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Fujiwara, S

Ku, Y

Saitoh, Y

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# SERUM BILE ACID MONITORING AS AN EARLY INDICATOR OF ALLOGRAFT FUNCTION IN CANINE ORTHOTOPIC LIVER TRANSPLANTATION

SUMIO FUJIWARA, YONSON KU, AND YOICHI SAITOH

First Division, Department of Surgery,
Kobe University School of Medicine

## INDEXING WORDS

liver transplantation; serum bile acid; graft viability

## SYNOPSIS

This study was undertaken to elucidate the correlation between early graft function and serum bile acids profile in canine orthotopic liver transplantation (OLT). The recipient dogs were categorized into four groups: group A (n=5); bile output over 10 ml during initial 6 hours, group B (n=4); bile output less than 1 ml during the same period, group C (n=5); transplanted immediately after graft harvesting, and group D (n=5); transplanted after 7-hour ice-cold preservation in lactated Ringer's solution. In all cases serum total bile acid (TBA) was markedly elevated during anhepatic phase, the value being 49.4±48.5 μmol/L. However, in group A, TBA decreased promptly after revascularization, the value at 4-hour being 14.7±12.7 μmol/L. In contrast, the corresponding TBA level in group B was 62.3±27.5 μmol/L 4 hours later ($p<0.01$ VS group A). In group C, TBA also decreased immediately after reperfusion. Furthermore, in comparison between groups C and D, TBA level was significantly different 4 and 6 hours after reperfusion ($p<0.01$). In the reduction rate of TBA (value at each time point after reperfusion the value immediately before reperfusion), the significant difference was observed after 2 hours between groups A and

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B ($p<0.05$). In addition, significant difference was recognized at 30 minutes between groups C and D ($p<0.05$). Although the changes of most bile acid fractions were proportional to the changes of TBA in all four groups, the detection of lithocolic acid at 6 hours was characteristic of both groups B and D. In conclusion, there was strong correlation between the TBA level and the graft function during early postoperative period. Therefore, TBA will be a specific and early indicator to differentiate the quality of the transplanted grafts in OLT.

## INTRODUCTION

The survival of patients with liver transplantation has been dramatically improved by technological advances in surgical procedures and organ preservation and with the advent of highly effective immunosuppressive agents such as cyclosporin A. However, retransplantation on patients with primary nonfunctioning graft (PNF) is still an important determinant to improve their survival. It is reported that PNF account for as much as 21% of all the causes that require retransplantation.[23] Therefore it is considered that accurate monitoring of the graft function is crucial to detect PNF as early as possible.[20] Under these circumstances, amino acid clearance[4] and ketone body ratio[25] have been proposed by others as good indicators reflecting graft function. Serum bile acids profile were evaluated as well using pigs by Visser.[27] Other investigators also reported its good correlation to the early graft function after liver transplantation.[8,9,13,16] However, these reports simply described serum bile acids profile after liver transplantation. Because of the lack of detailed analysis, the significance of serum bile acid measurement has not yet been fully elucidated in terms of evaluating the graft quality. In the present study, therefore, we investigated bile acids profiles with special reference to the duration of hypothermic ischemia, and bile production, both of which have been reported to be closely related to liver function.[3,28]

## MATERIALS AND METHODS

*Experimental animals and groups*

Mongrel dogs of both sexes weighing between 10 and 18 kg were used for 19 consecutive orthotopic liver transplantations (OLT). The donors

and the recipients were matched by body weight so that the ratio was about 1 : 1.3. During operations all animals were anesthetized with sodium pentobarbital and parenteral fluid support with lactated Ringer's solution was provided at a rate of 100 ml/hour. Antibiotics were given daily through an intravenous route in the postpoerative course. Immunosuppressive drugs were not given. Dogs were categolized into the following four groups depending on bile production and cold ischemic time:

Group A: High bile production group (n=5); bile was produced at a rate of more than 10 ml until 6 hours after reperfusion.

Group B: Poor bile production group (n=4); bile was produced at a rate of less than 1 ml until 6 hours after reperfusion.

Group C: Short cold ischemic time group (n=5); liver graft was implanted immediately after graft harvesting.

