

Increased exhaled carbon monoxide concentration during living donor liver transplantation

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Abstract. Exhaled carbon monoxide concentration (ExCO-C) has been reported to increase in oxidative tissue injuries such as systemic inflammation, and is thought to reflect increased heme breakdown in the affected organ. As a transplanted liver undergoes ischemia-reperfusion, we hypothesized that ExCO-C might also increase following liver transplantation and might serve as a measure of the severity of the graft tissue injury. We prospectively studied 67 living donor liver transplantation (LDLT) patients in a consecutive fashion. During anesthesia, ExCO-C was determined at 6 time points, ranging from anesthesia induction, to admission to the intensive care unit. We also measured two markers of endothelial cellular injury, i.e., serum soluble thrombomodulin (sTM) and intercellular adhesion molecule (ICAM)-1. At 5 min after reperfusion of the grafted liver, ExCO-C markedly increased from 5.69 ± 2.34 ppm at baseline, to 9.79 ± 4.72 ppm ($p < 0.0001$). There was an excellent correlation among an increase in CO concentration, arterial carboxyhemoglobin levels at the time of reperfusion ($r^2 = 0.19$, $p = 0.0003$), and postoperative total bilirubin levels (day 1, 2, and 3; $r^2 = 0.102$, 0.109 and 0.100 ; $p = 0.008$, 0.007 and 0.010 , respectively). Serum sTM and ICAM-1 levels were also significantly increased after reperfusion (sTM: 3.3 ± 0.8 to 5.1 ± 1.7 FU/ml, $p = 0.0001$; ICAM-1: 271.9 ± 86.3 to 515.0 ± 157.8 FU/ml, $p = 0.0001$). ExCO-C had a positive relationship with sTM ($r^2 = 0.16$, $p = 0.035$) and ICAM-1 ($r^2 = 0.12$, $p = 0.08$). There was, however, no correlation of ExCO-C with serum AST/ALT levels or clinical outcomes. This study demonstrated that ExCO-C significantly increased after reperfusion during LDLT. The increased ExCO-C may likely reflect increased heme breakdown and endothelial cell injury in the grafted liver.

Introduction

Currently, liver transplantation is the only therapeutic modality available for the treatment of end-stage liver diseases such as hepatocellular carcinoma, liver cirrhosis, and fulminant hepatitis (1). However, the shortage of donor organs is a serious problem, and, in order to expand transplant candidacy, the number of living donor liver transplantation (LDLT) has recently been increased (2). LDLT requires cold preservation and warm revascularization of liver grafts prior to transplantation. Thus, a graft liver inevitably suffers from insults due to ischemia-reperfusion (I/R), which is a well-known oxidative stress (3,4). There are, however, no available biomarkers that can be used to monitor I/R-induced tissue injury of the grafted liver during LDLT operation.

Endogenous carbon monoxide (CO) is principally produced by heme catabolism in humans (5,6). Heme oxygenase (HO)-1, the rate-limiting enzyme in heme catabolism, is induced by various types of oxidative stress which includes I/R (7-12). HO-1 induction leads to increased heme breakdown, resulting in the production of iron, CO, and biliverdin IX α which is subsequently reduced to bilirubin IX α by biliverdin reductase (13). CO synthesized through the HO reaction diffuses out of cells, enters the bloodstream to form carboxyhemoglobin (CO-Hb) and is transported to the lung where it is excreted in ambient air (14). We previously demonstrated that the exhaled carbon monoxide concentration (ExCO-C) is increased in critically ill patients with systemic inflammation, via increased heme breakdown (15), and it is suggested that ExCO-C may reflect the degree of oxidative tissue injuries in the affected organ. We thus hypothesized that ExCO-C might also increase during LDLT and reflect the severity of tissue injury of the graft due to I/R. In this study, we examined time course changes of ExCO-C during LDLT, and studied their relationship with the serum levels of two important molecular markers of endothelial injury, namely, soluble thrombomodulin (sTM) and intercellular adhesion molecule (ICAM)-1 (16-18).

