Effects of Halothane and Hypoxia on Hepatic Oxygen Metabolism in the Dog

Nobuyuki MATSUMOTO, Takao HORI, Takashi MIYAZAKI and Hiroshi NAGASAKA

Hepatic oxygen delivery and consumption were assessed in mongrel dogs receiving 2MAC of halothane combined with graded hypoxic hypoxemia (21–8% oxygen). Hepatic blood flow was measured using electromagnetic flowmetry; hepatic oxygen delivery and consumption were calculated from measured hepatic blood flow and oxygen content in hepatic arterial, portal venous and hepatic venous blood. In hypoxia-halothane group, total hepatic blood flow decreased at mild hypoxia (15% O_2) from control value, but recovered to control level at moderate hypoxia (10% O_2), then again decreased at 8% O_2. Oxygen supply to the liver was decreased with the augmentation of hypoxia in hypoxia-halothane and hypoxia-alone groups, and it was significantly lower in the hypoxia-halothane group at 15 and 12% O_2. Hepatic oxygen consumption also decreased from air control values with the increment of hypoxia, but there was no significant difference between the groups. Arterial ketone body ratio, which indicates mitochondrial energy charge level, decreased with the development of hypoxia but there was no significant difference in this ratio between the groups. These results show that halothane aggravated oxygen supply to the liver at mild to moderate hypoxia (15–12% O_2), but did not worsen it specifically at more serious hypoxia (10–8% O_2) compared with hypoxia alone. Hepatic hypoxia itself could not thus be a main cause of halothane hepatotoxicity. (Key words: halothane, hepatic blood flow, hepatotoxicity, ketone body ratio, oxygen metabolism in the liver)

(Matsumoto N, Hori T, Miyazaki T et al.: Effects of halothane and hypoxia on hepatic oxygen metabolism in the dog. J Anesth 3: 27–34, 1989)

The mechanism of halothane induced hepatotoxicity is not known at the present time. Toxic intermediate metabolites of halothane, chlorodifluoroethylene (CDE), chlorotrifluoroethane (CTE) or free radicals, have been suspected of producing hepatotoxicity[1], when phenobarbital-treated rats were exposed to halothane and hypoxia (less than 14% oxygen) simultaneously. However, hypoxia alone also produces hepatotoxicity when the inspired oxygen concentration is extremely low as 8% or less[2,3]. Furthermore, enflurane or isoflurane, which have relatively low metabolic rates, can be hepatotoxic in rats in the presence of 8% oxygen[2]. Therefore, it is difficult to define whether halothane metabolites or hypoxia is the main cause of halothane-induced hepatitis. However, mild hypoxia (14% oxygen) failed to produce hepatotoxicity by itself, while addition of halothane to such mild hypoxia can produce hepatic damage[2,4]. If hepatic hypoxia which is produced by respiratory and/or circulatory depression is the main cause of halothane hepatotoxicity, halothane with mild hypoxia should aggravate hepatic circulation and hepatic oxygen availability, and then should

Department of Anesthesiology, Saitama Medical School, Saitama, Japan

Address reprint requests to Dr. Matsumoto: Department of Anesthesiology, Saitama Medical School 38 Morohongo, Moroyama, Iruma-gun, Saitama, 350-04 Japan

bring on the imbalance of hepatic oxygen supply/uptake relationship equally to or worse than that in severe hypoxia (6–8% oxygen) which alone produces hepatotoxicity. This study examined: 1) how different the oxygen metabolism in the liver would be between mild hypoxia (12–15% oxygen) and severe hypoxia (6–8% oxygen); and 2) whether halothane with mild hypoxia could aggravate the hepatic oxygen metabolism compared with severe hypoxia alone in artificially ventilated dogs.

Methods

16 mongrel dogs, weighing 6.2–11.8 kg, were studied. Anesthesia was induced with ketamine 50–100 mg i.m. and thiamylal 20 $mg \cdot kg^{-1}$ i.v., and maintained with a supplemental dose (4–5 $mg \cdot kg^{-1}$) of thiamylal i.v. when required later in the investigation. Following trans-tracheal intubation, ventilation was maintained with a respirator at a rate of $15 \cdot min^{-1}$. Ventilation was adjusted according to arterial Pa_{CO_2} in order to achieve 30–40 mmHg. Pancuronium 0.2 $mg \cdot kg^{-1}$ was administered to induce neuromuscular blockade and additional dose was given when it was required.