Group D: Long cold ischemic time group (n=5); liver graft was implanted 7 hours after the graft harvesting.

*Surgical procedures*

Donor operation;

A midline abdominal incision is made from xyphoid to pubis. The common bile duct was transected close to the duodenum. The other harvesting procedures are essentially the same as those described by Jamieson and his associates.[10] Flushout solutions in groups A and B consist of 500 ml of ice cold Ringer's lactate with 3000 units of heparin and 1000 ml of Euro-Collins solution. In groups C and D, on the other hand, 1500 ml of Ringer's lactate alone was used for both flushout and preservation. During the final dissection on the back table, the suprahepatic inferior vena cava is cleaned and cut, leaving its length about 10 mm above the hepatic edge with all tributaries ligated.

Recipient operation;

Total hepatectomy is performed under a centrifugal pump assisted veno-venous bypass. Vascular reconstructions were subsequently performed according to the method described previously.[11] In groups A and B, external drainage of the biliary system was established by means of a catheter (5F) placement in the common bile duct whereas in groups C and D, it was reconstructed by anastomosis between the gallbladder and the duodenum.

*Blood sampling and analysis*

Peripheral blood samples were taken at the following time points: at the start of the recipient operation, immediately before reperfusion, and 30 minutes, 2, 4 and 6 hours after reperfusion. Each fraction of bile acids and total bile acids were measured by means of high-performance liquid chromatography (HPLC).[17] Total bile acids (TBA) represented the sum of all the fractions. In each group, the fractions were expressed as percentages of TBA. Firstly, in groups A and B, in which external drainage of the biliary tract was established to measure bile production, serum bile acid profiles were compared in terms of bile production. Secondly, in order to investigate the influence of cold ischemic time, serum bile acid profiles were compared between the groups C and D. As described above, the biliary tract was reconstructed in the groups C and D. The reduction rate of TBA at each time point was expressed as a percentage (%) of a TBA level immediately before reperfusion. Values were expressed as the mean±S.D., and student's T test was used for statistical analysis.

## RESULTS

*Cold and warm ischemic time and survival in each experimental group*

As indicated in Table 1, the warm ischemic time was almost the same in all four groups. The duration of survival in two groups, that is, the poor bile output group (group B) and the long cold ischemic time group (group D) was significantly shorter than that in the high bile output group (group A) and the short cold ischemic time group (group C). In group D, in particular, all animals died within 9 hours; their main cause of death was hemorrhage. The cause of death in group A animals, which survived for more than 7 days was due to rejection as judged by histological examination at autopsy. In two animals of group B, the cause of death was not determined, however, portal thrombosis and intra-abdominal hemorrhage was detected in two other animals in this group.

*Relationship between bile production and serum bile acids profile*

Serum bile acids at the start of the operation and immediately before reperfusion were 2.7±1.5 and 59.5±48.1 μmol/L, respectively, in group A, and 1.6±0.6 and 38.3±13.5 μmol/L, respectively, in group B, indicating no significant differences between these two groups. After

Table 1. Transplant outcome in each group of animals.

| Experimental group | Dog No | Cold ischemic time (min) | Warm ischemic time (min) | Survival | Cause of death |
|---|---|---|---|---|---|
| A | 1 | 104 | 24 | 10 hours | Cardiac failure |
| High bile | 2 | 125 | 22 | 7 days | Rejection |
| production | 3 | 100 | 31 | 3 days | Wound disruption |
| group | 4 | 110 | 40 | 8 days | Rejection |
| | 5 | 120 | 45 | 8 days | Rejection |
| B | 1 | 90 | 45 | 18 hours | Undetermined |
| Poor bile | 2 | 90 | 60 | 8 hours | Hemorrhage |
| production | 3 | 145 | 33 | 28 hours | Undetermined |
| group | 4 | 140 | 44 | 6 hours | Portal vein thrombosis |
| C | 1 | 64 | 38 | 7 days | Infection |
| Short cold | 2 | 95 | 40 | 2 days | Portal vein thrombosis |
| ischemic time | 3 | 100 | 27 | 3 days | Hepatic artery thrombosis |
| group | 4 | 105 | 26 | 7 days | Rejection |
| | 5 | 100 | 58 | 5 days | Infection |
| D | 1 | 420 | 37 | 8 hours | Hemorrhage |
| Long cold | 2 | 420 | 36 | 9 hours | Hemorrhage |
| ischemic time | 3 | 420 | 37 | 6 hours | Hemorrhage |
| group | 4 | 420 | 33 | 5 hours | Hemorrhage |
| | 5 | 420 | 35 | 7 hours | Hemorrhage |

