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Amino acids in rinse effluents as a predictor of graft function after transplantation of fatty livers in rats

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Abstract There are too few reliable markers by which one can predict future function of a liver before implantation. Consequently, the purpose of this study was to test the hypothesis that amino acids in rinse–effluents could predict transplant outcome in marginal fatty livers from rats. Amino acids were measured in the rinse effluent from the livers immediately after harvest and graft preparation or cold storage. Amino acids in the effluent were twice as high in ethanol-treated animals compared to those in nonfatty controls. Ethanol-treated fatty livers survived for no longer than 7 days after transplantation while 83% of nonfatty controls survived ($P < 0.05$). In subsequent studies, the cold-storage time was decreased to 6 h to determine whether failing fatty livers released more amino acid than grafts that would function normally. There was a significant increase in amino acids in the effluent of fatty grafts compared to controls. Moreover, the sum of the four selected amino acids (alanine, valine, histidine, leucine) was lower than 23 nmol/g liver in functional livers, whereas failing grafts had totals significantly higher than 25 nmol/g liver. The sum of the four amino acids correlated well with 24 h post-transplant serum AST levels ($r = 0.78$, $P < 0.0001$). So we can conclude that amino acid release can serve as a useful marker of graft viability and reliably predicts survival.

Key words Liver transplantation – Survival – Amino acids – Organ preservation – Primary nonfunction

Introduction

Despite enormous efforts to increase organ donation, and a concomitant 30% increase in liver transplant surgery, the number of patients needing organ transplantation has steadily increased. Currently, thousands of patients are awaiting a liver graft and approximately 20% of the patients on the waiting list die prior to transplantation [2, 4, 21]. Extending the donor pool by using marginal livers has been proposed to increase the number of available organs [2, 21]. Marginal organs are defined as having a greater risk of dysfunction and primary nonfunction, and represent approximately 35% of the donor pool [2, 21, 24, 28].

Many of these marginal organs are fatty livers [1,23,27]. Despite the fact that a marginal liver may function as a normal graft, the risk of complications after transplantation is considered unacceptable by most centers, and many of these organs are therefore not transplanted.

Several approaches have been attempted to distinguish between high- and low risk grafts but none can reliably predict future function prior to transplantation [21, 24, 25]. This is due to the fact that these tests of organ viability are based on evaluation of hepatocyte function or energy status, however, the target of ischemia-reperfusion injury after graft implantation is nonparenchymal cells [6]. Endothelial cells are destroyed within 20 min after reperfusion of a marginal organ, and activated Kupffer cells can release proteases, free radicals and cytokines that contribute to graft failure. Increased protease activity occurs in reperfusion injury of liver grafts and correlates well with storage time and graft function [7, 10]. Protease inhibitors improve survival, enhance microcirculation of transplanted livers [8, 10, 14, 22], and amino acids released from livers stored in University of Wisconsin (UW) solution reflect proteolytic activity during cold storage [7]. We therefore hypothesize that amino acids released by proteolytic activity in organ effluents collected immediately after harvest might be useful predictors of graft function. We tested this hypothesis by measuring amino acid release from fatty livers produced by chronic ethanol feeding. Only preliminary accounts of this work have already been published [11].

Methods

Production of fatty livers

Male Lewis rats weighing between 200–300 g were fed a liquid high-fat diet (Lieber-DeCarli) [19] containing 36% of its calories as alcohol for 4 weeks to produce fatty livers. Control rats received a high-fat maltose-dextrin-containing diet isocalorically. The level of fatty changes was determined by osmium staining [20]. In some experiments, gadolinium chloride ($GdCl_3$, 20 mg/kg), a specific Kupffer cell toxicant, was injected into the tail vein of donor rats 24 h before liver explantation.

Liver transplantation and collection of rinse effluents

Livers were transplanted using the method by Kamada et al. with arterial reconstruction [13, 18]. Briefly, the liver was explanted by cutting ligaments and isolating the bile duct and appropriate vessels. Prior to removal of the organ, heparin (500 U) was administered i.v. and donor livers were perfused with 8 ml ice-cold (2–4 °C) Ringer's solution followed by 3 ml ice-cold UW solution through the portal vein. The liver was explanted and the hepatic vessels were prepared for transplantation. At the end of the harvest procedure, not later than 25 min after removal, livers were placed on a nylon grid over a funnel and rinsed through the portal vein with 2.0 ml of ice-cold (2–4 °C) UW solution. This effluent was collected, centrifuged at 1000 g for 8 min, and frozen at −80 °C for subsequent measurement of amino acids (see below). Grafts were stored for 6 or 24 h at 4 °C, and liver transplantation was performed. Directly prior to implantation, the liver was perfused with 2.0 ml of ice cold Ringer's solution and the effluent was collected as above. Twenty-four hours following transplantation, 0.1 ml blood was drawn from the tail vein of recipients and analyzed for serum AST by standard enzymatic methods [5]. For all experiments the principles of laboratory animal care of NIH were followed.

