Changes in Plasma Concentrations of Free Amino Acids during and after the Anhepaic Period

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The liver plays regulatory roles in an inter-organ flux of amino acids (AAs) and energy metabolism[1]. The liver takes up alanine (Ala) and many other amino acids and utilizes them for energy production, gluconeogenesis, protein synthesis and ureagenesis. The changes in plasma AA concentrations after the removal of the liver have been investigated in animals[2–7]. Recently, the usefulness of plasma AA clearance to predict allograft functions after liver transplantation in animals[8] and also in humans[9,10] has been suggested. The concentrations of AAs in plasma, however, may be affected by many factors including pre-operative liver damage and nutrition: Fath et al. reported that the pre-operative concentrations of some glucogenic AAs and aromatic AAs in patients undergoing liver transplantation were above the values obtained in normal overnight-fasting subjects, and that those of isoleucine (Ile) and taurine (Tau) were below normal values[9].

We show here the changes in plasma AA concentrations in a patient undergoing bench surgery of the liver for a metastatic liver tumor. The patient was without her liver for 2 hours and 37 min. To our knowledge, this is the first report describing the changes in plasma AA concentrations during and after an anhepatic period in a patient whose pre-operative liver function was almost normal.

Case Report

A 49-yr-old woman (155 cm, 54 kg) underwent abdomino-perineal resection for rectal cancer five years ago. A metastatic mass was found, which occupied almost the whole right lobe of the liver beside the portal vein. Laboratory tests showed almost normal liver function: e.g. hepaplastin test 128% (normal range 67–138%), the degree of retention of indocyanine green at 15 min 6.4% (normal range less than 10%), serum lecithin cholesterol acyltransferase activity 99 $nmol \cdot ml^{-1} \cdot h^{-1}$ (normal range

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50–130 nmol·ml^{-1}·h^{-1}), and serum γ-glutamyltranspeptidase activity 105 IU·L^{-1} (normal range 5–55 IU·L^{-1}). Her nutritional state was as follows: serum albumin 3.7 g·dl^{-1}, peripheral lymphocyte count 1024 cells·mm^{-3}, and Hb concentration 10.5 g·dl^{-1}. A bench surgery was planned. She was required to fast overnight for 10 h. Anesthesia was induced with thiamylal and pancuronium, and was maintained by diazepam 18 mg, fentanyl 2.2 mg and isoflurane 0.8–2.0%. Dopamine at a rate of 3 μg·kg^{-1}·min^{-1} and prostaglandin E_1 (Prostandin 500®, Ono Pharmaceutical Co. Ltd., Osaka, Japan) at a rate of 14 ng·kg^{-1}·min^{-1} were infused intravenously to maintain hepatic blood flow. An Oximetrix-3® thermodilution fiberoptic catheter (Abbott, North Chicago, Ill) was introduced via the right internal jugular vein, and cardiac output (CO) was measured according to Fick's principle. Plasma volume (PV) was calculated from CO and hematocrit. During the anhepatic period, blood from the right femoral vein and from the portal vein was diverted to the left axillary vein using Anthron® tubes and a biopump. The flow rate via the pump was maintained at 2.3–2.7 L·min^{-1}. The liver was perfused with ice-cold University of Wisconsin solution on a backtable, and a partial resection of 225g of the liver was done. The anhepatic time and the operation time were 157 min and 12.3h, respectively. Blood loss was estimated to be around 3,000 ml. Thirty-four hundred ml of blood were transfused, and 3,000 ml of plasma protein fraction were infused during the operation. Serum alanine aminotransaminase activity increased to 284 IU·L^{-1} (normal range 5–33 IU·L^{-1}) at the first post-operative day (1POD). Her recovery was satisfactory, and she was discharged a month later.