Materials and methods

Patients. This study was conducted in conformity with the Declaration of Helsinki and was approved by the Institutional Review Board at Okayama University Medical School. After

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receiving the institutional approval and informed consent from study participants, we prospectively studied 67 (48 male, 19 female) LDLT patients between January 2004 and October 2005 at our institution in a consecutive manner.

Anesthetic procedure. All patients received general anesthesia with an endotracheal intubation. General anesthesia was induced by 2 mg/kg of propofol and 5 μ g/kg of fentanyl with 0.1 mg/kg of vecuronium to facilitate endotracheal intubation. Anesthesia was maintained using continuous infusion of fentanyl and vecuronium at a rate of 2-4 μ g/kg/h and 2-4 mg/kg/h, respectively, with 0.5 to 1.0% of isoflurane and oxygen/air mixture. For the first group of 35 patients (from January 2004 to December 2004) the fraction of inspired oxygen (F_iO_2) was increased from 0.6 to 1.0 at the time of graft reperfusion due to concerns about 'post reperfusion syndrome' (19). The level of $F_iO_2=1.0$ was maintained for 1 h and then returned to 0.6. Since the increase in inspired oxygen concentration may cause the elevation of CO concentration in exhaled air (20), for the second group of 32 patients (from January 2005 to October 2005), F_iO_2 was maintained at 0.6 throughout the procedure. However, we did not encounter any hypoxemic episode during the procedure in the second group.

Transplantation procedure. The donor hepatectomy was performed using the standard technique for living donation (21). The recipient hepatectomy was also performed using the standard technique without veno-venous bypass (21). The grafted liver was then weighed and perfused through the portal vein by the University of Wisconsin (UW) solution with methylprednisolone (20 mg/kg). The volume of the solution was determined according to the weight of the graft (1 ml/g). The portal flush was performed by gravity. After perfusion, the graft was immersed in the UW solution and kept at 4°C for storage. Transplantation of the graft was performed using the piggyback technique. Before completion of the caval anastomosis, the graft was flushed through the portal vein with an average of 250 ml of 5% albumin stored at 4°C. Hepatic artery reconstruction was performed by end-to-end anastomosis under an operating microscope, followed by duct-to-duct biliary reconstruction (22).

Exhaled CO measurement. ExCO-C was measured using a newly developed CO analyzer (Carbolyzer™ mBA-2000, Taiyo Instruments, Inc., Osaka, Japan) as described previously (15). This instrument is equipped with a gas sensor based on the controlled potential electrolysis method (23) with sensitivity to 0.1 ppm of CO and capability of continuous side-stream sampling. A sampling adaptor was attached to the respiratory circuit for exhaled air sampling. During anesthesia, ExCO-C was measured at the following 6 time points, i.e., i) after induction, (time 1); ii) pre-anhepatic phase (time 2); iii) anhepatic phase (time 3); iv) 5 min after reperfusion (time 4); v) 1 h after reperfusion (time 5); and vi) admission to the intensive care unit (ICU) (time 6). The measurement was carried out for 1 min at each time point and a mean ExCO-C was calculated using the special software package (Carbolyzer™ DataBox, Taiyo Instruments, Inc.). Respiratory status was corrected based on PaCO₂, PaO₂, and

Table I. Patient characteristics.

Age (in years)	51.5±4.9
Gender ratio (F/M)	19/48
Liver disease	
Viral disease	39
Non-viral disease	22
Fluminant hepatitis	6
Operation time (min)	549.3±173.2
CIT (min)	61.5±4.9
WIT (min)	36.0±8.5
Bleeding (ml)	3000±1414
GRW (%)	1.16±0.20
MELD score (point)	18±4.2

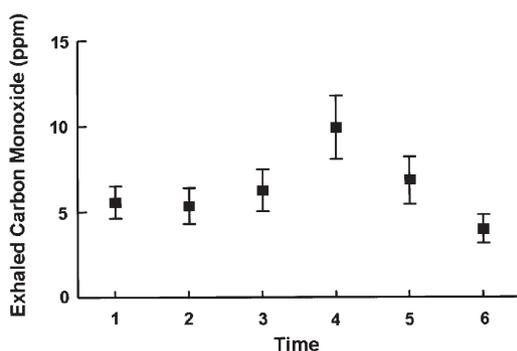
Values are the means ± SD, or number of patients. CIT, cold ischemic time; WIT, warm ischemic time; GRW, graft volume/recipient body-weight ratio; and MELD, Mayo end-stage liver disease.

the respiratory rates that were also determined at the time of CO measurements.