Following laparotomy, the hepatic artery was dissected free adventitiae, but the nerve plexus was left intact at a site 1–2 cm from the coeliac axis. The gastroduodenal artery was ligated close to the junction between the proper hepatic arteries and the common hepatic artery, in order to allow proper hepatic arterial blood flow measurement. A 3 or 4 mm electromagnetic flowmeter probe was applied on the common hepatic artery, and a 6 or 7 mm electromagnetic flowmeter probe was applied on the portal vein for the blood flow measurement by electromagnetic flowmeters (Nihon Koden, Tokyo, MFV-1200). Catheters for collection of blood samples were placed in the abdominal aorta, portal vein, and left hepatic vein. A Swan-Ganz catheter was introduced to the pulmonary artery from the right external jugular vein through the right ventricle, and cardiac output was measured by thermodilution with a cardiac output computer (Nihon Koden, Tokyo, AH and EQ-611V). Systemic arterial pressure was monitered through the catheter placed in the abdominal aorta continuously, and core temperature was maintained at 37–38°C by means of a heating lamp.

After preparation of the animal had been completed, an interval of 1–2 hours was allowed to elapse in order to establish a circulatory steady state. Animals were divided into two groups (group B and group H). Group B (or hypoxia-alone group) consisted of eight dogs that served as control group and were given a small dose of thiamylal (4–5 $mg \cdot kg^{-1}$), but were not given halothane as well as any other inhalational anesthetics during the experiment. In group H, eight dogs received 2 MAC (approximately 1.8%) of halothane during every course of the experiment. After the recovery period, inspired oxygen concentration was gradually reduced from 21% to 15%, 12%, 10%, 8% and 6% which was achieved by mixing air and nitrogen and maintained for 30 min. at each inspired oxygen concentration in group B. In group H, 2 MAC of halothane was continuously inhaled, and inspired oxygen concentration was changed in the same fashion as in group B.

In both groups, MAP, CO, HABF, PVBF, blood gas tension, Hb, and oxygen saturation in arterial, portal venous and hepatic venous blood were measured before hypoxia and at each period of hypoxia. $\dot{D}hep_{O_2}$ and $\dot{V}hep_{O_2}$ were calculated by the following formulae:

$$\dot{D}hep_{O_2} = Cao_2 \times HABF + Cpv_{O_2} \times PVBF$$

$$\begin{aligned}\dot{V}hep_{O_2} &= (Cao_2 - Chv_{O_2}) \times HABF + (Cpv_{O_2} - Chv_{O_2}) \times PVBF \\ &= Cao_2 \times HABF + Cpv_{O_2} \times PVBF - Chv_{O_2} \times THBF\end{aligned}$$

[5,6]

Arterial ketone body ratio (acetoacetate/β-hydroxybutylate, KBR) was measured before operative procedure, before hypoxia, and immediately after 30 min. of inhalation of 12% and 8% oxygen as an index of the energy charge in the liver, i.e. as an index of redox potential of the liver mitochondria during hepatic hypoxia[7,8].

The data presented were the mean values

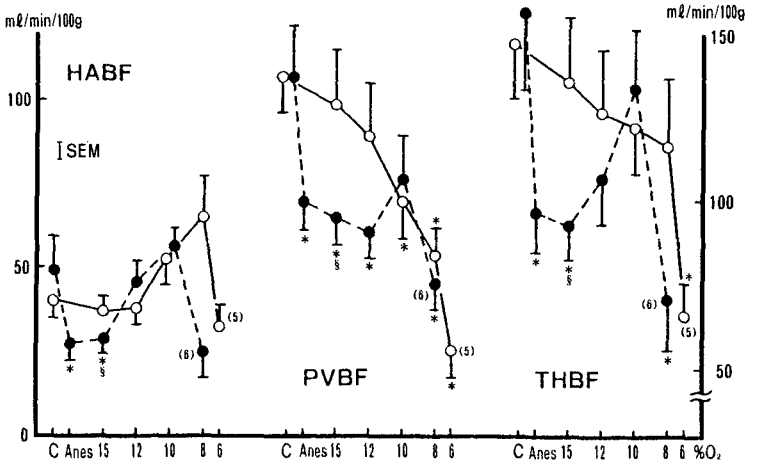

Fig. 1. Changes of HABF (hepatic arterial blood flow), PVBF (portal venous blood flow) and THBF (total hepatic blood flow) by graded hypoxia concomitant with or without halothane. o: hypoxia alone (+thiamylal) ●: hypoxia + halothane, *P <0.01 vs. control, §P <0.05 vs. hypoxia alone; n=8 in each group except the number shown in ( ).