reperfusion, however, TBA promptly decreased in group A, whereas TBA did not profoundly decrease in group B, the values at 4 hours being 14.7±12.1 and 62.3±27.5 μmol/L in groups A and B ($p<0.01$, Fig. 1). Moreover, the rate of TBA reduction gave the better separation between groups A and B as early as at the time point immediately after reperfusion ($p<0.05$). The difference in the rate of TBA reduction became more significant 4 and 6 hours after reperfusion between the two groups ($p<0.01$, Table 2).

When fractions of bile acid were compared (Table 3), both groups showed a decrease in the percentage of free cholic acid (F-CA) during the anhepatic phase. However, there was no significant difference in this parameter between these two groups. Nevertheless, in group B the trace fractions such as glycine-conjugated ursodeoxycholic acid (G-UDCA) and taurine-conjugated lithocholic acid (T-LCA) were detected 4 hours after reperfusion. Glycine-conjugated chenodeoxycholic acid (G-CDCA) was also detected 6 hours after reperfusion. In contrast, none of these trace fractions was detected 2 hours after reperfusion in group A. In the percentages of other bile acid fractions, there were no significant differences between these two groups.

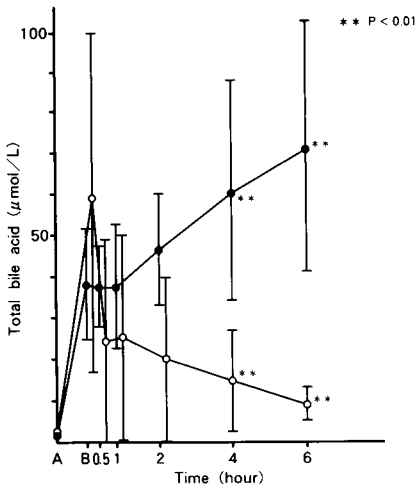


Fig. 1
Time course of total bile acid in high and low bile production groups.
-○-: high bile production group.
-●-: poor bile production group.
A: a time point at the start of operation.
B: a time point immediately before reperfusion.

Table 2. Reduction rate (%) of total bile acid after reperfusion in high and poor bile production groups.

| | Time after reperfusion (hr) | | | | |
|---|---|---|---|---|---|
| | 0.5 | 1 | 2 | 4 | 6 |
| A High bile producion group (n=5) | 38.4±39.8 | 41.0±34.5 | 45.9±43.6 * | 39.9±24.0 ** | 23.0±16.7 ** |
| B Low bile producion group (n=4) | 86.1±9.7 | 106.4±53.0 | 123.7±13.9 * | 159.8±18.4 ** | 176.9±43.0 ** |

* P<0.05, ** P<0.01

$$\text{The reduction rate of TBA (\%)} = \frac{\text{TBA at each time point}}{\text{TBA immediately before reperfusion}} \times 100$$

Table 3. Serum profiles of bile acid fractions.

