Journal of Investigative Surgery, 20:35–40, 2007

ISSN: 0894-1939 print / 1521-0553 online
DOI: 10.1080/08941930601126165

informa
healthcare

ORIGINAL RESEARCH

Evaluation of Postinjury Hepatocyte Function by Central Amino Acid Clearance

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ABSTRACT It has been demonstrated by other investigators that central plasma clearance of amino acids accurately predicts hepatocyte function in patients with liver disease and correlates with clinical outcome. This methodology has not heretofore been studied in the trauma patient. It is our hypothesis that central amino acid clearance in trauma patients is more reflective of hepatocyte function than traditional liver function tests. We examined the plasma amino acid clearance rates using L-[1-^{13}C]phenylalanine. Clearance rates were compared to standard liver function tests (LFTs) and the sensitivity and predictability of the technique were determined. The study was conducted on uninjured control subjects and in seriously injured patients, both with and without significant liver injuries. Compared to baseline values in the control group, initial phenylalanine breath scores were reduced in the injured, but exceeded control levels at 7 days postinjury. These changes were statistically significant. There was no difference between those with and without liver trauma. LFTs showed inconsistent and conflicting results. Thus, central amino acid clearance measured by L-[1-^{13}C]phenylalanine oxidation is depressed immediately following injury but reaches supranormal levels at 7 days postinjury. Compared to LFTs, amino acid clearance suggests initial hepatocyte suppression followed by hyperactivity and is a more accurate determinant of hepatocyte function.

KEYWORDS amino acid clearance, hepatocyte function, liver function tests

The liver is the metabolic workhorse of the body. Because of its strategic anatomic location at the termination of the portal circulation, the liver processes virtually all products absorbed from the intestine. Therefore, the liver has a lead role in the metabolism of carbohydrates, fats, protein, and other nutrients. The end products of dietary protein absorption are amino acids, which enter the portal circulation in their ionized form. Amino acids are taken up by the hepatocytes but are not stored in the liver. Instead, they are rapidly converted to plasma and other acute-phase proteins, heme, and hormones. Because this is a dynamic process, amino acid clearance is thought to provide a more accurate reflection of the functional state of the liver.

The methodology to study amino acid clearance by breath analysis was originally described by Schneider et al. in 1978 [1]. The $^{13}CO_2$ enrichment technique

Received 20 March 2006; accepted 9 October 2006.

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was later refined by Scrimgeour and Rennie [2]. However, central amino acid clearance has not been studied in the acutely injured. In 1997, Burke et al. [3] introduced new methodology to determine phenylalanine oxidation and thereby assess hepatocyte functional capacity. They showed a high correlation between the clearance rate of phenylalanine and both Child Pugh classification and outcome in patients with end-stage liver disease. Using Burke's technique, we sought to determine phenylalanine clearance in control subjects to obtain a normal reference, then study acutely injured patients both with and without liver injury, to assess the role of phenylalanine clearance in defining liver function in the seriously injured.

MATERIALS AND METHODS

The study consists of a control group of 10 normal subjects (Group 1); 8 seriously injured patients, defined as an Injury Severity Score (ISS) > 20, with liver injuries classified as American Association for the Surgery of Trauma (AAST) grade 3 or greater (Group 2); and 9 seriously injured patients without liver injuries (Group 3). All subjects were enrolled in the study after obtaining informed consent. The study received favorable action by the Institutional Review Board, which confirmed that the conduct of the study was in accordance with ethical standards established by the Helsinki Declaration of 1975. Control subjects were instructed not to consume food after 9 o'clock the evening before testing. The next morning, each subject was intravenously administered 100 mg L-[1-^{13}C]phenylalanine. Duplicate breath samples were collected at rest and following a normal exhalation into a disposable, modified Halane–Priestly tube connected to a plastic bag (Quintron, Milwakee, WI). A side hole in the mouthpiece was connected to an adapter and syringe needle holder. A sample of breath was collected by piercing the syringe needle with a 10cc breath collection vacuum tube (Labco, Ltd, UK). Samples were collected at 0-, 5-, 10-, 15-, 20-, 30-, 40-, 50-, and 60-min intervals. $^{13}CO_2$ enrichment was measured by isotope mass spectrometry.