Measurement of amino acids

Amino acids in rinse effluents were measured by reverse-phase high performance liquid chromatography (HPLC). Quantitative analysis of total free amino acids was carried out using the PICO · TAG Amino Acid Analysis System (Waters Corp., Milford, MA). Effluent samples were first deproteinized by ultrafiltration and then derivatized with phenylisothiocyanate (PITC) to produce phenylthiocarbamyl (PTC) amino acids. Amino acids were determined by automated gradient reverse phase HPLC and ultraviolet detection at 254 nm [16].

Statistics

Student's t-test, Mann-Whitney rank sum test, or Fisher's exact tests (for survival experiments) were used for determination of significance. The correlation between amino acid concentrations and AST levels were calculated using Pearson's Moment Product correlation, and between amino acid concentrations and survival using the Spearman Rank Order correlation. A P value less than 0.05 was selected prior to the study as the level of significance.

Results

Serum transaminases and mortality after transplantation of fatty livers

Donor livers from rats fed ethanol-containing high fat diet demonstrated large fatty droplets in 50–60% of hepatocytes as determined by osmium staining. Rats fed control diet had fatty changes in less than 10% of the hepatocytes.

Serum AST levels 24 h after transplantation and rates of survival after 7 days are shown in Figure 1. Serum AST levels in recipients of fatty livers were nearly tripled compared to non-fatty controls (2234 ± 308 vs. 768 ± 144 U/l; respectively). Furthermore, rats receiving fatty livers survived for no longer than 7 days after transplantation, whereas mortality was only 17% in the non-fatty control group. Swollen and congested livers, ascites, and hemoperitoneum were the major pathological findings.

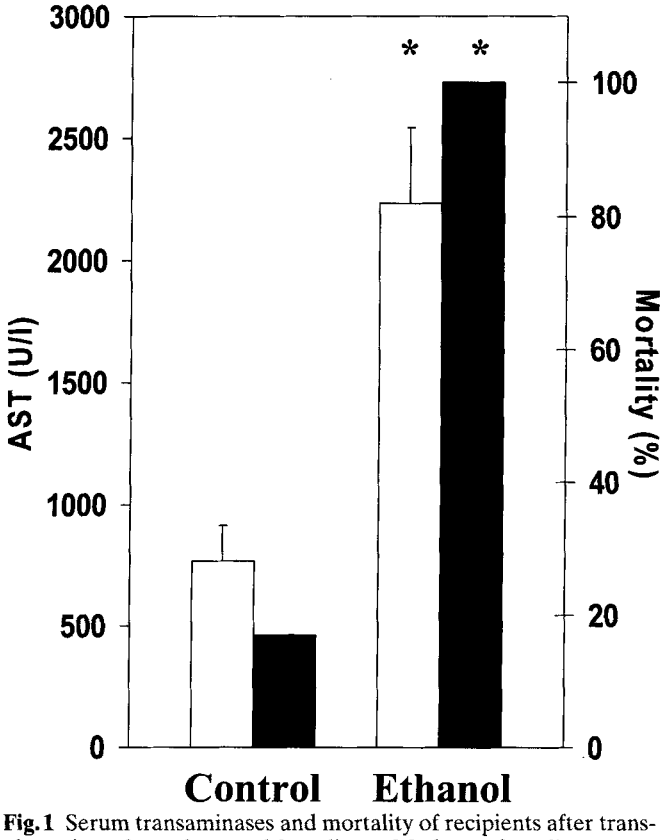

Fig. 1 Serum transaminases and mortality of recipients after transplantation of non-fatty and fatty livers. Moderate fatty livers were produced by feeding rats a liquid high-fat diet containing 36% of calories from ethanol as described in Methods. Controls received a liquid, high-fat diet with isocaloric calories from maltose-dextrin. Livers were transplanted after 24 h of cold storage in UW solution. AST (open) was determined at 24 h and mortality (closed) was evaluated at 7 days after transplantation. Data are mean ± SEM ($n = 8$). *, $P < 0.05$ using Student's t-test (AST) or Fisher's exact test (mortality)

