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Changes in blood ketone body ratio with reference to graft viability after liver transplantation in rats

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Abstract. Arterial blood ketone body ratio (acetoacetate/3-hydroxybutyrate; KBR), which reflects hepatic mitochondrial redox potential, was measured during a 2-week period after orthotopic liver transplantation in three groups of rats: group 1, the isogenic combination of LEW ($RT1^l$) graft to LEW recipient as control; group 2, the allogenic combination of ACI ($RT1^a$) graft to LEW recipient without immunosuppressive treatment; and group 3, the allogenic combination of ACI to LEW with immunosuppressive treatment using cyclosporin (CyA). Isogenic recipients survived indefinitely. Allogenic recipients in group 2 had severe rejection with a mean survival of 10.3 ± 0.54 days, while 77.8% of the allogenic recipients in group 3 survived more than 30 days. KBR of rats surviving more than 2 weeks in groups 1 and 3 gradually increased post-transplantation and was maintained at a high level. By contrast, though KBR in group 2 was restored at 3 days, it gradually fell and remained at a significantly low level ($P < 0.001$). It is suggested that KBR provides an accurate indicator for evaluating metabolic viability of the critically deteriorating liver graft accompanied by severe rejection.

Key words: Ketone body ratio, in liver transplantation - Rejection in liver transplantation - Liver transplantation and ketone body ratio - Liver transplantation, experimental - Viability, liver graft.

In recent years, there has been a marked improvement in the survival rate of patients undergoing orthotopic liver transplantation, especially since the introduction of the immunosuppressive agent cyclosporin A (CyA). The failure of the allograft itself, however, is a major life-threatening risk, requiring hepatic retransplantation [14]. To improve the chances of patient survival, an accurate indicator is needed to assess the metabolic viability of the allograft. Attention has recently been focused on the fact that cellular viability of the liver graft may ultimately depend on the capacity for ATP synthesis and energy status in the liver mitochondria [6, 18]. In studies from our laboratory, the ratio of acetoacetate to 3-hydroxybutyrate in the arterial blood (KBR), which reflects the mitochondrial redox potential, was found to be positively correlated with hepatic energy charge level in jaundiced [17], hepatectomized [10], hemorrhagic-shocked [22], and hemodiluted animals [16]. In addition, the crisis in hepatic energy balance has been shown to contribute to the incidence of hepatic failure [11], hepatic coma [12], and multiple organ failure [9]. In other clinical and experimental studies, it has been reported that KBR may serve as an accurate means for assessing the cellular viability of allografts after liver transplantation [3, 15].

The present study explores the changes in KBR in the acute rejecting phase after allogenic liver transplantation in rats and compares the results with those from allografts under CyA treatment as well as with isogenic grafts. In this study, evidence will be

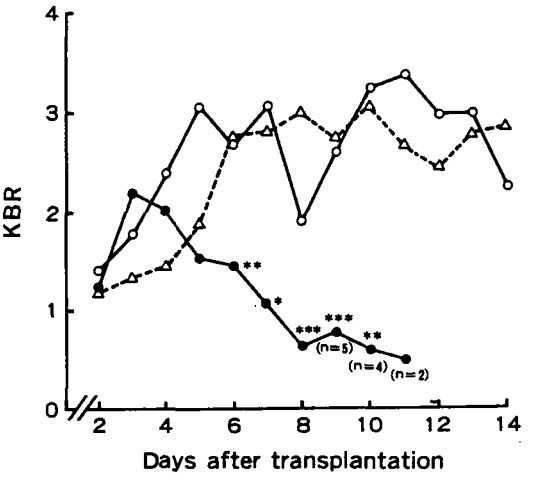

Fig. 1. Changes in ketone body ratio *(KBR)* after liver transplantation. *n*-values indicate numbers of rats that survived in group 2. -O-, Group 1; -●-, group 2; --△--, group 3; $*P < 0.025$, $**P < 0.01$, $***P < 0.005$, compared to group 3