Injured patients (Groups 2 and 3) were studied in like manner on hospital day 1, and at 12 h postinjury. Both sequential breath isotope studies were collected and standard liver function tests (LFTs) were drawn. LFTs included serum values for albumin, total bilirubin, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), and lactic dehydrogenase (LDH). The injured patients were allowed a diet as tolerated during their hospitalization, and all studies were repeated at 7 days post admission.

The phenylalanine breath score was quantitated by determining the percentage of the phenylalanine dose metabolized per hour using an under-the-curve method and an assumed CO_2 production rate of 5 mmol/min/m^2 body surface area, as originally described by Schneider [1]. All data are expressed as means $\pm$ standard error of the mean (SEM) at the 95% confidence interval. Differences between the two trauma groups and between the two trauma groups and control subjects were compared using the standard Student's *t*-test; $p \leq .05$ defined a significant statistical difference between groups.

RESULTS

The percentage of dose of intravenously administered phenylalanine oxidized within 1 h on the initial hospital day was compared among groups. Group 1 showed a phenylalanine breath score of 3.79 ± 1.3, that is, 3.79% of the intravenously administered phenylalanine was oxidized to $^{13}CO_2$ within the first hour. By comparison, Group 2 and Group 3 showed depressed metabolism of phenylalanine at 2.23 ± 0.37 and 2.27 ± 0.28, respectively. This difference was statistically significant, $p = .04$ for Group 2 and $p = .02$ for Group 3, when compared to the control group. There was no difference between the study groups; that is, liver injury did not appear to further impede oxidation of phenylalanine compared to serious injury alone. Table 1 denotes the results of the phenylalanine breath tests in the control and study groups.

At 7 days postinjury, there was enhanced oxidation of phenylalanine to $^{13}CO_2$ in the study groups compared with the control group. The percentage of dose expired was 5.6 ± 0.72 in Group 2 and 5.1 ± 0.81 in Group 3. Again, there was no apparent difference between the injured groups.

TABLE 1 **Comparison of L-[1-^{13}C]phenylalanine Clearance in Control and Injured Subjects**

Group	Baseline	12 h Postinjury	7 days Postinjury	Student's *t*-test
1	3.79 ± 1.3	—	—	—
2	—	2.23 ± 0.4	5.6 ± 0.72	$p < .04$
3	—	2.27 ± 0.3	5.1 ± 0.81	$p < .02$

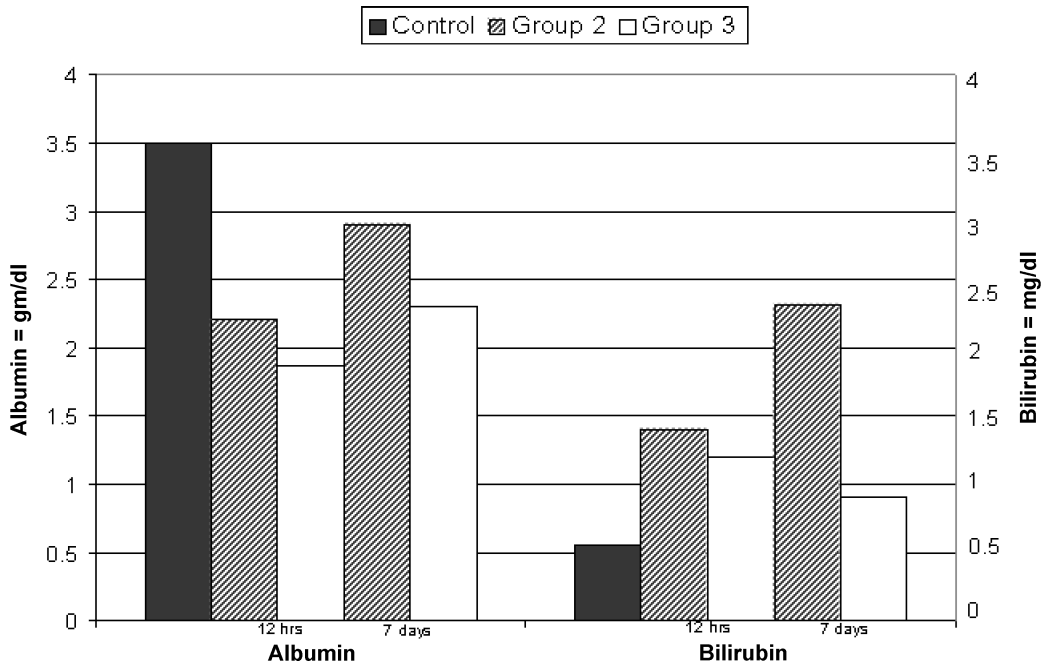

FIGURE 1 **Results of albumin and bilirubin determinations in controls and injured subjects at 12 h and 7 days postinjury.**