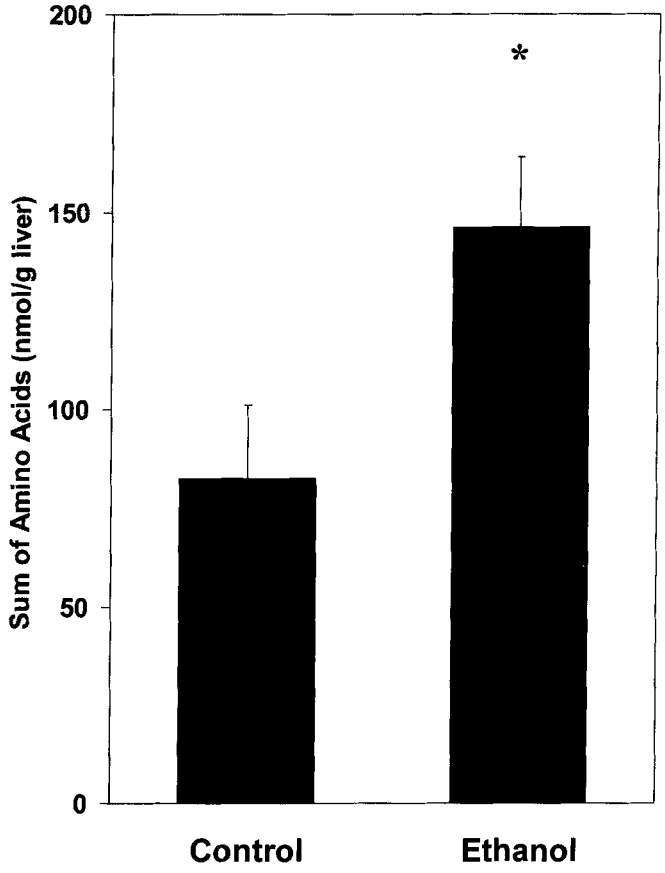

Fig. 2 Amino acid release from non-fatty and fatty livers after harvest and graft preparation. Rinse effluent was collected from explanted livers and amino acids were measured as described in Methods. Data are mean ± SEM ($n = 6$–8). *, $P < 0.05$ in non-fatty vs. fatty grafts, using Student's t-test

Amino acids in rinse effluents from control and fatty livers

Amino acid release directly after graft harvest (0.5 h) and after 24 h cold storage is shown in Figure 2. Amino acid release was twice as high during harvest and graft preparation in fatty grafts compared to that of nonfatty controls. Interestingly, amino acid concentration did not differ between fatty and non-fatty controls after 24 h of cold storage (4535 ± 352 and 4465 ± 305 nmol/g liver, respectively). Amino acids were below the limits of detection in fresh UW storage solution. Individual amino acids measured in the rinse effluent of fatty and nonfatty livers after harvest and graft preparation are shown in Table 1. Four individual amino acids with control values greater than 1.5 nmol/g liver, a 190% or greater increase in fatty livers compared to nonfatty controls and a P value of 0.008 or less were retrospectively identified as the most reliable indicators of marginal viability of the grafts (alanine, histidine, leucine and valine) and the sum was increased about two-fold in fatty livers compared to the nonfatty controls.

Serum AST, mortality and amino acid release in marginal livers

Since rats receiving marginal (fatty) livers after 24 h cold storage did not survive after transplantation, a second set of experiments was completed with fatty livers stored for 6 h to rigorously test the hypothesis that amino acid release could be used to distinguish between marginal livers that would fail or survive after transplantation. Livers from ethanol-fed rats were stored for 6 h after pretreatment with the Kupffer cell toxicant $GdCl_3$ or vehicle, as it is well known that the destruction of Kupffer cells decreases mortality after experimental transplantation [15].

Table 1 Concentrations of amino acids in graft rinse effluents collected after liver harvest[a]