± standard errors of the means. The data among the groups were compared using unpaired t test and the data within each group were compared using paired t test. Differences were declared significant with $P<0.01$ for the values within each group, and with $P<0.05$ for the values between both groups.

Results

MAP, CO, HABF, PVBF, THBF, $\dot{D}hep_{O_2}$, $\dot{V}hep_{O_2}$, $\dot{D}hep_{O_2}/\dot{V}hep_{O_2}$, Chv_{O_2} and ketone body ratio were not significantly different between group B and group H before experiment (table). Table also shows data of both groups during hypoxic experimental procedure.

Pa_{O_2} values were not significantly different between both groups at each inspired oxygen concentration. In group B, MAP gradually increased as the increment of hypoxia from 21% to 10% oxygen, and was significantly higher at 12% and 10% oxygen, then decreased, compared with control values at 21% oxygen. In group H, MAP significantly decreased from control values at 21%, 15%, 12%, 10% and 8% oxygen concomitant with halothane. Therefore, MAP was significantly lower in group H at 15%, 12%, 10% and 8% oxygen compared with group B. CO did not change by the increment of hypoxia in group B, but in group H it decreased at 21%, 15% and 12% oxygen by administration of halothane, then recovered toward the control level just before the abrupt decrease at 8% oxygen. CO was significantly lower at 15% and 8% oxygen in group H compared with group B (table).

Changes of HABF, PVBF and THBF are shown in table and figure 1. In group B, HABF did not change at 15% and 12% oxygen from control value, and increased at 10% and 8% oxygen, then decreased at 6% oxygen. However, these changes were not significant compared with control value of 21% oxygen. In group H, HABF decreased during halothane anesthesia concomitant with 21% and 15% oxygen, but recovered near the control value at 12% and 10% oxygen before decreasing at 8% oxygen. Thereby HABF was significantly lower only at 15% oxygen in group H compared with group B. PVBF decreased by the increment of hypoxia from 21% to 6% oxygen, and this decrease was significant at 10%, 8% and 6% oxygen in group B. In group H, PVBF significantly decreased during halothane anesthesia with 21%, 15%, 12% and 8% oxygen compared with control value, and these decreases were significant only at 15% oxygen compared with group B. THBF gradually decreased with the augmentation of hypoxia and significantly decreased at 6% oxygen in group B. In group H, THBF significantly decreased during halothane anesthesia with 21% and 15% oxygen compared with the control value, but recovered to near the control level when inspired oxygen concentration was decreased to 12% and 10%, then suddenly decreased at

Table 1. Comparable data for each group (X ± SEM)

	FI_{O_2}						
	0.21	Anes (0.21)	0.15	0.12	0.10	0.08	0.06
Group B							
MAP	128±9		139±11	144±10*	153±9*	116±10	79±14
CO	251±23		250±27	229±31	263±35	286±26	147±43
HABF	40.5±5.0		37.5±4.7	37.9±5.4	51.7±7.5	63.5±13.1	31.7±4.8
PVBF	105.8±13.5		97.8±16.1	88.2±16.8	69.5±11.0*	52.5±7.6*	25.4±7.8*
THBF	146.4±16.0		135.3±18.5	126.1±18.5	121.1±14.4	116.0±18.4	65.6±8.1*
$\dot{D}hep_{O_2}$	18.3±1.8		14.7±2.5*	9.8±1.6*	4.8±0.8*	1.8±0.4*	0.7±0.6*
$\dot{V}hep_{O_2}$	5.2±0.4		3.8±0.3	3.3±0.4*	2.9±0.4*	1.6±0.4*	0.6±0.6*
$\dot{D}/\dot{V}$	3.9±0.5		3.7±0.5	3.0±0.5*	1.7±0.2*	1.2±0.1*	1.1±0.1
Chv_{O_2}	9.31±1.24		7.90±1.09*	5.03±1.18*	1.58±0.45*	0.23±0.05*	0.14±0.04
Pa_{O_2}	84±4		53±3*	36±2*	25±2*	20±2*	
Group H							
MAP	148±6	93±4*	93±5*§	104±11*§	97±10*§	52±6*§	
CO	278±26	184±19*	180±15*§	214±23*	263±30	134±34§	
HABF	49.7±9.4	26.9±4.6*	28.1±4.6*§	45.6±6.4	55.4±5.9	23.8±8.5	
PVBF	106.3±14.9	69.1±9.3*	63.8±7.9*§	60.2±8.3*	76.6±13.3	45.2±7.5*	
THBF	156.0±23.0	96.0±12.8*	91.9±11.1*§	105.8±14.0	133.2±18.2	70.2±15.3*	
$\dot{D}hep_{O_2}$	21.2±3.8	11.6±1.8*	8.7±1.2*§	5.4±0.6*§	3.6±0.4*	2.2±0.5*	
$\dot{V}hep_{O_2}$	5.6±0.8	3.7±0.6*	3.3±0.7*	2.9±0.4*	2.7±0.3*	1.8±0.2	
$\dot{D}/\dot{V}$	3.5±0.5	2.8±0.3	2.2±0.3§	2.0±0.3*	1.3±0.1*	1.2±0.1	
Chv_{O_2}	9.48±1.25	8.03±1.15	6.10±1.12*§	2.52±0.54*§	0.65±0.3*§	0.36±0.02	
Pa_{O_2}	77±3	79±2	50±2*	33±3*	25±2*	25±1*	