Arterial CO-Hb measurement. Arterial CO-Hb concentration was measured as a percentage using a co-oximeter blood gas analyzer (ABL 735 System™, Radiometer Medical A/S, Copenhagen, Denmark). This instrument was specifically adjusted to the absorption wavelength of CO-Hb (SAT 100), to permit the most accurate measurement of CO-Hb concentration (24). The instrument was pre-calibrated by the manufacturer, based on heparinized blood samples that met with strict criteria for pH, pCO₂, Hb, FCO-Hb, and FMet-Hb (25). Routine arterial blood gas analysis was performed at the same time of the measurements of ExCO-C.

Serum sTM and ICAM-1 measurement. In 14 patients, serum was obtained from the arterial blood sampled at the same time as the CO measurement. Blood samples were drawn into 0.11 mol/l sodium citrate (9:1), placed immediately on melting ice, and centrifuged within 30 min. Serum was kept frozen at -70°C until tested. Serum sTM and ICAM-1 concentrations were measured by microenzyme-linked immunosorbent assay (micro-ELISA), using the micro-ELISA plate coated with monoclonal antibodies (Human Thrombomodulin Immunoassay kit, Daiichi Fine Chemical Co. Ltd., Toyama, Japan and CellFree™ Human sICAM-1 ELISA, Pierce Endogen, Rockford, IL), according to the manufacturer's protocol.

Postoperative biochemical determinations and clinical outcomes. Serum concentrations of total bilirubin (T-Bil), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained from routine laboratory results at postoperative day 1, 2, 3 and 7. Data of clinical outcomes including acute cellular rejection, reintubation, bleeding required re-laparotomy, length of intensive care unit



B

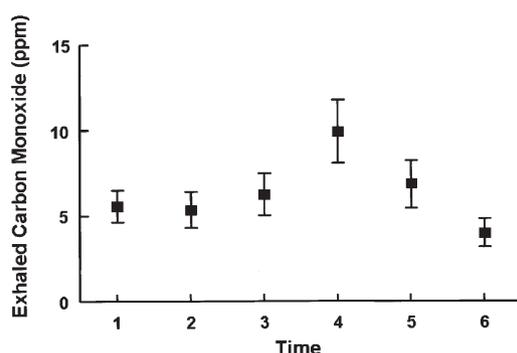


Figure 1. Changes in exhaled carbon monoxide concentrations during living donor liver transplantation with fraction of inspired oxygen ($F_{I}O_2=1.0$ (A) and $F_{I}O_2=0.6$ (B)). Exhaled carbon monoxide concentrations markedly increased and reached a maximum at 5 min after reperfusion (time 4) and gradually decreased to baseline by the time of admission to the ICU (time 6) regardless of inspired oxygen concentration. (A) $F_{I}O_2$ was increased from 0.6 to 1.0 at the time of graft reperfusion, which was maintained for 1 h and then returned to 0.6. (B) $F_{I}O_2$ was maintained at 0.6 throughout the procedure. Data are expressed as the mean \pm SD. The timing of measurement of exhaled carbon monoxide concentrations: time 1, after induction of anesthesia; time 2, pre-anhepatic phase; time 3, anhepatic phase; time 4, 5 min after reperfusion; time 5, 1 h after reperfusion; and time 6, admission to the intensive care unit.

stay, length of hospital stay, and hospital survival were also collected.

Statistical analysis. Data were expressed as the mean with standard deviation. Analyses were made using the Student's t-test, Pearson's correlation coefficients or analysis of variance as appropriate. $p < 0.05$ was considered statistically significant.