Amino Acids	Non-Fatty Livers (nmol/g liver)	Fatty Livers	% of Control	Correlation to AST	*P* value
Ala	5.0 ± 0.5	9.7 ± 0.8	194	0.91	0.00009
His	2.9 ± 0.6	5.6 ± 0.5	193	0.86	0.0006
Val	2.3 ± 0.4	5.6 ± 0.5	243	0.75	0.008
Leu	1.9 ± 0.5	4.8 ± 0.3	253	0.78	0.005
Sum of Ala, His, Val and Leu	12.1 ± 1.1	25.6 ± 1.7	212	0.93	0.00004
Arg	0.1 ± 0.1	0.7 ± 0.3	700	0.75	0.008
Asn	1.9 ± 0.9	4.4 ± 1.4	232	0.28	0.389
Asp	2.3 ± 2.1	5.0 ± 4.5	217	0.22	0.517
Cys	0.0 ± 0.0	0.5 ± 0.4	–	0.46	0.151
Gln	17.6 ± 4.1	31.0 ± 2.5	176	0.78	0.005
Glu	13.7 ± 6.1	26.2 ± 11	191	0.19	0.576
Gly	15.2 ± 4.1	26.5 ± 3.0	174	0.63	0.038
Ile	1.4 ± 0.2	3.1 ± 0.3	221	0.79	0.004
Lys	6.1 ± 3.1	5.7 ± 0.7	93	0.18	0.607
Met	0.8 ± 0.2	1.9 ± 0.5	238	0.65	0.029
Phe	2.0 ± 0.5	2.5 ± 0.4	125	0.48	0.134
Pro	0.8 ± 0.3	2.2 ± 0.7	275	0.74	0.009
Ser	0.8 ± 0.7	2.1 ± 1.8	263	0.26	0.443
Thr	5.0 ± 2.0	5.1 ± 1.0	102	0.11	0.739
Trp	1.0 ± 0.2	1.2 ± 0.2	120	0.05	0.872
Tyr	1.6 ± 0.3	2.3 ± 0.2	144	0.66	0.026
Sum	82.4 ± 18.7	146.0 ± 17.8	177	0.65	0.031

[a] Numbers are the mean amino acid concentrations in rinse effluents from at least 5 grafts. Selected amino acids are defined as the sum of the amino acids with values greater than 1.5 nmol/g liver in the control, an increase of 190% or greater in fatty livers, compared to control and a *P* value of 0.008 or less (alanine, histidine, leucine and valine). Correlation was determined using the Pearson's moment product correlation

Table 2 Serum AST, mortality and amino acid release from fatty livers transplanted after 6 h cold-storage[a]

Donor	Serum AST (U/l)	% Mortality	Amino Acids (nmol/g liver)
High-fat, ethanol diet	1447 ± 190	63% (10/16)	198 ± 14
High Fat, Ethanol Diet + $GdCl_3$ treatment	905 ± 149[b]	20% (2/10)	141 ± 10[b]

[a] Livers were transplanted after 6 h cold storage as described in methods. One group of animals was treated with $GdCL_3$ (20 mg/kg, i.v.) 24 h before liver explantation. Amino acids in rinse effluents were determined after graft harvest and preparation, serum AST was determined 24 h after implantation and mortality was determined at 7 days. [b] Significantly different from high fat, ethanol fed rats; $P < 0.05$ by Student's t-test

Table 2 shows the serum AST 24 h after operation, the rate of mortality, and the amino acid concentration in the rinse effluent of recipients of fatty livers stored cold for 6 h with and without $GdCl_3$ treatment. The mortality of transplanted fatty livers was 63% and the plasma AST level was 1447 ± 190 U/l. As shown previously, $GdCl_3$ treatment of the fatty liver donor decreased mortality (20%) and reduced postoperative (24 h) serum AST levels (905 ± 149 U/l) significantly. Moreover, fatty livers released 40% more total amino acids than fatty livers treated with $GdCl_3$ (198 ± 14 vs. 142 ± 10 nmol/g liver; Table 2) and significant changes were found in the sum of the four most different amino acids (Figure 3). Significantly, the increase in these amino acids was reduced by pretreatment of fatty liver donors with $GdCl_3$ (17 ± 2.4 nmol/g liver) compared to fatty livers (27.0 ± 1.5 nmol/g liver).

The sum of the four most different amino acids reliably predict graft survival before implantation of marginal livers

The sum of the four amino acids (alanine, histidine, leucine and valine) in the effluent from fatty livers of each animal study are shown in Figure 4. Overall, the concentration of these four amino acids increased from 16 ± 1 nmol/g liver in functioning fatty livers to 31 ± 1 nmol/ g liver in failing fatty livers ($P < 0.001$). Furthermore, all survivors had concentrations lower than 23 nmol/g liver, whereas concentrations in nonsurvivors were always higher than 25 nmol/g liver. The correlation coefficient between the sum of the four amino acids and survival was –0.84 ($P < 0.005$). Figure 5 shows the correlation between the sum of the four amino acids and 24 h postoperative AST. The sum of the four amino