* $P < 0.01$, significant difference vs control for each group (FI_{O_2} 0.21), and §$P < 0.05$, vs Group B values at same FI_{O_2}; Anes = anesthesia; MAP = mean arterial pressure (mmHg); CO = cardiac output ($ml \cdot min^{-1} \cdot kg^{-1}$); HABF = hepatic arterial blood flow ($ml \cdot min^{-1} \cdot 100g^{-1}$); PVBF = portal venous blood flow ($ml \cdot min^{-1} \cdot 100g^{-1}$); THBF = total hepatic blood flow ($ml \cdot min^{-1} \cdot 100g^{-1}$); $\dot{D}hep_{O_2}$ = hepatic oxygen delivery ($ml\ O_2 \cdot min^{-1} \cdot 100g^{-1}$); $\dot{V}hep_{O_2}$ = hepatic oxygen consumption ($ml\ O_2 \cdot min^{-1} \cdot 100g^{-1}$); $\dot{D}/\dot{V}$ = hepatic oxygen delivery/consumption; Chv_{O_2} = hepatic venous oxygen content (ml O_2/dl); Pa_{O_2} = arterial oxygen tension (mmHg); Group B = N_2–O_2–thiamylal; Group H = N_2–O_2–halothane.

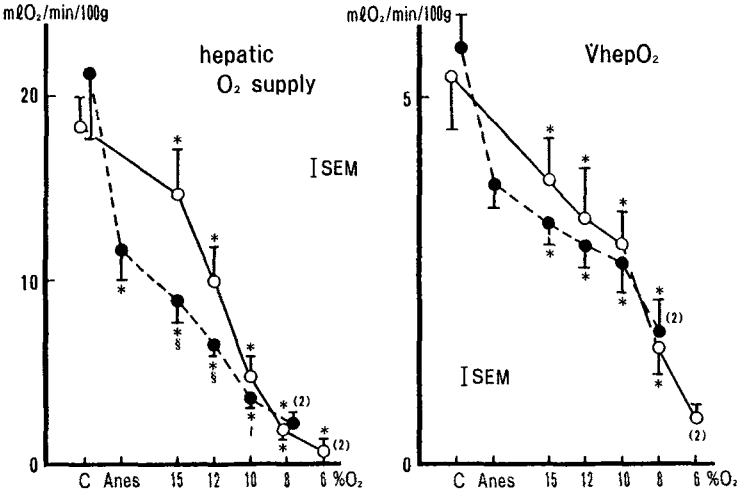

Fig. 2. Changes of hepatic oxygen supply and $\dot{V}hep_{O_2}$ (hepatic oxygen consumption) by graded hypoxia concomitant with or without halothane. o: hypoxia alone (+thiamylal), ●: hypoxia + halothane, *P <0.01 vs. control, §P <0.05 vs. hypoxia alone, +P <0.05 vs. hypoxia alone at 8% oxygen; n=8 in each group except the number shown in ( ).

