three groups showed the following results: biopsies of organs with good postoperative function had a fat content of 8.45 ± 4.2 g/100 g FFS, biopsies of organs with fair function, 7.25 ± 2.6 g/100 g FFS, and biopsies of organs which were complicated by postoperative non-function had a fat content of 19.55 ± 5.1 g/100 g FFS. Concentrations of amino acids in the liver grafts after cold storage showed the following results. Total amino acids allocated to the three groups: good function, 8.717 ± 2.162 mmol/100 g FFS; fair function, 8.021 ± 3.454 mmol/100 g FFS; PNF, 11.140 ± 0.211 mmol/100 g FFS. Essential amino acids (tyrosine, valine, methionine, tryptophan, isoleucine, phenylalanine, leucine, lysine), divided into the three groups: good function, 1.539 ± 0.555 mmol/100 g FFS; fair function, 1.357 ± 0.781 mmol/100 g FFS; PNF, 1.734 ± 0.305 mmol/100 g FFS. Aromatic amino acids (tyrosine, phenylalanine), distributed among the three groups: good function, 0.095 ± 0.05 mmol/100 g FFS; fair function 0.13 ± 0.029 mmol/100 g FFS; PNF, 0.12 ± 0.016 mmol/100 g FFS. Branched-chain amino acids, divided into the three groups: good function, 0.301 ± 0.16 mmol/100 g FFS; fair function, 0.376 ± 0.071 mmol/100 g FFS; PNF, 0.419 ± 0.127 mmol/100 g FFS.

In addition to these results of the liver biopsies we found the following correlation to the ICU-stay of the donor and the intraoperative anhepatic period. Divided into the three groups, ICU-stay of the donor showed the following results: good function, 1.9 ± 1.3 days; fair function, 2.8 ± 2.1 days; PNF, 4.5 ± 0.7 days. Good working organs were transplanted with an anhepatic period of 84 ± 17 min, organs showing fair function with an anhepatic period of 90 ± 10 min, and the organs which were classified as having no postoperative function had an anhepatic period of 125 ± 43 min.

Discussion

The increasing demand of organs for liver transplantation has led to the utilization of older donors, of minimal steatotic livers, of livers from donors after a longer period of hypotension and of donors after a longer ICU-stay. Therefore the risk of development of a PNF of the transplanted organ remains high. Since preservation injury is not completely understood, we want to know if there is any predicting factor which could be identified by the examination of a biopsy taken after cold storage. High donor liver fat content has been reported to be associated with the development of PNF [1, 12], and our investigation confirmed this finding; as in other reported analyses we were able to identify a strong correlation between fat content of the liver and development of postoperative PNF. Although the differences were not statistically significant, there was a trend towards higher water content in the livers developing PNF. Cell injury and subsequent cell swelling may be an explanation for the development of PNF. Amino acid content in the liver has not been evaluated as a possible predictor for postoperative graft function so far. Other investigators have examined amino acids in the preservation fluid and found a strong correlation between the amount of amino acids and the development of graft dysfunction [4]. Another group has investigated the normalization of plasma amino acids immediately after liver transplantation and found that abnormalities of the amino acid content should be restored within 3 h after transplantation, in other cases, PNF should be strongly suspected [8]. Our own data showed a significantly higher amino acid content in the biopsies obtained from livers which developed postoperative PNF. As a possible explanation for these findings we could assume an incapacity of the liver to utilize available substrates for gluconeogenesis and anaerobic energy charge. The donor-

related risk factor of length of ICU stay before organ retrieval could again be identified as an independent factor associated with the appearance of PNF if the donor stayed for longer than 4 days in the ICU [6, 7, 10]. Another results of our previous study, which was associated with a higher rate of PNF, the prolonged anhepatic period, was again clearly above the critical time of 90 min in the PNF group.

Although we were able to confirm previous studies and their findings of indicators for postoperative organ failure, we could not identify a new predictor of PNF by these analyses of liver biopsies obtained after cold storage of the organ.

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