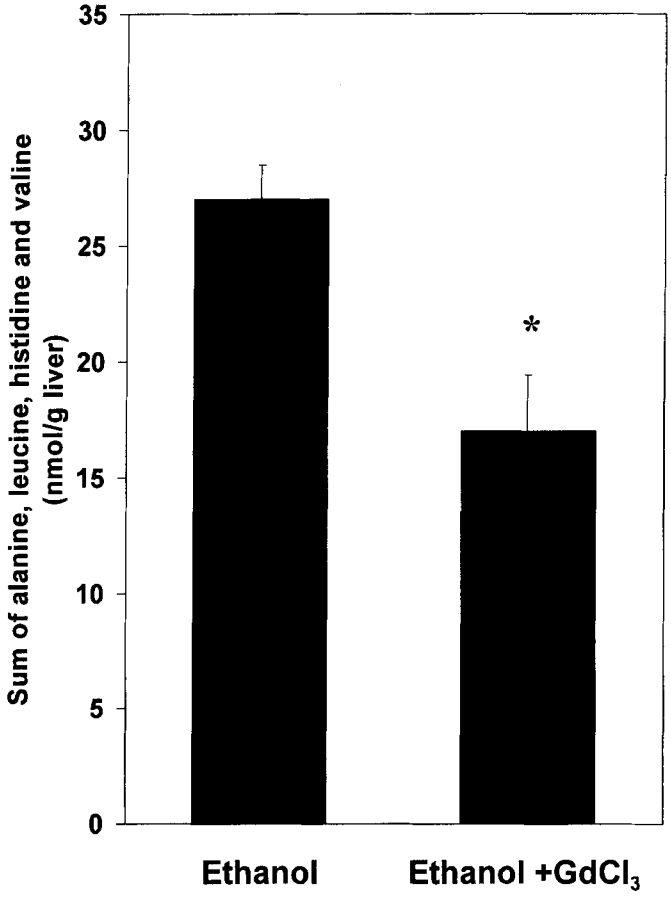

Fig. 3 Concentrations of amino acids in rinse effluents of fatty livers from control and $GdCl_3$-treated donors. Amino acids were measured in rinse effluents collected as described in Methods 25 minutes after explantation. One group of fatty liver donors were given $GdCl_3$ (20 mg/kg body weight) i.v. 24 h before explantation. Graph depicts the sum of the selected four amino acids alanine, histidine, leucine and valine as defined in Results. Data are mean ± SEM (n = 16/10). *, $P < 0.01$, by Student's t-test

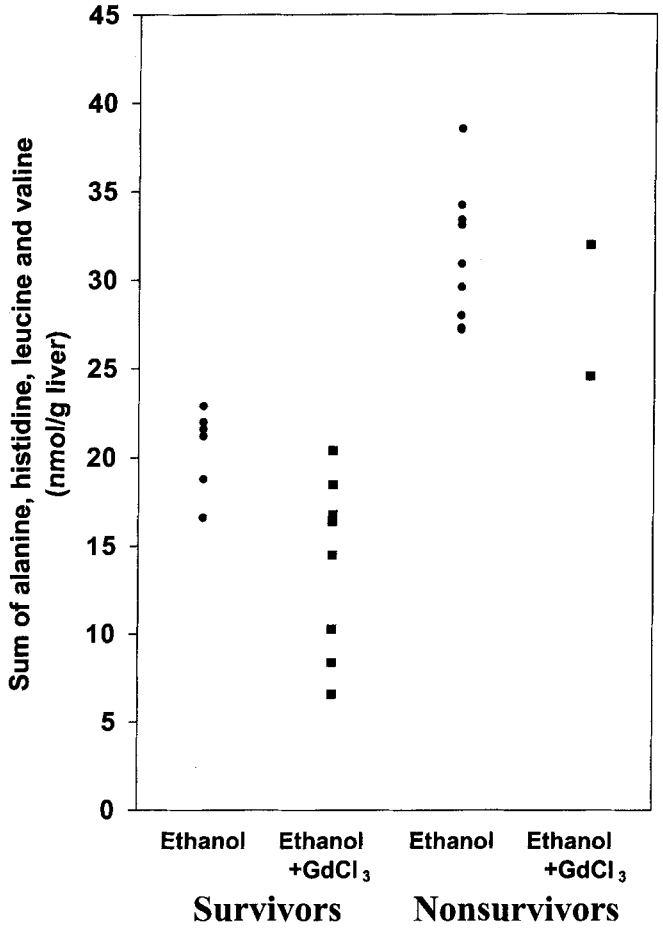

Fig. 4 Concentration of amino acids in rinse effluents of survivors and nonsurvivors. Rinse effluents were collected after graft preparation. Livers were transplanted after 6 h of cold storage. The graph shows the sum of alanine, histidine, leucine and valine from fatty livers (filled circles) or $GdCl_3$-treated fatty livers (filled squares)

acids in the rinse effluent of all livers correlated well with serum AST 24 h after transplantation (Pearson Product Moment correlation coefficient = 0.78, $P < 0.0001$).