A. high bile production group (n=5)

| | | At laparotomy | Immediately before reperfusion | Time (hr) after reperfusion | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 0.5 | 1 | 2 | 4 | 6 |
| Taurine conjugated | CA | 38.7±25.8 | 47.0±3.6 | 47.7±10.9 | 51.1±12.5 | 53.3±15.3 | 52.5±16.4 | 47.3±12.9 |
| | DCA | 36.6±14.5 | 36.7±3.5 | 33.9±4.1 | 33.4±4.1 | 29.4±2.4 | 26.9±5.2 | 21.1±3.3 |
| | CDCA | 0 | 5.7±5.3 | 5.7±2.8 | 5.6±2.3 | 4.7±1.2 | 4.0±1.2 | 6.1±7.4 |
| | LCA | 0 | 0.4±0.3 | 0.9±0.6 | 0.4±0.5 | 0 | 0 | 0 |
| | UDCA | 0 | 4.0±3.5 | 0.8±0.7 | 0 | 0.7±0.7 | 0.5±0.5 | 1.4±1.5 |
| Glycine conjugated | CA | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | DCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | CDCA | 0 | 0.1±0.2 | 0 | 0 | 0 | 0 | 0 |
| | LCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | UDCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Free | CA | 19.4±22.4 | 5.6±7.3 | 8.1±11.1 | 7.0±11.0 | 8.5±10.7 | 11.3±16.3 | 11.3±18.5 |
| | DCA | 5.3±9.2 | 3.5±2.4 | 2.5±1.8 | 2.0±2.6 | 2.9±2.3 | 4.4±5.3 | 7.4±8.7 |
| | CDCA | 0 | 0.4±0.6 | 0.4±0.5 | 0.4±0.8 | 0.4±0.7 | 0 | 0 |
| | LCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | UDCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

B. poor bile production group (n=4)

| | | At laparotomy | Immediately before reperfusion | Time (hr) after reperfusion | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 0.5 | 1 | 2 | 4 | 6 |
| Taurine conjugated | CA | 57.3±11.8 | 67.5±12.3 | 68.6±2.8 | 61.1±12.2 | 60.2±13.0 | 60.8±11.5 | 62.0±11.5 |
| | DCA | 22.1±12.1 | 19.7±7.0 | 21.7±4.4 | 26.9±14.1 | 25.5±10.5 | 24.5±11.2 | 23.5±11.4 |
| | CDCA | 2.4±4.8 | 6.0±1.6 | 4.5±0.1 | 5.1±0.8 | 5.4±1.0 | 5.8±1.2 | 5.3±1.9 |
| | LCA | 0 | 0.2±0.3 | 0.2±0.3 | 0.7±0.7 | 0.6±0.5 | 0.5±0.5 | 0.5±0.2 |
| | UDCA | 0 | 2.9±1.4 | 2.8±0.8 | 2.0±0.9 | 2.0±9.0 | 1.8±0.9 | 2.4±1.1 |
| Glycine conjugated | CA | 0 | 0 | 0 | 0.8±1.6 | 0.5±1.0 | 0.9±1.8 | 0.6±1.3 |
| | DCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | CDCA | 0 | 0 | 0 | 0 | 0 | 0 | 5.3±9.1 |
| | LCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | UDCA | 0 | 0 | 0 | 0.1±0.1 | 0 | 0.2±0.3 | 0.1±0.2 |
| Free | CA | 18.2±21.7 | 2.9±3.0 | 1.5±0.5 | 2.7±3.7 | 4.1±2.9 | 4.8±3.0 | 5.0±4.1 |
| | DCA | 0 | 1.3±1.7 | 0.8±0.1 | 0.4±0.3 | 0.8±0.8 | 0.7±0.8 | 0.6±0.4 |
| | CDCA | 0 | 0.9±1.7 | 0 | 0 | 0 | 0.1±0.1 | 0.1±0.2 |
| | LCA | 0 | 0.2±0.4 | 0 | 0 | 0 | 0 | 0 |
| | UDCA | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

CA: cholic acid, DCA: deoxycholic acid, CDCA: chenodeoxycholic acid, LCA: lithocholic acid, UDCA: ursodeoxycholic acid

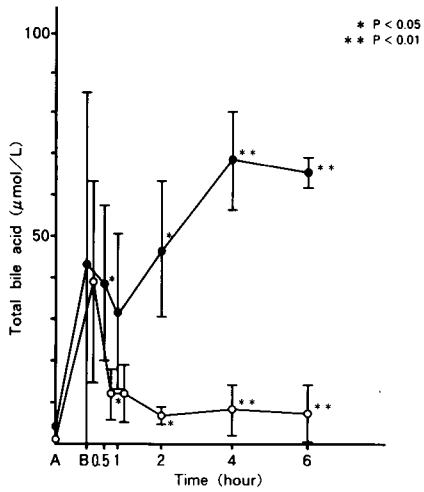


Fig. 2
Time course of total bile acid in short and long ischemic time groups.
-○-: short cold ischemic time group.
-●-: long cold ischemic time group.
A: a time point at the start of operation.
B: a time point immediately before reperfusion.