Results of the LFTs varied. Albumin and bilirubin values are shown in Figure 1. Compared to the control albumin level of 3.5 gm/dL, albumin levels were suppressed in both injured groups: Group 2 had levels of 2.2 ± 0.02 and Group 3 had levels of 1.87 ± 0.1. These were highly significant decreases. Both injured groups showed improvement at 7 days, 2.9 ± 0.18 and 2.3 ± 0.14, respectively. Compared to a control total bilirubin of 0.55 ± 0.09 mg/dL, initial bilirubin levels were increased in Group 2 (1.4 ± 0.45) and in Group 3 (1.2 ± 0.32). At 7 days postinjury, Group 2, the seriously injured with liver trauma, showed a persistent elevation in total bilirubin of 2.31 ± 1.02, which contrasted with a value of 0.9 ± 0.16 in Group 3.

AST, ALT, LDH, and ALP results are represented in Figure 2. AST levels in the control group were

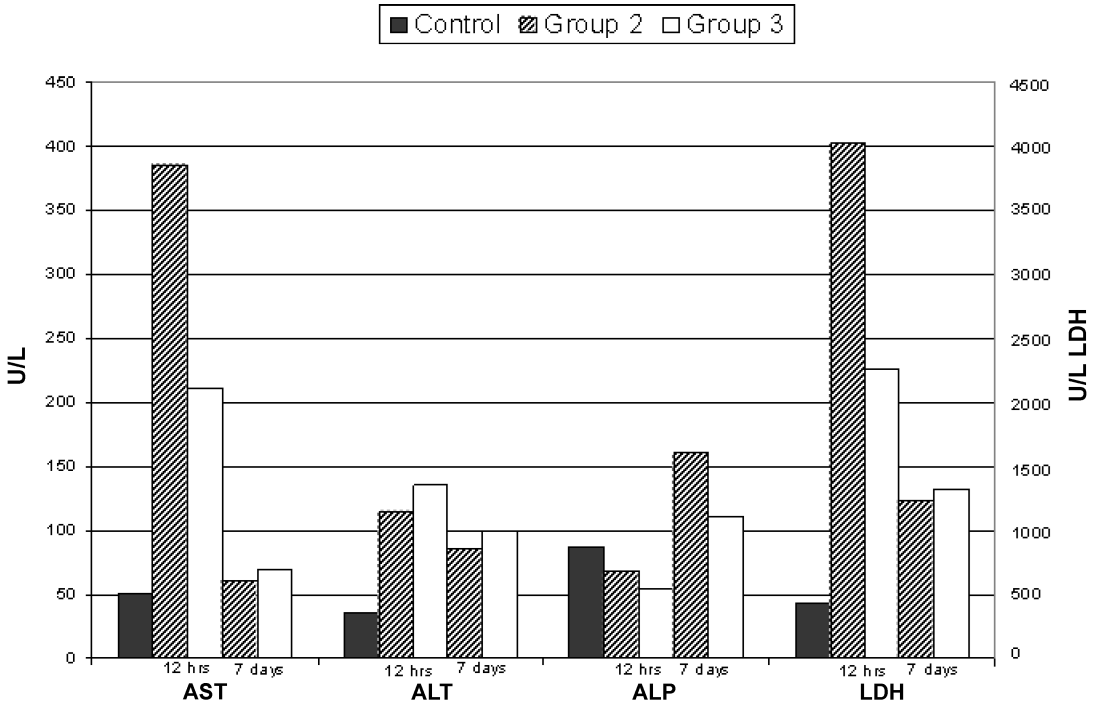

FIGURE 2 **Results of AST, ALT, ALP, and LDH determinations in controls and injured subjects at 12 h and 7 days postinjury.**