Results

From January 2004 to October 2005, 67 patients, 48 men and 19 women, mean age 51.5 ± 4.9 years, underwent LDLT. Underlying diseases of the transplant recipients included liver cirrhosis caused by viral hepatitis ($n=39$), fulminant hepatitis ($n=6$), and others ($n=22$). Average Mayo end-stage liver disease score of these patients was 18 ± 4.2 (Table I). The donors included 36 men and 31 women; their mean age was 35 ± 15.6 . Preoperative evaluation of the donors revealed

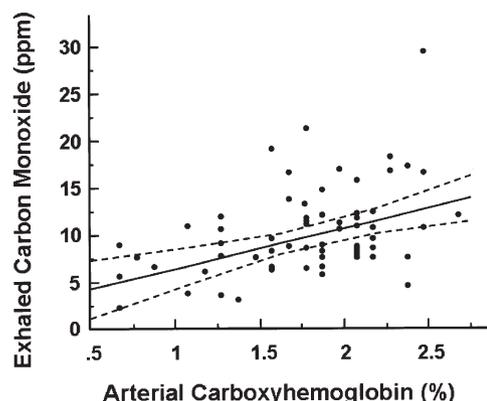


Figure 2. Relationship between exhaled carbon monoxide concentrations and arterial carboxyhemoglobin levels at 5 min after reperfusion (time 4). Exhaled carbon monoxide concentrations correlated positively with arterial carboxyhemoglobin levels ($r^2=0.19$, $p=0.0003$). Linear regression line (solid line) with 95% confidence intervals (dotted lines) is presented.

that all of them had normal liver biochemical findings and bilirubin levels. The types of liver grafts were right lobe ($n=43$), lateral lobe ($n=7$), extended left lobe ($n=5$) and left lobe ($n=12$). The operation time of recipients was 549.3 ± 173.2 min; cold and warm ischemia times of the graft were 61.5 ± 4.9 min and 36.0 ± 8.5 min, respectively. The graft volume/recipient body weight ratio was 1.16 ± 0.20 and blood loss during the recipient operation was 3000 ± 1414 ml (Table I).

We kept the respiratory condition constant during LDLT using anesthetic drugs with muscle relaxants. Throughout the entire anesthetic period, we controlled respiratory rate, PaO_2 , $PaCO_2$ and pH to maintain the normal range by sequential measurements of arterial blood gas samples. Although there were still small fluctuations after normalization, it was essentially constant and stable, and time courses were comparable among all individuals, and between two groups of patients with different $F_{I}O_2$ levels (Table II).

Although ExCO-C following LDLT (time 1) was significantly higher than those of healthy volunteers (14), CO concentration was essentially constant until the anhepatic phase (time 3) (Fig. 1). In the first 35 patients with $F_{I}O_2=1.0$, ExCO-C significantly increased from 5.60 ± 2.82 ppm at the baseline (time 1) to 9.95 ± 5.58 ppm 5 min after reperfusion (time 4) ($p < 0.0001$) (Fig. 1A). The increased ExCO-C returned to the baseline (to 4.01 ± 2.48 ppm) at the time of ICU admission (time 6) (Fig. 1A). Similarly, in the next 32 patients with $F_{I}O_2=0.6$, ExCO-C increased from 5.77 ± 1.61 ppm at time 1 to 9.60 ± 3.52 ppm at time 4 ($p < 0.0001$) (Fig. 1B). Thus there was no difference in ExCO-C at any point between the two groups of patients with different $F_{I}O_2$ levels.