Table 4. Reduction rate (%) of total bile acid (TBA) after reperfusion in short and long cold ischemic time groups.

| | Time after reperfusion (hr) | | | | |
|---|---|---|---|---|---|
| | 0.5 | 1 | 2 | 4 | 6 |
| C Short cold ischemic time group (n=5) | * 41.0±31.7 | * 40.6±30.4 | * 35.7±36.4 | * 28.4±27.9 | * 22.6±22.9 |
| D Long cold ischemic time group (n=5) | * 148.7±79.3 | * 129.3±73.4 | * 105.9±52.3 | * 122.9±73.3 | * 121.9±84.6 |

* P<0.05

$$\text{The reduction rate of TBA (\%)} = \frac{\text{TBA at each time point}}{\text{TBA immediately before reperfusion}} \times 100$$

*Influence of cold ischemic time on the serum bile acid profile*

TBA prior to the start of the operation was 1.6±1.2 μmol/L and 4.0±2.1 μmol/L in groups C and D, respectively. Although TBA was higher in group D than those in group C, there was no significant difference between these two groups at this time point. On the other hand, TBA immediately before reperfusion was 38.9±24.4 μmol/L and 41.0±44.5 μmol/L in groups C and D, respectively, showing a marked increase in both groups. Nevertheless, TBA 30 minutes after reperfusion was 11.6±6.0 μmol/L and 38.1±19.4 μmol/L in groups C and D, respectively, and there was a significant difference between these two groups ($p<0.05$). The difference between these groups became more marked 4 and 6 hours after reperfusion ($p<0.01$, Fig. 2). As indicated in Table 4, moreover, TBA decreased rapidly immediately after reperfusion in group C, whereas there was no such tendency in group D. There was a marked difference between these two groups as early as 30 minutes after reperfusion ($p<0.05$).

The main fractions of bile acids in groups C and D showed almost the same time courses as did groups A and B. There was no marked difference between the two groups. However, as described in the results of groups A and B, T-LCA disappeared 4 hours after reperfusion in group C, whereas it was still detected at 4 hours in group D.

## DISCUSSION

There is a broad agreement that graft function is affected by several factors such as preservation time, surgical procedure during transplantation and reperfusion injuries.[14] Therefore, graft function can be evaluated only after reperfusion. In this regard, it is an important issue to establish more sensitive marker to assess graft function in an early stage after transplantation. Nevertheless, it has already been reported that the conventional liver function tests such as serum transaminases and bilirubin involve difficulties in estimating graft function in an early stage after transplantation.[19] Meanwhile, Visser[27] and Okanoue[16] reported on serum bile acids in pigs, while Herrera[8,9] and Mora[13] et al. documented these bile acids in humans, although both of their studies were only concerned with the measurement of bile acids in peripheral blood prior to and after liver transplantation, and thus, none of them undertook precise investigation in elucidating the correlation between graft function and serum bile

acid profile. In the present study, therefore, we evaluated the relationship between the serum bile acid profile and bile production which has long been used as an important criterion of liver function after transplantation.[1,7] It was reported that daily bile output after OLT in dogs was about 30 to 60 ml. Based on these observations, we compared the TBA profile of high bile output animals whose bile flow was more than 10 ml up to 6 hours after reperfusion with those of poor bile producing animals whose bile flow was as low as 1 ml or less. As to the duration of survival, some animals in group A survived 5 days or longer, which represented a marked difference from the survival in group B. Furthermore, there were no deaths resulting from liver insufficiency even in an early stage after OLT in group A. Since bile production after liver transplantation is a valuable marker to assess graft function, the time course of TBA after reperfusion was compared between these two groups. Four of five animals in group A showed an extremely rapid decrease in TBA. The remaining animal (Dog No. 3) exhibited a gradual decrease when compared to other animals. In group B, there were some animals showing a decrease in TBA immediately after reperfusion. However, all the animals in this group consequently showed a marked increase in TBA. Thus, prolonged high TBA levels were invariably associated with poor bile production. However, there were four other animals in which bile production up to 6 hours after transplantation was between 1 ml and 10 ml. These animals did not show any consistent tendency in the time course of TBA; that is, some animals exhibited a decrease in TBA after reperfusion, some continued to show increased levels, and some showed a tendency of decreased TBA levels 4 hours after reperfusion. Therefore, TBA profiles in animals which belonged to the "intermediate" group should be accumulated and compared with other available viability markers such as keton body ratio.