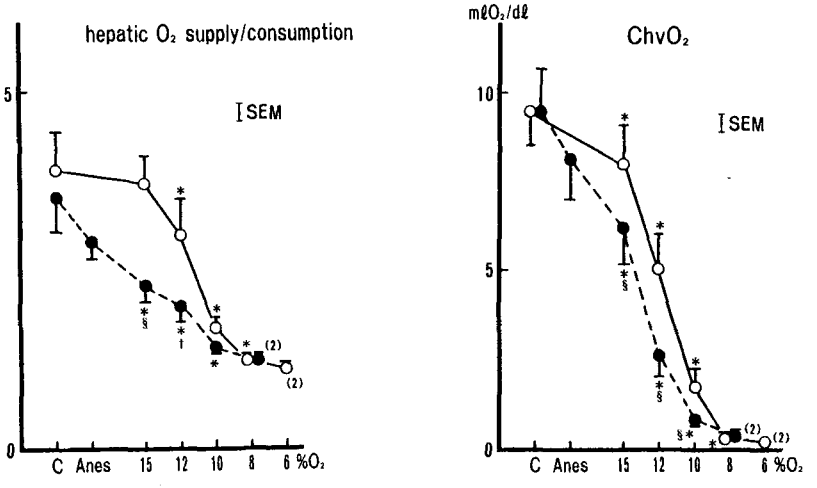

Fig. 3. Changes of hepatic oxygen supply/consumption and Chv_{O_2} (hepatic venous oxygen content) by graded hypoxia concomitant with or without halothane. o: hypoxia alone (+thiamylal), ●: hypoxia + halothane, *P <0.01 vs. control, §P <0.05 vs. hypoxia alone, +P <0.05 vs. hypoxia alone at 8% oxygen; n=8 in each group except the number shown in ( ).

8% oxygen. Only at 15% oxygen, THBF was significantly lower in group H compared with group B.

Changes of $\dot{D}hep_{O_2}$ and $\dot{V}hep_{O_2}$ were shown in table and figure 2. $\dot{D}hep_{O_2}$ gradually decreased with the increment of hypoxia in both groups, and was significantly lower at 15% and 12% oxygen in group H compared with group B. However, value of group H at 10% oxygen was significantly higher than that of group B at 8% oxygen. $\dot{V}hep_{O_2}$ also gradually decreased as hypoxia advanced in both groups, and there was no significant difference between both groups. As shown in figure 3, $\dot{D}hep_{O_2}/\dot{V}hep_{O_2}$ significantly decreased at 12%, 10% and 8% oxygen in group B, and at 15%, 12% and 10% oxygen in group H, compared with the control values. This ratio is significantly lower only at 15% in group H compared with group B as shown by mark §, but the value of group H at 12% oxygen was significantly higher than that of 8% oxygen in group B as shown by mark †. Changes of $\dot{D}hep_{O_2}/\dot{V}hep_{O_2}$ were well reflected by the changes of Chv_{O_2} (fig. 3). Chv_{O_2} in group H was significnatly lower

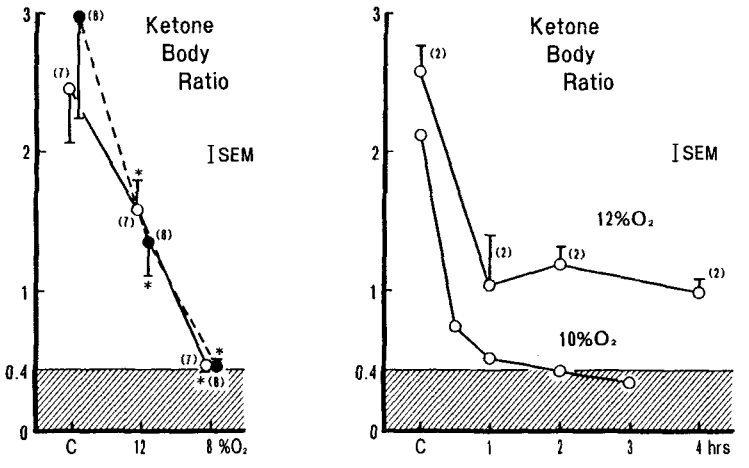

Fig. 4. Changes of arterial ketone body ratio by graded hypoxia concomitant with or without halothane; and prolonged hypoxia. o: hypoxia alone (+thiamylal), ●: hypoxia + halothane, $*P < 0.01$ vs. control; numbers are shown in ( ) except 10% O_2 of prolonged hypoxia.

at 15%, 12% and 10% oxygen than that of group B as shown by mark §.