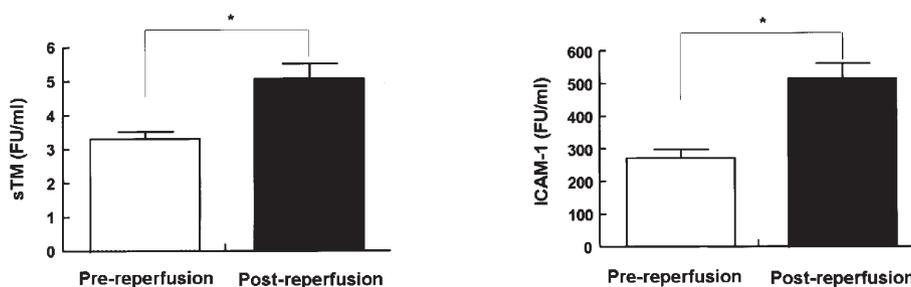
Changes in arterial CO-Hb concentration during LDLT were similar to those of ExCO-C (Table II). At time 4, CO-Hb concentration was significantly correlated with ExCO-C ($r^2=0.19$, $p=0.0003$) (Fig. 2). Both serum sTM and serum ICAM-1 concentration markedly increased after reperfusion from the pre-reperfusion level (sTM: 3.3 ± 0.8 FU/ml to 5.1 ± 1.7 FU/ml, $p=0.0001$; ICAM-1: 271.9 ± 86.3 FU/ml to 515 ± 157.8 FU/ml, $p=0.0001$) (Fig. 3A). We also found that

Table II. Respiratory conditions.

	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
$F_{I}O_2=1.0^a$						
PaCO ₂ (mmHg)	35.3±3.7	35.3±2.9	34.3±2.9	36.2±3.4	38.7±3.4	36.7±3.6 ^c
PaO ₂ (mmHg)	267±106	225±57	256±58	455±70 ^c	357±130 ^c	210±61
RR	12.2±1.3	12.1±0.9	12.5±1.5	12.7±1.5	12.6±1.6	14.0±2.3 ^c
CO-Hb (%)	1.93±0.56 ^c	1.93±0.53	1.95±0.46	2.12±0.50	1.65±0.42 ^c	1.63±0.40
$F_{I}O_2=0.6^b$						
PaCO ₂ (mmHg)	37.0±3.2	36.1±2.6	35.1±2.7	36.5±2.9	38.7±2.8	39.8±3.2 ^c
PaO ₂ (mmHg)	244±73	237±40	247±37	257±65 ^c	245±62 ^c	169±61
RR	12.2±1.4	12.0±1.6	12.0±1.6	12.1±1.4	12.1±1.0	12.6±1.4 ^c
CO-Hb (%)	1.53±0.44 ^c	1.95±0.48	1.91±0.41	2.14±0.34	1.94±0.45 ^c	1.8±0.29

Values are the mean ± SD. ^aFraction of inspired oxygen ($F_{I}O_2$) was increased from 0.6 to 1.0 at the time of graft reperfusion, which was maintained for 1 h and then returned to 0.6. ^b $F_{I}O_2$ was maintained at 0.6 throughout the procedure. RR, respiratory rate. The timing of measurements: time 1, after induction of anesthesia; time 2, pre-anhepatic phase; time 3, anhepatic phase; time 4, 5 min after reperfusion; time 5, 1 h after reperfusion; and time 6, admission to the intensive care unit. ^c $P<0.05$ in comparisons between the group of $F_{I}O_2=1.0$ and the group of $F_{I}O_2=0.6$.