Blood samples were taken through a radial arterial cannula, and the concentrations of free AAs in plasma were determined by the ninhydrin method after separation by high performance liquid chromatography using a Hitachi L-8500 AA analyzer (Hitachi, Tokyo, Japan). Because cysteine is oxidized to cystine spontaneously, the total amount of cysteine and cystine was obtained through this assay. Serum ketone body concentrations were determined spectrophotometrically using the Ketone Test Sanwa (Sanwa Chemical Lab., Nagoya, Japan). Blood glucose and lactate were determined using Dextrostix (Miles-Sankyo, Tokyo, Japan) and Diagluca (Toyobo, Osaka, Japan), respectively. Blood pyruvate concentrations were assayed enzymatically using the Determiner PA (Kyowa Medics, Tokyo, Japan). Serum free fatty acid concentrations were assayed spectrophotometrically using a Hitachi automatic analyzer 712 (Hitachi, Tokyo, Japan). Serum immunoreactive insulin (IRI) and glucagon were determined by radioimmuno assay using the Insulin RIA Kit II (Dinabott, Osaka, Japan) and the Glucagon Kit Daiichi (Daiichi Radioisotope, Tokyo, Japan), respectively.

Results and Discussion

The changes in plasma concentrations of free AAs, derivative AA data, and plama or serum concentrations of other fuels and hormones are summarized in figure 1. Most AAs increased during the anhepatic period, and then decreased after reperfusion. During and after the anhepatic period, the concentrations of Ala, glutamate (Glu), phenylalanine (Phe), tryptophane (Try) and tyrosine (Tyr) were above the values obtained in normal overnight-fasting subjects. Those of Ile and serine (Ser) became below normal values after the anhepatic period.

We observed two patterns of the increase in AA concentrations during the anhepatic period. The first pat-