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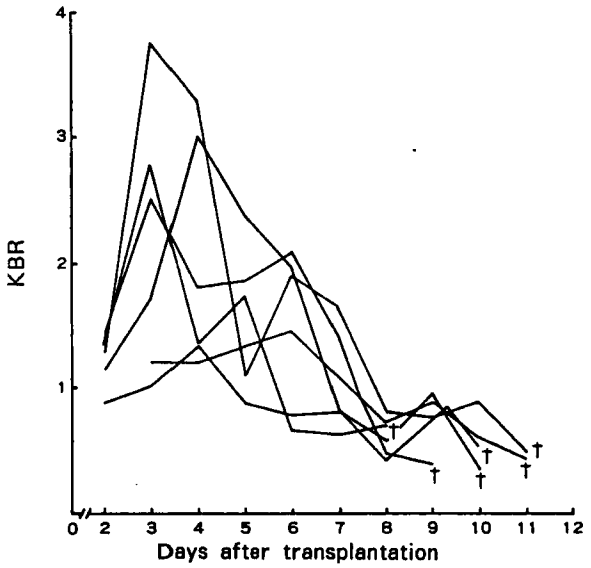

Fig. 2. Changes in ketone body ratio *(KBR)* after liver transplantation in each case in group 2 ($n=6$). †, death of rats

presented showing that markedly decreased KBR indicates irreversible deterioration of metabolic liver function, and that KBR may serve as a useful criterion in determining hepatic retransplantation.

Materials and methods

In this study, orthotopic liver transplantation was performed in male LEW ($RT1^l$) and ACI ($RT1^a$) rats, weighing 200–380 g, using the modified technique described by Kamada et al. [5]. Recipients were divided into three groups: group 1, the isogenic combination of LEW graft to LEW recipient as control ($n=7$); group 2, the allogenic combination of ACI graft to LEW recipient without immunosuppression as the acutely rejecting model ($n=6$); and group 3, the allogenic combination of ACI to LEW with CyA treatment ($n=6$). CyA was administered subcutaneously in a daily concentration of 3 mg/kg body weight from the day of operation to 14 days post-transplantation. In each group, on the day following transplantation, a silastic rubber catheter was inserted into the internal carotid artery or femoral artery of the recipient under ether anesthesia. A glucose solution was administered with a microinfusion syringe pump at a constant rate of 0.1 g/kg per hour to maintain an adequate blood sugar level. Aminobenzyl penicillin was also infused at dosages of 60 mg/day. Rats were maintained on a diet of laboratory chow and water ad libitum postoperatively. Without anesthesia or fasting, arterial blood samples were taken daily and replaced with the same volume of isogenic blood through the catheter from 2 days to 4 days post-transplantation. Arterial blood ketone bodies were measured enzymatically [8, 20] using 3-hydroxybutyrate dehydrogenase (Sanwa Kagaku Kenkyusho, Nagoya, Japan) [4]. Histologically, light microscopic and electron microscopic examinations of liver allografts were also performed in groups 2 and 3.

Values are expressed as means ± SEM. Statistical significance between mean values was determined by the Wilcoxon rank-sum test.

Results

Survival rates at 30 days in groups 1 and 3 were 91.7% and 77.8%, respectively, while all rats in group 2 died within 12 days (mean survival 10.3 ± 0.54 days). The present study thus focuses upon the rats surviving more than 14 days in groups 1 and 3. Rats that died due to technical failure were also excluded in all groups.

Figure 1 shows the changes in KBR post-transplantation. In groups 1 and 3, KBR gradually increased in the early postoperative phase; thereafter, its mean value was maintained at a high level (Table 1). KBR in group 2 rose to 2.18 ± 0.39 at 3 days and then gradually decreased and remained at a significantly low level. Figure 2 shows the changes in KBR in recipients in group 2. The level of KBR in all cases was under 1.0 in the few days prior to death; mean values were 0.58 ± 0.05, 0.80 ± 0.07, and 0.76 ± 0.13 at 1, 2, and 3 days prior to death, respectively.

On light microscopic examination, histological section at autopsy in group 2, shown in Fig. 3a, revealed marked edema and mononuclear cell infiltration, not only in the expanded portal tracts but also in the central parts of lobules. Liver cells were extensively destroyed. On the other hand, though the section by biopsy at 14 days in group 3 showed slight expansion of portal tract with a few excess bile ducts and mononuclear cells, the portal tract outline and hepatocytes were generally intact (Fig. 3b).

On electron microscopic examination of group 2 at 7 days, the hepatocytes showed a nuclear clumped chromatin pattern and swollen and pleomorphic mitochondria with an unclear matrix (Fig. 4a). By contrast, the hepatocytes of the recipients in group 3 at 7 days appeared almost normal (Fig. 4b).