51 ± 13.5 U/L. Initial levels in the study groups showed 385 ± 97 in Group 2 and 211 ± 79 in Group 3. Both groups showed a return toward normal with values at 7 days of 60 ± 10 and 70 ± 19, respectively. ALT levels in the control group were 35.7 ± 8.6 U/L. Initial levels in the study groups showed 115 ± 51 in Group 2 and 135 ± 68 in Group 3. At 7 days postinjury, both groups showed a return toward normal of 85 ± 10 and 100 ± 25, respectively. LDH levels in the control group were 422 ± 82 U/L. Both study groups showed acute elevations of the LDH with Group 2 having 4022 ± 1041 and Group 3 having 2259 ± 865 but returning toward normal at 7 days, 1232 ± 176 and 1319 ± 313, respectively.

ALP levels averaged 88 ± 29 U/L in the control group. Acutely, alkaline phosphatase levels in the injured subjects differed little from controls: Group 2 showed levels of 68 ± 9 and Group 3 showed levels of 54 ± 5. At seven days, however, levels in both groups were elevated compared to controls: 160 ± 25 and 110 ± 26. Figures 1 and 2 graphically demonstrate the comparative values of the LFTs in the control group and both study groups.

DISCUSSION

Traumatic effects on the liver may be related to (1) direct injury to the liver substance, causing disruption of liver parenchyma, destruction of hepatocytes, and tearing of biliary canaliculi; (2) the effects of shock, which, if not promptly reversed, may lead to varying degrees of hepatic parenchymal ischemia and cellular malfunction; or (3) the effects of sepsis, which generally occur in a delayed manner, compromise multiple hepatic metabolic pathways, and contribute to multiorgan system failure syndrome, a common preterminal clinical event.

The most commonly used tools to assess liver function consist of a battery of tests grouped together as liver function tests (LFTs) [7]. Albumin is an important measure of liver function because 90% of protein synthesis occurs in the liver and 100% of albumin undergoes hepatic synthesis. As liver tissue is destroyed, albumin levels fall. As a measure of liver function, albumin is not ideal because albumin levels are also affected by renal disease, malnutrition, protein-losing enteropathies, and other conditions that have little bearing on the functional capacity of the liver. Acutely, falling albumin levels may also represent the effect of hemorrhage. Bilirubin likewise provides an indirect reflection of liver function. Bilirubin is the primary breakdown product of hemoglobin. The rate of bilirubin synthesis and the rate of excretion exist in a balance that depends on the amount of hemoglobin presented to the liver and the conjugation of bilirubin by the hepatocyte. ALT and AST reflect the hepatocyte's role in the amino acid–carbohydrate interconversion pathways. These enzymes are usually good markers for liver injury or necrosis. In direct trauma to the liver, most of the parenchyma is unaffected, but there may be sufficient hepatocytes injured or destroyed to allow spillage of intracellular contents into the local circulation, leading to a rise in these values. It is arguable whether, under these circumstances, the ALT and/or AST accurately reflect the true functional state of the liver. The LDH is sensitive to cellular injury, and large increases in serum LDH reflect significant trauma. Unfortunately, LDH is not liver specific and considerable amounts may be released from skeletal muscle and cardiac muscle, and LDH is also present in erythrocytes and the kidney. Lastly, ALP, although useful, is confined in greatest concentrations to the hepatocytes lining the bile canaliculi. It is a good measure of bile duct injury or obstruction. Most serum ALP is derived from the liver, but ALP is also found in bone, kidney, and intestine. Therefore, it is not liver specific. With healing, ALP tends to return more slowly to normal levels than the other hepatic enzymes.

In contradistinction to the LFTs, which, collectively, provide useful information in determining levels of hepatocellular damage, the clearance of phenylalanine provides the more useful assessment of functional hepatic reserve. Since liver disease is associated with abnormal levels of amino acids and elevated levels of phenylalanine in particular, this study sought to assess the role of phenylalanine clearance in the detection of hepatic function both acutely (within 12 h of injury) and at 7 days postinjury. Phenylalanine is naturally occurring, and because the test uses no radioactivity, it poses no harm to patients as an investigational tool. The test is easy to set up and easy to analyze. The utility of this test has not been studied heretofore in the acutely injured.