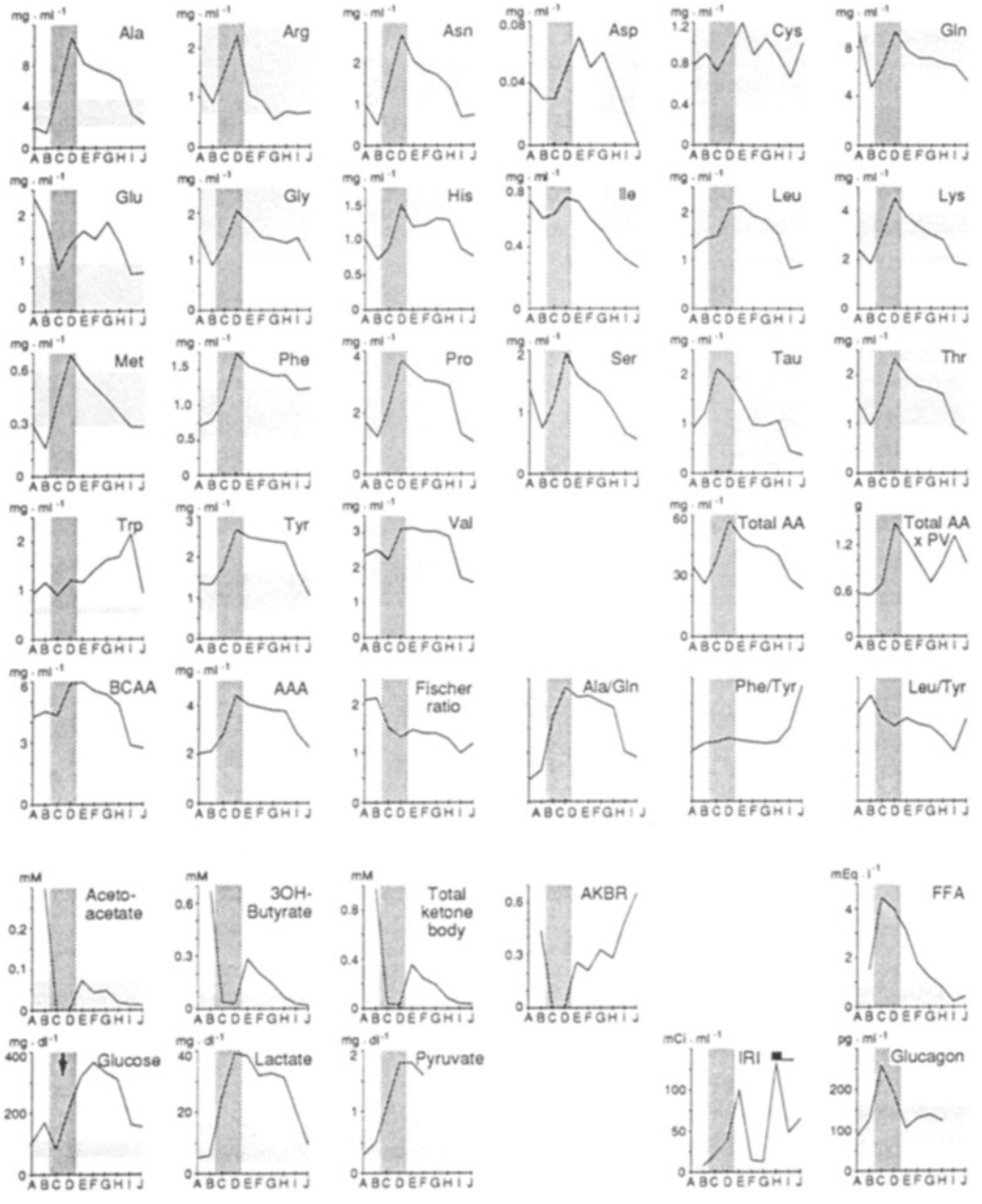

Fig. 1. The changes in plasma concentrations of free amino acids, derivative amino acid data, and plasma or serum concentrations of other fuels and hormones during and after bench surgery of the liver.

Amino acids are shown in alphabetical order. Heavily and lightly shaded areas indicate the anhepatic period and the normal range of each parameter in healthy, and overnight-fasted subjects, respectively. A, before operation; B, just before the anhepatic period; C and D, 60 and 120 min after the start of the anhepatic period, respectively; E, F, G and H, 15, 30, 60 and 120 min after reperfusion, respectively; I and J, 9 AM at the 1POD and the 2POD, respectively. Total AA, total concentrations of 21 AAs calculated by summing the individual amino acid concentrations; Total AA × PV, total amounts of AAs in plasma. The arrow in Glucose indicates an intravenous infusion of glucose, 10g over 30 min. The black bar in IRI indicates continuous infusion of insulin started at a rate of 8 $IU \cdot h^{-1}$.

tern was that the onset of the increase was rapid and the degree of the increase was marked. Ala, arginine (Arg), asparagine (Asn), glutamine (Gln), glycine (Gly), lysine (Lys), methionine (Met), Phe, proline (Pro), Ser, threonine (Thr), Tyr, and aromatic AA (AAA, Tyr+Phe) showed this pattern. The second pattern was that the onset of the increase was delayed and the degree of the onset was modest. Aspartate (Asp), Ile, leucine (Leu), valine (Val), and branched chain AA (BCAA, Val+Ile+Leu) showed this pattern. The increase in BCAA concentration during the anhepatic period might indicate that BCAA was utilized in the liver as well as in muscles and the kidney[11].

The concentrations of AAs which increased during the anhepatic period started to decline to the pre-operative values after reperfusion. The concentration of Arg decreased rapidly and sharply after reperfusion. Arg is a precursor of nitric oxide, and a component of the urea cycle, which is localized in hepatocytes. On the other hand, the decrease in Phe, Tyr, Val, AAA and BCAA concentrations was retarded The concentrations of ketogenic AAs (Leu, Lys, Phe, Trp and Tyr) tended to ramaine high after reperfusion.