Discussion

Failing fatty livers release increased amounts of amino acids after organ harvest

Ethanol has been shown to cause fatty liver and is a risk factor for increased primary nonfunction and liver failure after transplantation [1, 23, 27]. Chronic ethanol feeding and cold storage of the donor liver caused a significant increase in AST in the recipient and enhanced mortality compared to nonfatty controls (Figure 1), as shown in other studies [12, 29]. Based on experiments showing that proteolysis contributes to graft injury after transplantation of livers [7, 10] and that protease inhibitors improve graft function [8, 22], we hypothesized that amino acids released from grafts during graft preparation and cold storage could be used to predict graft function after implantation. Indeed, fatty grafts released more amino acid than nonfatty grafts during harvest and graft preparation (Figure 2), but released the same amount of amino acids during the cold storage period. Why similar amounts of amino acids were released during the 24 h cold-storage period in fatty and nonfatty livers is unclear, however, this may be due to a temperature dependent decrease in proteolysis at 4 °C during cold storage, compared to an organ temperature of 8–10°C

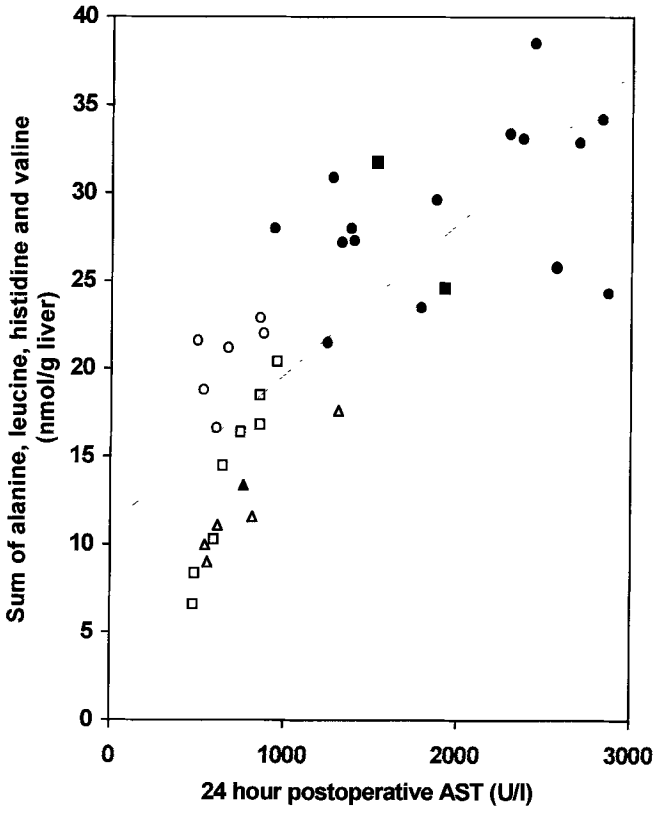

Fig. 5 Correlation of amino acids and AST levels. In control and fatty grafts with and without $GdCl_3$ treatment of the donors, rinse effluent was collected after graft preparation and amino acids were measured as described in Methods. Livers were transplanted after 24 and 6 h of cold storage, AST levels were measured 24 h after transplantation and mortality was evaluated at 7 days. Pearson's product moment correlation coefficient was used to calculate the correlation of the sum of concentrations of alanine, histidine, leucine and valine in the rinse effluent with AST ($r = 0.78$, $P < 0.0001$). Survivors (open symbols); Nonsurvivors (closed symbols); Control livers (triangels); Fatty livers (circles); $GdCl_3$-treated fatty livers (squares)

during graft preparation. The fact that amino acids are probably released by enhanced proteolytic activity during harvest shows the importance of the early stage of organ harvest for graft injury. The increase in amino acid release from fatty livers during the harvest period may be due to the fact that alcohol makes Kupffer cells easier to activate, most likely by increasing the sensitivity of calcium channels [17]. Destruction of Kupffer cells with $GdCl_3$ blocks reperfusion injury and enhances survival after liver transplantation [3, 29]. Warm ischemia occures during the harvest procedure, and early storage might also result in activation of Kupffer cells with the subsequent release of proteases. These data are consistent with the observation that protease inhibitors added to the storage solution reduce ischemia-/reperfusion injury and increase survival after transplantation [8, 22].