Arterial ketone body ratio was higher than 2.0 at control state in both groups, and significantly decreased to 1.56 ± 0.28 at 12% oxygen and 0.42 ± 0.04 at 8% oxygen in group B, and to 1.33 ± 0.20 at 12% oxygen and 0.39 ± 0.03 at 8% oxygen in group H. There was no significant difference between both groups at any oxygen concentration (fig. 4). The changes of ketone body ratio when the inspired oxgen concentration was maintained at 12% and 10% for more than three hours, are also shown in figure 4.

Discussion

Hepatic failure which occasionally developes following halothane anesthesia, has been postulated to relate to hepatic ischemia due to anesthesia-induced decrease in hepatic arterial and/or portal venous blood flows.

In this study, HABF was rather well maintained in graded hypoxia in both groups. On the other hand, PVBF significantly decreasd in hypoxia-halothane group with 15%, 12% and 8% oxygen, whereas in hypoxia-alone group it remained unchanged with 15% and 12% oxygen and decreased consistently with 10%, 8% and 6% oxygen. Thus in hypoxia-alone group, THBF did not change significantly until the concentration of inhaled oxygen reached 8% or less. In halothane group, THBF decreased significantly with 21% and 15% oxygen, and remained almost the same level as in hypoxia-alone group with 12%, 10% and 8% oxygen.

It is stated that there exists a physiological principle of "reciprocity of total hepatic blood flow" between the hepatic artery and portal vein[9]. The higher proportion of HABF in THBF induced by increased hypoxia, could be a physiological mechanism for adaptation to hypoxia which sustains the supply of oxygen-rich blood to the hepatic cells. Halothane seems to disturb the reciprocity of total hepatic blood flow at normoxia and mild hypoxia i.e. 15% oxygen in the present study, while the compensatory mechanism during more severe hypoxia could overwhelm the disturbing effect of halothane on this reciprocity.

$\dot{D}hep_{O_2}$ calculated as the sum of hepatic arterial and portal venous oxygen delivery, decreased with hypoxia in two groups. In hypoxia-halothane group, a significantly lowered $\dot{D}hep_{O_2}$ was found at a mild hypoxia of 15% and 12% oxygen compared with hypoxia-alone group, mainly because of significantly low THBF resulting from circulatory depression by halothane. However, at more severe hypoxia with less than 10% oxygen, the difference of $\dot{D}hep_{O_2}$ between the two groups became insignificant.

$\dot{V}hep_{O_2}$ consistently decreased as hypoxia became more severe, and no significant difference was found in $\dot{V}hep_{O_2}$ between the two groups. These results indicated that

halothane did not inhibit oxygen uptake in the liver, because $\dot{V}hep_{O_2}$ was almost equivalent in two groups.

Hepatic oxygen supply/consumption ratio in hypoxia-alone group was maintained with 15% oxygen, lowered significantly with 12% oxygen, and further markedly lowered with 10%, 8% and 6% oxygen. In hypoxia-halothane group, hepatic oxygen supply/consumption ratio was significantly lowered with 15% oxygen compared with that of hypoxia-alone group. However, no significant difference was found between the two groups at more severe hypoxia with 12%, 10% and 8% oxygen. Furthermore, the ratio of hepatic oxygen supply and consumption at 10–12% oxygen in hypoxia-halothane group was not worse than that of hypoxia-alone group at 8% oxygen, even at which concentration it is not always possible to produce hepatotoxicity by hypoxia per se[2].

Therefore, hepatic hypoxia which is brought on by low concentration of inspired oxygen and/or decreased hepatic blood flow during halothane anesthesia would not produce hepatotoxicity by itself at more than 10% inspired oxygen concentration.

The vital function of the cells, essential to survival, depends on the continuous supply of energy. The mitochondrial energy production, crucial for the well-being of a cell, can be sustained with a continuous supply of adequate oxygen and metabolic substrates to the cells. It is reported that a hepatic energy charge (ATP + 1/2ADP/ATP + ADP + AMP) is directly related to the mitochondrial free $NAD^+/NADH$ ratio, which is in equilibrium with the hepatic tissue ketone body ratio (the ratio of acetoacetate to β-hydroxybutyrate). Since acetoacetate and β-hydroxybutyrate, both exclusively produced in the liver, can freely penetrate the cell membrane, the ketone body ratio measured in arterial blood reflects that in hepatic mitochondria[7,8].