The concentrations of some AAs changed differently from the AAs mentioned above during and after the anhepatic period. The concentration of Glu decreased after removal of the liver, which coincides with the observation by Fath et al.[9], but returned to the pre-operative value after reperfusion. Because pre-operative Glu concentrations were higher than those in normal, fasting subjects, the physiological significance of the reduction of Glu during the anhepatic period is unknown. A possible explanation of the decrease in Glu concentration at the initiation of the anhepatic period is that Glu converted to Gln: the conversion plays a role in the detoxification of ammonia. Cystine (Cys), seemed to show no significant change until the end of the anhepatic period, but increased transiently after reperfusion. This rise in Cys and the concomitant decrease in Tau and Met after reperfusion might be partly due to ischemia/reperfusion-induced oxidative stress. This is supported by the following observations: the reduced/oxidized glutathione ratio and the granulocyte elastase concentration in plasma increased after reperfusion in another patient undergoing bench surgery of the liver. The concentration of Tau increased during the first hour of the anhepatic period, but decreased during the second hour, indicating an increased utilization of Tau or a suppressed influx into plasma under a prolonged anhepatic condition. The concentration of Try did not change until reperfusion, and increased gradually until the 1POD.

The restoration of the BCAA/AAA ratio, the Fischer ratio, has been reported to correlate well with a longer term outcome of liver transplantation[12]. In our patient, the Fischer ratio remained low until the second post-operative day (2POD), although the early restoration of her bile secretion and the following uneventful clinical course indicated that the re-implanted liver functioned fairly well. The other molar ratios of plasma AAs, Ala/Gln, Phe/Tyr, and Leu/Tyr ratio, have been proposed to correlate with liver damage or muscle protein breakdown[13,14]. In our patient, the Ala/Gln ratio, which increased during the anhepatic period, did not decrease until the 1POD, and neither the Phe/Tyr nor the Leu/Tyr ratios changed during and after the anhepatic period.

Among the changes in plasma concentrations of free AAs and those in derivative amino acid data, the reduc-

tion of the increased concentration of Arg and that of total amounts of AAs in plasma (Total AA × PV) seemed more useful to predict sufficient blood flow and immediate functional restoration of the implanted liver promptly after reperfusion in our patient. However, plasma concentrations of free AAs are known to be influenced by many factors other than the hepatic function: nutrition, starvation, plasma volume, blood transfusion, blood product infusion, blood sugar levels , hormones, surgical stress and anesthetic management[15,16]. The liver plays crucial roles in ketone body metabolism as well as AA metabolism. The liver is the only organ which supplies ketone bodies into plasma. In our patient, the restoration of ketone body production and the arterial ketone body ratio (AKBR) rather than the changes in plasma AAs and derivative AA data seemed to correlate better with the functional restoration of the reimplanted liver. The reciprocal changes in the concentration of serum free fatty acid (FFA) seemed to be partly explained by the cessation of FFA utilization for ketone body production during the anhepatic period. The AKBR is not affected by the changes in plasma volume which fluctuates easily during and after major surgery. Furthermore, the analytical procedures are easier and data is obtained more quickly in ketone body analysis than in AA analysis. The usefulness of the AKBR has been also suggested in liver transplantation[17]. Although the restoration of blood lactate concentration has been proposed to predict the extent of ischemic liver injury after liver transplantation[18], it was not immediate in our patient.

The examination of plasma AA dynamics during and after the anhepatic period is important to elucidate the metabolism of each AA and of other fuels. Although data obtained from patients undergoing liver transplantation has been reported, the pre-operative conditions of these patients are critical in view of liver functions and nutrition, and they sometimes require circulatory support. For example, the patients who underwent liver transplantation had already low Fischer ratios from the start of the operations[9]. We have presented data obtained from a patient undergoing bench surgery of the liver, whose pre-operative liver damage and malnutrition seemed minimal. The difference between our patient and healthy subjects is that she bore a malignant tumor: protein turnover might be accelerated in tumor-bearing subjects[19].