Table 1. Changes in ketone body ratio (KBR) after liver transplantation. Results shown are expressed as means ± SEM. * $P<0.01$, ** $P<0.005$, compared to group 3

	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Group 1 ($n=7$)	1.36 ± 0.13	2.38 ± 0.39	2.68 ± 0.45	1.89 ± 0.23	3.24 ± 0.44	2.96 ± 0.29	2.25 ± 0.30
Group 2 ($n=6$)	1.22 ± 0.09	1.99 ± 0.34	1.47 ± 0.23*	0.62 ± 0.05**	0.61 ± 0.10* ($n=4$)		
Group 3 ($n=6$)	1.18 ± 0.09	1.45 ± 0.11	2.73 ± 0.16	2.99 ± 0.15	3.06 ± 0.27	2.43 ± 0.25	2.84 ± 0.33

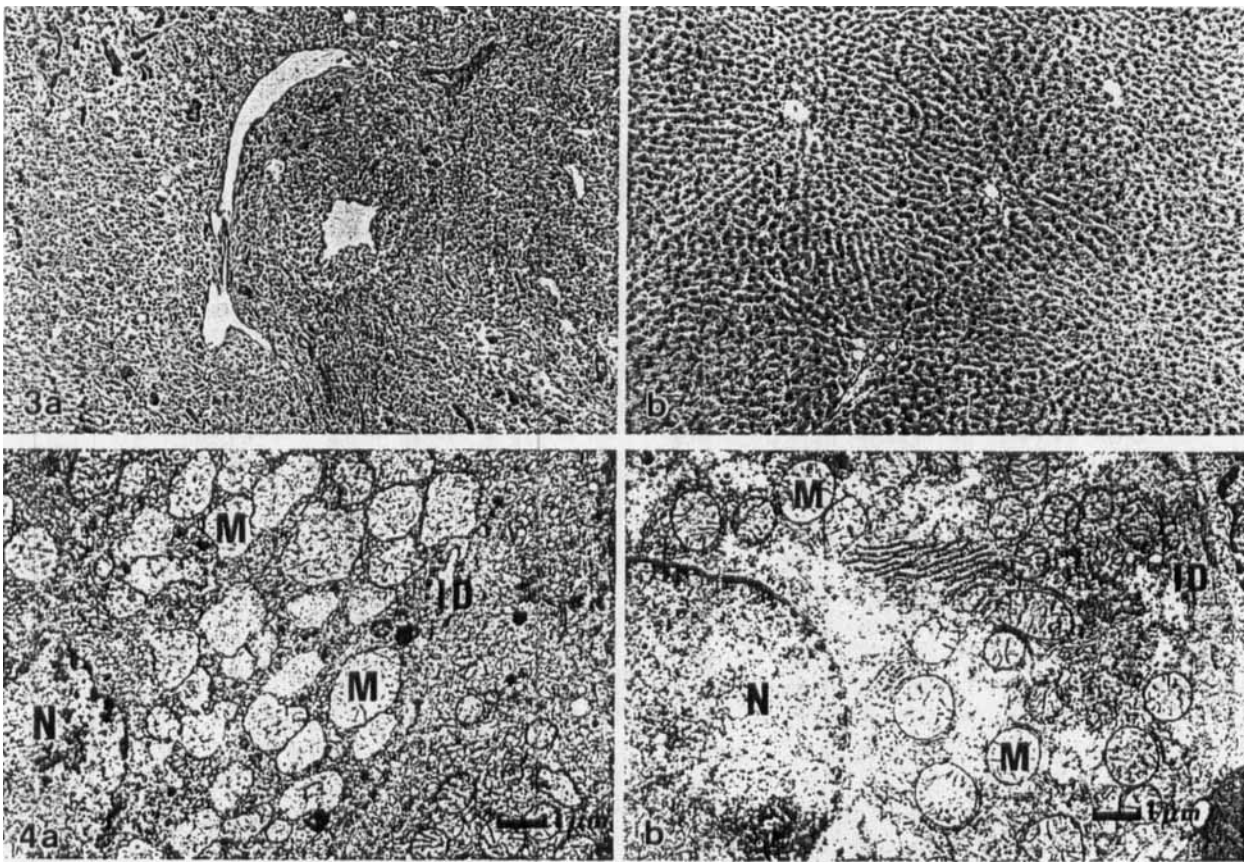

Fig. 3a, b. Histological response in ACI liver, grafted to LEW recipients, *on light microscopic examination*: **a** liver section at autopsy in group 2 showing marked mononuclear cell infiltration, expanded portal tract and liver cell necrosis; **b** liver section by biopsy at 14 days post-transplantation in group 3 showing only slight mononuclear cell infiltration and slight bile duct proliferation (H&E, × 100)