Individual amino acids

Of the individual amino acids in the rinse effluent collected after harvest and graft preparation (Table 1), four amino acids (alanine, histidine, leucine and valine) met the criteria of values greater than 1.5 nmol/g liver. This is an increase of 190% or more in fatty livers, compared to non-fatty controls and a P-value of 0.008 or less. The sum of these four amino acids, termed for convenience the selected amino acids, approximately doubled in fatty livers when compared to controls. These data suggest that measurement of these specific amino acids could be used to predict function of fatty liver grafts after implantation. Because of high mortality in the recipients of fatty livers after 24 h of cold storage (Fig. 1), the claim that amino acid release could help to distinguish between functioning and failing marginal livers could not be tested without shortening the cold storage time or treating fatty marginal grafts with agents known to improve survival.

Amino acid concentrations are increased in failing marginal grafts

When fatty livers had been stored for 6 h, 63% of the recipient animals died (Table 2). This time point was selected to evaluate whether specific amino acids could predict graft outcome. Furthermore, treatment with $GdCl_3$ destroyed 90% of the resident Kupffer cells, improved survival, and decreased the sum of four selected amino acids, alanine, histidine, leucine, and valine significantly (Figure 3). When the most different amino acids from rats that survived and died were compared (Figure 4), amino acids in the rinse-effluent of grafts that failed were significantly higher. These data also show that there is a maximal value of the sum of alanine, leucine, histidine and valine of about 25 nmol/g liver which, if exceeded, correlates with 100% mortality. We can also conclude that the release of amino acids from the harvested liver is Kupffer cell–dependent. Furthermore, there is a significant correlation between recipient AST level at 24 h and the concentration of amino acids determined after graft harvest (Figure 5). We can therefore conclude that the selected four amino acids can reliably predict graft function of the liver before implantation of the graft. These data suggest that measuring amino acids in the rinse–effluents of donor organs could be used as a reliable test to predict the function of marginal fatty grafts before implantation.

Clinical significance

At present, most transplant surgeons rely on the overall appearance of the donor liver as an indicator of organ viability [9, 24]. The major problem with this method of evaluation is that it is highly subjective and depends on the surgeon's experience and perception of the explant. Many grafts that are subjectively evaluated as "poor in physical appearance" may function well on implantation. Providing a sensitive, reliable and objective tool for this critical decision would decrease the risk in using a marginal graft. Several facts clearly advocate transferring the experimental technique described here to a clinical setting. First, rinsing the graft with UW solution after harvest and at the time of final packaging is a routine task with little risk of damaging the organ. Second, amino acids can be quantified in less than 1 h [26], this amount of time being sufficient to make a decision before a potential recipient is selected and notified. Third, with this test, the donor pool would be utilized better because grafts with high risk of primary nonfunction would not be implanted, thereby minimizing the frequency of retransplantation. Finally, measurement of amino acid release after harvest and preparation of liver might serve as a useful marker for evaluating graft viability prior to transplantation, and may predict subsequent host graft survival. Based on the data presented in this study, clinical trials are called for to determine whether a similar set of criteria can be defined in human liver transplantation that correlates well with the overall appearance of the donor liver to the explant surgeon or to biopsies taken before explantation, by which future graft function can be predicted early enough to influence the transplant decision.