A



B

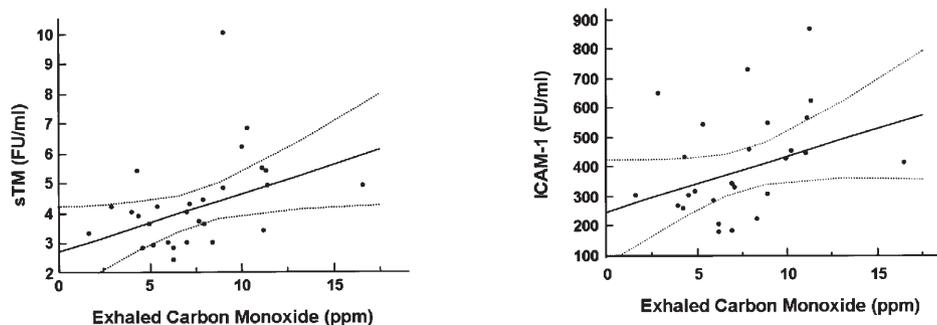


Figure 3. Serum concentrations of soluble thrombomodulin (sTM) and intercellular adhesion molecule-1 (ICAM)-1 at the time of pre- and post-reperfusion in 14 patients (A) and their relationship to exhaled carbon monoxide concentrations (B). (A) Both sTM (left) and ICAM-1 (right) levels significantly increased after reperfusion (sTM, $*p=0.0001$; ICAM-1, $*p=0.0001$, respectively). Data are expressed as the mean ± SD. (B) Exhaled carbon monoxide concentrations positively correlated with serum sTM ($r^2=0.16$, $p=0.035$) (left) and ICAM-1 ($r^2=0.12$, $p=0.08$) (right) levels, respectively. Linear regression line (solid line) with 95% confidence intervals (dotted lines) is presented.



A, Biochemical markers

	Day ^a 1	Day ^a 2	Day ^a 3	Day ^a 7
T-Bil (mg/dl)	6.49±3.20	5.34±2.76	4.81±2.93	4.69±3.73
r ²	0.102	0.109	0.100	0.040
p value	0.008	0.007	0.010	0.107
ALT (IU/dl)	282±199	273±407	177±293	92±115
r ²	0.015	0.042	0.056	0.001
p value	0.320	0.090	0.050	0.815
AST (IU/dl)	256±192	308±298	271±348	202±250
r ²	0.028	0.004	0.024	0.001
p value	0.180	0.616	0.203	0.906

B, Clinical outcomes

	Yes	No	p value
Acute cellular rejection	9.67±0.90	9.87±0.76	0.86
Reintubation	10.32±1.50	9.70±0.63	0.70
Bleeding	10.92±1.58	9.61±0.62	0.44
Survival	9.83±0.62	9.49±1.58	0.84
ICU stay	r ² =0.021		0.23
Hospital stay	r ² =0.023		0.22

Values of T-Bil, ALT and AST are the mean ± SD. ^aPostoperative day. T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Values of exhaled carbon monoxide concentration are the mean ± SD.

ExCO-C had a significant positive correlation with serum sTM and ICAM-1 levels (sTM: r²=0.16, p=0.035; ICAM-1: r²=0.12, p=0.08, respectively) (Fig. 3B).

Serum T-Bil was highly elevated on postoperative day 1, while it decreased gradually thereafter. We found there was a significant correlation between the peak of ExCO-C and serum T-Bil at postoperative day 1, 2, 3, but not at day 7 (Table IIIA). Serum AST and ALT levels were also markedly increased on postoperative day 1 and 2, followed by the gradual decrease thereafter. However, there was no significant correlation between ExCO-C and AST, or ALT levels, at all time points (Table IIIA). There was also no correlation between ExCO-C and postoperative complications and clinical outcomes (Table IIIB).

Discussion

There are no studies which have examined ExCO-C in LDLT, and this study is the first to describe significant changes in ExCO-C occurring immediately after LDLT. We found that ExCO-C markedly increased and reached a maximum at 5 min after reperfusion of the donor liver. Thereafter, it gradually decreased, and reached the baseline level by the time of admission to the ICU. Similar changes in ExCO-C were observed for two groups of patients with different F₁O₂ conditions, i.e., F₁O₂=1.0 and F₁O₂=0.6. The increased ExCO-C was also correlated with the levels of serum sTM and ICAM-1,

the two markers of endothelial cell damages. These findings strongly suggest that increased ExCO-C may reflect the degree of endothelial injury of the grafted liver.

As shown in Fig. 1, ExCO-C markedly increased immediately after reperfusion of the grafted liver during LDLT, suggesting that hepatic I/R must have also resulted in a systemic increase in CO concentration prior to its increase in exhaled air. ExCO-C was also well correlated with arterial CO-Hb concentration (Fig. 2). Previous studies reported that there was an increase in ExCO-C in inflammatory airway diseases, such as asthma, chronic obstructive pulmonary disease and cystic fibrosis (26-28). It has also been reported that ExCO-C was increased in systemic inflammatory diseases such as sepsis and critical illness (15,29-31). Our findings in this study have additionally shown that I/R during LDLT led to an increase in ExCO-C, and indicate that I/R in LDLT is a significant oxidative stress on the grafted liver.