In this study, the ketone body ratio in arterial blood in dogs before hypoxia was above 2.0, decreased to around 1.5 with 12% oxygen, and to 0.4 with 8% oxygen. No significant difference was found between the two groups. It was also found that the ketone body ratio remained above 1.0 with inhalation of 12% oxygen for more than 3 hours, whereas it was lowered below 0.4 when 10% oxygen was inspired for 3 hours.

According to Ozawa et al.[8] the clinical courses were uneventful in the first 2–4 days following surgery, when their blood ketone body ratio remained over 0.7. In the patients whose blood ketone body ratio was below critical level, that is, 0.4–0.25, the incidence of multiple organ system failure was very high. Thus, hypoxia per se induced by less than 10% inspired oxygen could be deleterious to the liver. Halothane decreased hepatic oxygen supply/consumption ratio compared with hypoxia alone at a mild hypoxia with 15% inspired oxygen. This relationship between oxygen supply and uptake in the liver was exaggeratedly represented by the change of hepatic venous oxygen content. However, the blood ketone body ratio was above 1.0 with 12% oxygen, and no significant difference was found between hypoxia-halothane and hypoxia-alone groups.

It was reported that hypoxia per se produced hepatotoxicity only when the inspired oxygen concentration reached 8% or less[2,3]. It was also stated that halothane could produce hepatotoxicity with 14% oxygen in enzyme induced rats[2,4] as well as with 21% oxygen in guinea pigs. In these conditions hepatic oxygen supply decreased to 1/2 of control level in rats and 1/3 in guinea pigs[10]. These were corresponding to 15–12% oxygen in our dog models. In the present study, hepatic oxygen supply at 10% oxygen and supply/consumption ratio at 12% oxygen in hypoxia-halothane group were both maintained better than those in hypoxia-alone group at 8% oxygen.

From these results, it was concluded that the main cause of halothane-induced hepatotoxicity might not be hepatic hypoxia per se produced by the disturbance of hepatic circulation due to halothane anesthesia, even though hepatic hypoxia contributed to halothane-induced hepatotoxicity.

(Received Apr. 27, 1988, accepted for publication Oct. 21, 1988)

References

1. Cousins MJ, Sharp JH, Gourlay GK, Adams JF, Haynes WDG, Whitehead R: Hepatotoxicity and halothane metabolism in an animal model with application for human toxicity. Anesth Intensive Care 7:9–24, 1979
2. Van Dyke RA: Hepatic centrilobular necrosis in rats after exposure to halothane, enflurane, or isoflurane. Anesth Analg 61:812–819, 1982
3. Shingu K, Egar EI, Johnson BH: Hypoxia may be more important than reductive metabolism in halothane-induced hepatic injury. Anesth Analg 61:824–827, 1982
4. Jee RC, Sipes IG, Brown BR: Factors influencing halothane hepatotoxicity in the rat hypoxic model. Toxicol Appl Pharmacol 52:267–277, 1980
5. Hughes RL, Mathie RT, Campbell D, Fitch W: Effect of hypercarbia on hepatic blood flow and oxygen consumption in the greyhound. Br J Anaesth 51:289–296, 1979
6. Irestedt L, Andreen M: Effects of neurolept anaesthesia (NLA) on haemodynamics and oxygen consumption in the dog with special reference to the liver and preportal tissues. Acta Anaesthesiol Scand 23:1–12, 1979
7. Ukikusa M, Ozawa K, Shimahara Y, Asano M, Nakatani T, Tobe T: Changes in blood ketone body ratio. Their significance after major hepatic resection. Arch Surg 116:781–785, 1981
8. Ozawa K, Aoyama H, Yasuda K, Shimahara Y, Nakatani T, Tanaka J, Yamamoto M, Kamiyama Y, Tobe T: Metabolic abnormalities associated with postoperative organ failure. A redox theory. Arch Surg 118:1245–1251, 1983
9. Richardson PDI, Withrington PG: Liver blood flow. I. Intrinsic and nervous control of liver blood flow. Gastroenterology 81:159–173, 1981
10. Lunam CA, Cousins MJ, Hall P de la M: Guinea-pig model of halothane-associated hepatotoxicity in the absence of enzyme induction and hypoxia. J Pharmacol Exp Ther 232:802–809, 1985