In summary, we have presented the changes in plasma concentrations of free AAs that occurred during bench surgery of the liver. AAs accumulated in plasma during the anhepatic period, and were cleared after reperfusion, indicating that the uptake of many AAs ceased during the anhepatic period and resumed after reperfusion. The Fischer ratio showed no value to predict the restoration of blood flow and functions in the liver. The changes in the arterial ketone body concentrations and the AKBR were more affected than those in plasma AAs by the removal and the reimplantation of the liver.

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References

1. Abumrad NN, Williams P, Frexes-Steed M, et al: Inter-organ metabolism of amino acids *in vivo*. Diabetes/Metabolism Rev 5:213–226, 1989
2. Bollman JL, Mann FC, Magath TB: Studies on the physiology of the liver: XV. Effect of total removal of the liver on deaminization. Am J Physiol 78:258–269, 1926

3. Miller LL: The role of the liver and the non-hepatic tissues in the regulation of free amino acid levels in the blood. Amino acid pools. Edited by Holden JT. Amsterdam, Elsevier, 1962, pp 708–721
4. McMenamy RH, Vang J, Drapanas T: Amino acid and α-keto acid concentrations in plasma and blood of the liverless dog. Am J Physiol 209:1046–1052, 1965
5. Blackshear PJ, Holloway PAH, Alberti KGMM: Factors affecting amino acid release from extrasplanchnic tissues in the rat: Interactions of alanine and glutamine. Biochem J 150:379–387, 1975
6. Onitsuka A, Matsunami H, Senga S, et al: Changes of plasma amino acid profiles following total hepatectomy under veno-veno bypass in dogs (in Japanese). J Jap Surg Soc 89:376–380, 1988
7. Hisanaga M, Nakajima Y, Segawa M, et al: Evaluation of initial hepatic allograft function: Changes of arterial ketone body ratio and plasma free amino acids (in Japanese). Transplant J 25:477–485, 1989
8. Goto T, Asano T: Experimental studies on hepatic clearance rate of amino acids as an initial function test for the liver graft (in Japanese). Transplant J 25:271–278, 1990
9. Fath JJ, Ascher NL, Konstantinides FN, et al: Metabolism during hepatic transplantation: Indicators of allograft function. Surgery 96:664–674, 1984
10. Jenkins RL, Bosari S, Khettry U, et al: Survival from hepatic transplantation. Ann Surg 204: 364–374, 1986
11. Ichihara A: Interorgan relationship of amino acid metabolism: Special reference to branched chain amino acid and glutamine (in Japanese). Jap J Parent Ent Nutr 11:795–799, 1989
12. Reilly JJ, Halow JGM, Gerhardt AL, et al: Plasma amino acids in liver transplantation: Correlation with clinical outcome. Surgery 97:263–270, 1985
13. Wannemacher RW, Klainer JAS, Dinterman RE, et al: The significance and mechanism of an increased serum phenylalanine-tyrosine ratio during infection. Am J Clin Nutr 29:997–1006, 1976
14. Imler M: Study of muscular glutamic and alanine release in cirrhotics with hyperammonemia and in ammonium infused rats, Aminosäuren und Ammonia akstoffwechsel bei Leberinsuffizienz. Edited by Holm E. Heidelberg, Boek Holm, 1982, pp 33–39
15. Smith R, Williamson DH: Biochemical effects of human injury. Trends Biochem Sci 8:142–146, 1983
16. Wolfe RR, Jahoor F, Hartl WH: Protein and amino acid metabolism after injury. Diabetes/Metabolism Rev 5:149–164, 1989
17. Taki Y, Gubernatis G, Yamaoka Y, et al: Significance of arterial ketone body ratio measurement in human liver transplantation. Transplantation 49:535–539, 1990
18. Koller J, Wieser Ch, Furtwängler W, et al: Orthotopic liver transplantation and perioperative lactate metabolism. Transplant Proc 23:1989–1990, 1991
19. Knox LS, Crosby LO, Feurer ID, et al: Energy expenditure in malnourished cancer patients. Ann Surg 197:152–162, 1983