References

1. Adam R, Reynes M, Johann M, Morino M, Astarcioglu I, Kafetzis I, Castaining D, Bismuth H (1991) The outcome of steatotic grafts in liver transplantation. Transplant Proc 23: 1538–1440
2. Alexander JW, Vaughn WK (1991) The use of "marginal" donors for organ transplantation. Transplantation 51: 135–141
3. Arii S, Monden K, Adachi Y, Furutani M, Mise M, Fujita S, Ishiguro S, Nakamura T, Harada T, Niwano M, (1995) Suppression of the reperfusion injury of cold-preserved livers by Kupffer cell blockade. Transplant Proc 27: 765–767
4. Belle SH, Beringer KC, Detre KM (1996) Recent findings concerning liver transplantation in the United States. Clin Transplant 1996: 15–29
5. Bergmeyer HU (1988) Methods of Enzymatic Analysis. Academic Press, New York
6. Caldwell-Kenkel JC, Currin RT, Tanaka Y, Thurman RG, Lemasters JJ (1989) Reperfusion injury to endothelial cells following cold ischemic storage of rat liver. Hepatology 10: 292–298
7. Calmus Y, Cynober L, Dousset B, Lim SK, Soubrane O, Conti F, Houssin D, Giboudeau J(1995) Evidence for the detrimental role of proteolysis during liver preservation in humans. Gastroenterology 108: 1510–1516
8. Currin RT, Reinstein LJ, Lichtman SN, Thurman RG, Lemasters JJ (1993) Inhibition of tumor necrosis factor release from cultured rats Kupffer cells by agents that reduce graft failure from storage injury. Transplant Proc 25: 1631–1632
9. D'Alessandro AM, Kalayoglu M, Sollinger HW, Hoffmann RM, Reed A, Knechtle SJ, Pirsch JD, Hafez GR, Lorentzen D, Belzer FO (1991) The predictive value of donor liver biopsies for the development of primary nonfunction after orthotopic liver transplantation. Transplantation 51: 157–163
10. Ferguson DM, Gores GJ, Bronk SF, Krom RA (1993) An increase in cytosolic protease activity during liver preservation. Inhibition by glutathione and glycine. Transplantation 55: 627–633
11. Frankenberg Mv, Forman DT, Frey W, Bunzendahl H, Lemasters JJ, Thurman RG (1997) Amino acids in storage solution predict primary nonfunction in fatty liver grafts. Transplant Proc 29: 1131–1132
12. Gao W, Connor HD, Lemasters JJ, Mason RP, Thurman RG (1995) Primary nonfunction of fatty livers produced by alcohol is associated with a new, antioxidant-insensitive free radical species. Transplantation 59: 674–679
13. Gao W, Lemasters JJ, Thurman RG (1993) Development of a new method for hepatic rearterialization in rat orthotopic liver transplantation: reduction of liver injury and improvement of surgical outcome by arterialization. Transplantation 56: 19–24
14. Gores GJ, Krom AF (1995) Is proteolysis a critical mechanism of hepatic preservation injury? Gastroenterology 108: 1594–1596
15. Hardonk MJ, Dijkhuis FWJ, Hulstaert CE, Koudstaal J. (1992) Heterogeneity of rat liver and spleen macrophages in gadolinium chloride-induced elimination and repopulation. J Leukoc Biol 52: 296–302
16. Heinrikson RI, Meredith SC (1984) Amino acid analysis by reverse-phase high-performance liquid chromatography: Precolumn derivation with phenylisothiocyanate. Anal Biochem 136: 65–74
17. Hijioka T, Goto M, Lemasters JJ, Thurman RG (1993) Effect of short-term ethanol treatment on voltage-dependent calcium channels in Kupffer cells. Hepatology 18: 400–405
18. Kamada N, Calne RY (1979) Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. Transplantation 28: 47–50
19. Lieber CS, DeCarli LM (1989) Liquid diet technique of ethanol administration: 1989 update. Alcohol Alcohol 24: 197–211
20. Luna L (1968) Manual of Histologic Staining Method of the Armed Forces Institute of Pathology. McGraw-Hill Book Co., New York
21. Mirza DF, Gunson BK, DaSilva RF, Mayer AD, Buckels AC, McMasters P (1994) Policies in Europe on "marginal quality" donor livers. Lancet 344: 1480–1483
22. Oldhafer KJ, Hauss J, Spiegel HU, Gubernatis G, Pichlmayr R (1993) Tissue PO2 and reperfusion injury in the transplanted liver after application of aprotinin. Transplant Proc 25: 2555

23. Ploeg RJ, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD,Hoffmann RM, Sasaki T, Sollinger HW, Belzer FO, Kalayoglu M (1993) Risk factors for primary dysfunction after liver transplantation–A multivariate analysis. Transplantation 55: 807–813
24. Pruim J, Klompmaker IJ, Haagsma EB, Bijleveld CM, Slooff MJH (1993) Selection criteria for liver donation: a review. Transpl Int 6: 226–235
25. Strasberg SM, Howard TK, Molmenti EP, Hertl M (1994) Selecting the donor liver: Risk factors for poor function after orthotopic liver transplantation. Hepatology 20: 829–838
26. Teerlink T, Leeuwen van PAM, Houdijk A. (1994) Plasma amino acids determined by liquid chromatography within 17 minutes. Clinical Chemistry 40: 245
27. Todo S, Demetris AJ, Makowa L, Teperman L, Podesta L, Shaver T, Tzakis A, Starzl TE (1989) Primary nonfunction of hepatic allografts with preexisting fatty infiltration. Transplantation 47: 903–908
28. Wood RP, Ozaki CF, Katz SM, Monsour HP, Dyer CP, Johnston TD (1994) Liver transplantation – the last ten years. Surg Clin North Am 74: 1133–1154
29. Zhong Z, Connor H, Mason RP, Qu W, Stachlewitz RF, Gao W, Lemasters JJ, Thurman RG (1996) Destruction of Kupffer cells increases survival and reduces graft injury after transplantation of fatty livers from ethanol-treated rats. Liver Transpl Surg 2: 383–387