Fig. 4a, b. Electron microscopic examination of liver grafts at 7 days: **a** though liver section in group 2 showed swollen and pleomorphic mitochondria **b** the hepatocyte in group 3 appeared almost normal (× 6800). *N*, Nuclear; *M*, mitochondrion; *ID*, interdigitation of cytoplasm

Discussion

In liver mitochondria, acetoacetate undergoes reduction to 3-hydroxybutyrate by 3-hydroxybutyrate dehydrogenase localized in the mitochondrial cristae [1]. KBR, the ratio between acetoacetate and 3-hydroxybutyrate in the arterial blood, reflects the ratio of free-oxidized nicotinamide adenine nucleotide (NAD^+) to reduced nicotinamide adenine nucleotide (NADH) in the mitochondria, as shown by the following formula [21]:

acetoacetate + NADH + H^+ ⇌ 3-hydroxybutyrate + NAD^+.

Thus, the free $NAD^+/NADH$ = acetoacetate/3-hydroxybutyrate × 1/K, where K indicates the equilibrium constant of 3-hydroxybutyrate dehydrogenase. Since 3-hydroxybutyrate dehydrogenase activity is exceptionally high in the liver [7], and since acetoacetate and 3-hydroxybutyrate freely penetrate the cell membrane, KBR can be said to reflect the $NAD^+/NADH$ ratio in the liver mitochondria, with a decreasing KBR being indicative of a progressive reduction in the mitochondrial $NAD^+/NADH$ ratio. Citrate synthase, which determines the turnover rate of the Krebs cycle, is inhibited by the reduced mitochondrial redox potential, and enzymatic processes requiring NAD^+ in the mitochondria, such as pyruvate dehydrogenase, glutamate dehydrogenase, isocitrate dehydrogenase, and a-ketoglutarate dehydrogenase, are also inhibited [19]. These inhibitions would thus work to prevent the entrance of plasma amino acids or pyruvate into the Krebs cycle, resulting in a hepatic energy deficit and an elevation of plasma amino acids or lactate levels. Previous reports from our laboratory have shown that the decreases in the hepatic energy charge level are positively correlated with decreases in ketone body ratio in animals in shock [22] and in jaundiced [17] and hepatectomized animals [10].

Although Fath et al. [2] have reported that the amino acid clearance rate should reflect the initial function of allografts and Reilly et al. [13] have reported that a persistent low molar ratio after liver transplantation is an indication of graft failure, it is well known that the changes in amino acid and lactate reflect not only hepatic function but also the condition of nonhepatic tissues such as skeletal muscle. It is also known that an increase in aromatic amino acids in blood can be caused by liver dysfunction and an accelerated degradation of muscle protein. By contrast, since the liver is the only organ with the capacity to produce ketones and to convert the ratio between the two ketones, the evaluation of KBR may ultimately provide the most basic information about the metabolic viability of the allograft. Clinically, Gubernatis et al. [3] have reported that the security in diagnosing the irreversibility of liver damage is enhanced by measuring the total oxygen consumption and KBR.

In this study, there was a marked difference in the changes in KBR between group 2 and the other groups after liver transplantation. While KBR in groups 1 and 3 was maintained at high levels for 2 weeks post-transplantation, it gradually decreased and remained at a significantly low level in group 2, allograft recipients without CyA treatment, who died within 12 days due to acute rejection, thus corroborating the results of histological examinations. The histological section of the allografts in group 2 showed the typical pattern of severe acute rejection and pleomorphic mitochondria. These findings suggest that a decrease in KBR indicates the reduced viability of the allograft in the advanced state of acute rejection.

Consequently, the present study leads us to suggest that the accurate assessment of the viability of the allograft after liver transplantation may be enhanced by KBR.

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