In 1984, Clowes et al. [4] were the first to show that in patients with severe liver disease, the prognosis was poorest in those patients who had suppressed amino acid clearance. However, until the current study, the effects of acute trauma on amino acid clearance were unknown. In fact, limited amino acid clearance data is available in acute illness. In 1989, Vente et al. [5]

prospectively studied 65 stressed and/or septic patients and demonstrated suppression of all amino acid levels except for cystine and phenylalanine which were significantly elevated. However, he showed no correlation with outcome and commented that changes in amino acid metabolism could not distinguish septic subjects from those with metabolic stress alone. In 1992, Mori et al. [6] examined plasma phenylalanine changes in stressed rats and confirmed the findings of previous investigators that phenylalanine accumulates in the plasma of stressed and septic subjects who have no apparent intrinsic hepatic disease. Whether this represents the effects of increased plasma loading, presumably from muscle release, or impaired metabolism or a combination of effects, remains unclear.

Our approach was based on the work of previous investigators who showed that the rate of hepatic phenylalanine metabolism could be determined from the appearance of $^{13}CO_2$ in the breath using the nonradioactive tracer L-[1-^{13}C]phenylalanine [1]. The first pass through the liver results in the conversion of phenylalanine to tyrosine, which is subsequently metabolized, releasing $^{13}CO_2$ from position 1. The actual extent of metabolism is reflected in the amount of exhaled CO_2. In their study, Burke et al. [1] confirmed the work of others that showed that the increased levels of phenylalanine were caused by decreased metabolism. Burke et al. showed that the phenylalanine breath test could accurately distinguish between those with and without liver disease. As liver failure progressed, the ability to metabolize phenylalanine worsened. There was a striking correlation with both Child Pugh classification and albumin and bilirubin levels. Interestingly, there was no correlation with ALP, AST, or ALT. In a more recent study by Tugtekin et al. [8] the true CO_2 production rate was compared between normal control and cirrhotic patients. The study found that cirrhotic patients had lower total CO_2 production rates than controls, making the phenylalanine oxidation difference actually larger than using the 5 mmol CO_2/min/Body Surface Area (BSA) rate. It is possible that the differences between groups in this study might have been even greater if the true CO_2 production rate was used.

In the current study, phenylalanine clearance is suppressed in the early postinjury period compared to uninjured controls. The reason for this is unclear. Clearance of phenylalanine was compared between seriously injured patients both with and without direct injury to the liver, and no differences were found. Therefore, whatever affects central amino acid clearance in the early postinjury period is not related to direct liver trauma but rather to some systemic insult that impairs the liver's ability to process phenylalanine. These are not long-term effects, because by 7 days postinjury, the phenylalanine clearance reaches supranormal levels. Further investigation is needed to determine how this new liver function assessment tool may be used to determine prognosis in the acutely injured patient, presumably to restore homeostasis.

There are limitations to this study. First, because the intent of this study was to determine whether L-[1-^{13}C]phenylalanine could detect changes in central amino acid clearance in injured patients and whether such changes persisted, there are gaps in what was learned. The initial metabolic suppression was followed by what appears to be metabolic overshoot, but it is not clear when this occurred or how long it persisted. These are questions that require further study. Second, the study design was unable to control for phenylalanine flux from skeletal muscle, which may act as a confounding variable. The inhibition of phenylalanine clearance at 12 h suggests that muscle injury plays little or no contributing role. Lastly, because none of the study patients suffered from preexisting liver disease, the role of phenylalanine clearance in the injured patient with preexisting liver disease remains unknown but of interest. Further investigation is needed to determine how this new liver function assessment tool may be used to determine prognosis in the acutely injured patient and, hopefully, improve management.

CONCLUSIONS

Plasma amino acid clearance rates using L-[1-^{13}C] phenylalanine were studied in 27 subjects. Group 1 members ($n = 10$) were normal controls. Group 2 members ($n = 8$) were injured seriously (ISS > 20) and had significant liver injuries (AAST > Grade III); Group 3 members ($n = 9$) were seriously injured (ISS > 20) but did not have liver injuries. Initial phenylalanine breath scores were reduced in both Group 2 and Group 3 compared to controls but exceeded control levels at 7 days postinjury. These changes were statistically significant ($p < .05$). There was no difference between those with and without liver trauma. Further investigation is needed to determine the specificity of this new liver function assessment tool and how it may be used in the clinical management of the acutely injured patient.

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