It has previously been suggested that changes in F₁O₂ might influence ExCO-C (20,32). However, our findings showed that the peak concentration of exhaled CO after reperfusion was similar for two groups of patients with different F₁O₂ levels, i.e., F₁O₂=1.0 and F₁O₂=0.6 (Fig. 1). Consistent with our findings, a recent study also reported that an increase in F₁O₂ from 0.5 to 1.0 did not influence end-tidal CO concentration (32). These recent findings thus indicate that a <2-fold change in F₁O₂ does not influence CO concentration in exhaled air. Other potential variables that might influence

ExCO-C such as changes in respiratory status have been adjusted and rectified in our study, based on sequential measurements of PaCO₂, PaO₂, and respiratory rate throughout the entire period of observation (Table II).

It is known that cytochrome P450, a major heme protein in the liver, undergoes a rapid degradation by ischemia (33), which results in the release of free heme, followed by the production of CO, iron, and biliverdin by the HO-mediated enzymatic process (34,35). It is likely that a grafted liver may also undergo a similar change during LDLT, and may result in the production of CO from heme breakdown. Increased CO concentration from the graft would then be diffused into general circulation, ultimately resulting in exhaled air. A significant correlation between peak ExCO-C and serum T-Bil levels also strongly suggests that increased CO concentration in exhaled air may be due to heme breakdown in the grafted organ (Table IIIA).

While heme is required as the prosthetic group for hemo-proteins that are essential for life, an excess amount of free heme is highly toxic to cells, as it catalyzes the production of oxygen radicals (34,35). Free heme can also readily intercalate into the lipid bilayer, resulting in an oxidative damage of the cytoskeleton (34-36). Exposure of endothelial cells to hemin, an oxidized form of heme that is available as a chemical, is also known to stimulate the expression of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin, indicating that it elicits significant tissue damages (37,38). In order to counteract against such insults, the body is equipped with a mechanism to rapidly activate the HO-1 gene to increase HO activity, which then eliminates free heme by converting it to CO, iron and biliverdin IX α (7-10). Recent evidence suggests that all metabolites of heme can significantly contribute to the cellular defense mechanism, e.g., CO by suppressing apoptosis via activation of p38 MAPK, iron by inducing an acute phase reactant ferritin, and biliverdin by its anti-oxidant property (7-10). Our findings also showed that serum levels of sTM and ICAM-1 after reperfusion were increased, suggesting that there was in fact significant endothelial cell damages in the grafted liver (Fig. 3), and that HO-1 induction took place as judged by an increase in ExCO-C, as cellular defense against the I/R-mediated insult (Fig. 1).

A significant relationship between peak ExCO-C and serum T-Bil levels was observed on day 1, 2, and 3 after LDLT, but not on day 7 (Table IIIA). This finding suggests that changes in ExCO-C may be very rapid but reversible, and related only to changes reflecting heme breakdown such as serum T-Bil and arterial CO-Hb concentration (Table IIIA, Fig. 2). There was also no significant correlation of elevated CO concentration with serum AST/ALT, or clinical outcomes (Table III). These results thus suggest that ExCO-C might be a very sensitive and reversible marker for early changes, but may not be related to prolonged changes such as serum ALT and AST levels.

In conclusion, our finding in this study demonstrated that ExCO-C significantly increased immediately after reperfusion during LDLT. The increased ExCO-C may likely reflect heme breakdown in the grafted liver, as it was also associated with increases in the serum T-Bil concentration and in arterial CO-Hb concentration. While ExCO-C might be an attractive marker for certain changes reflecting graft injury

that can be monitored in a non-invasive manner, its value as a useful clinical marker is yet to be demonstrated with respect to other aspects associated with LDLT.

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