In order to further clarify the relationship between the extent of graft dysfunction and serum bile acid profile, we compared TBA profile between the short and long cold ischemic time groups. Consequently, almost the same results as those obtained by the comparison between the groups A and B were obtained. However, there was a significant difference between these two groups in terms of TBA levels and the reduction rate of TBA in an extremely early stage after liver transplantation, that is, 30 minutes after reperfusion. Unlike animals which were compared in terms of bile production, the difference between these two groups was more marked. These results clearly

indicated that 7-hour cold ischemia of the canine liver in lactated Ringer's solution exceeds the storage limit. Taken together, serum bile acid profile would serve as an extremely valuable criterion for evaluating the efficacy of storage method for liver grafts.

O'Maille[18] and Pries[21] reported the hepatic extraction of bile acids from portal vein blood in normal dogs to be 92±5% and 61-98%, respectively. Based on these observations, the amount of bile acids that pass through the liver is very small. Therefore, systemic bile acid levels are kept low under physiologic conditions.[2,24] However, the hepatic extraction of bile acids decreases significantly in patients with chronic liver diseases.[5,6] It is likely that the clearance of serum bile acids in the liver graft after reperfusion sensitively represents liver function after transplantation. The reduction rate of TBA at each time point to a TBA level immediately before reperfusion gave better separation between the groups A and B as well as the groups C and D, as compared to the levels of TBA per se. There was a marked difference in a much earlier stage after reperfusion. Since TBA immediately before reperfusion in transplanted animals reflects differences between individual animals and the length of the anhepatic phase, the difference between groups became more clear when the reduction rate of TBA levels were compared. Thus, the reduction rate of TBA reflects graft function more accurately than do TBA levels per se in individual animals.

Some reports suggest that amino acid clearance[4] and ketone body ratio in arterial blood[14,25] are valuable as a marker of the graft function in an early stage after transplantation. Taki et al. reported on serum ketone body ratio after transplantation. They compared the levels between immediate transplanted graft after the harvest and the graft transplanted after the storage in cold saline solution for 12 hours. However, they did not clearly indicate the time points when serum keton body ratio could discriminate the functioning and the nonfunctioning grafts. Although some reports indicated that amino acid clearance[4] is useful in the determination of graft function, they did not evaluate the time course of these parameters after reperfusion. In this regard, the present study clearly demonstrated that TBA profile gave the clear separation between the functioning and nonfunctioning grafts as early as 4-hour after reperfusion. In addition, the measurement of bile acids by an HPLC technique requires a relatively short time of approximately 2 hours, which indicated that the bile acids measurement may serve as

a timely, valuable diagnostic marker in the determination of the graft function.

The fractions of bile acids vary with animal species.[12] For instance, glycine-conjungated bile acids are present in even healthy individuals in human.[22] In the present study using dogs, however, the glycine conjugate was hardly detected as in the case of a previous study.[18] It is speculated that the time course of each bile acid fraction after liver transplantation in dogs slightly differs from that in humans. Visser et al. reported[27] that bile acids in the peripheral blood increased linearly with time after total hepatectomy in P-V shunt models. This seems to be a clear reflection of the time-dependent transmigration of bile acids in the intestinal tract into the portal vein. Absorption in the intestinal tract varies depending on bile acid fraction. It is known that the non-conjugtate has an absorption efficiency lower than that of the conjugate.[15] In fact, the percentage of F-CA tended to decrease immediately before reperfusion in the present study. In addition, T-LCA became not detected in groups A and C by the time 6-hour after reperfusion, whereas it was still detected in groups B and D. However, as Herrera et al.[9] reported, these results may simply reflect changes in TBA per se rather than a decrease in the hepatic extraction of T-LCA. Thus, the significance of measurement of each bile acid fraction remains unclear in the present study.

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