PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

WJG, 1999 February; 5(1):28-30
World Journal of Gastroenterology
 ISSN 1007-9327

Experimental research on phospholipids variation of halothane on liver mitochondria

SUI Bo[1], ZHANG Guang-Ming[2], YU Wei-Feng[3], WANG Xue-Min[4], MA Yong-De[1] and LIU Shu-Xiao[5]

Subject headings halothane; sevoflurane; liver mitochondria; HPLC; hepatotoxicity

Abstract

AIM To study the pathogenesis of hepatotoxicity of halothane.

METHODS The effect of different concentration of halothane and sevoflurane on mitochondrial membrane phospholipids composition of rat liver were analyzed using high performance liquid chromatography (HPLC) technology.

RESULTS Halothane at low concentration could degrade mitochondr ial membrane major phospholipids and increase lysophosphatidylcholine.

CONCLUSION The pathogenesis of halothane hepatotoxicity was the phospholipids variation on liver mitochondria.

[1]Department of Anesthesia, General Hospital of Jianan Commanding Area, Jinan 250031,Shandong Province,China
[2]School of Pharmacy, the Second Military Medical University, Shanghai 200433
[3]Department of Anesthesia, East Liver Surgical Hospital, the Second Military Medical University, Shanghai 200433
[4]Department of Biochemistry, Basic Section, the Second Military Medical University, Shanghai 200433
[5]Department of Anesthesia, Changhai Hospital, the Second Military Medical University, Shanghai 200433
Dr. SUI Bo, male, born in 1958 in Shandong Province, graduated from the Second Military Medical University as a postgraduate in 1995, now an attending aesthetist, East Hepatobilliary Surgery Hospital, the Second Military Medical University, Shanghai 200433, having 12 papers published.
***Project supported by the National Natural Science Foundation of China, No.94300126.**
Correspondence to: Dr. SUI Bo, Department of Anesthesia, General Hospital of Jinan Commanding Area, Jinan 250031,China
Tel. +86·531·5946536
Received 1998-08-27

INTRODUCTION

The effect of traditionally inhalational anesthetic halothane and new drug sevof lurane on mitochondrial membrane is reported below in an attempt to study the pa thogenesis of halothane hepatotoxicity.

MATERIALS AND METHODS

Preparation of liver mitochondria and pretreatment of specimen

According to modified Estabook's velocity gradient method[1], the mitochondria of male rat weighing 150g-200g was separated. Seventy mmol sucrose and 220mmol bovine serum albumin were used as isolation medium. Albumin was assayed by biuret reaction. The mitochondria concentration was adjusted to 10g/L-30g/L. Phospholipids except for ganglioside and acetal phospholipid were extracted using improved Higgins' method[2]. The mito chondrial suspension was mixed well with the extraction solvent (1:10, V/V), and stood for 15min. The albumin was removed by centrifugation. $CaCl_2$ 0.05mL/L was added to the supernatant, and stood for centrifugation (3000 r/ min). The lower layer was evaporated to dryness under nitrogen at 40℃ - 50℃. After added with diluent accurately to the residue, the solution was sealed to protect from light and stored at -20℃ for HPLC analysis. The whole procedure was carried out at 4℃ in the airtight ice-bath.

Preparation of solvent

The standard control phospholipids of phosphatidylethanolamine (PE), phosphatidy linositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), cardiolipin (CL), sphingomyclin (SPH) and lysophosphatidylcholine (LPC) were purchased from Sigma Co.

Extracting solution: chloroform:methanol:hydrochloric acid (2:1:0.01,V/V/V).

Moving phase: n-hexane:isopropanol:ethanol: potassium dihydrogen phosphate (25 mnol/ L) : glacial acetic acid (370 : 485 : 100 : 562 : 0.1 V/V/V/V). The solution was evenly mixed and stood overnight for separating phosphoric acid crystal. After ultrafiltration and deoxygenation the supernatant was used as moving phase.

Standard solution: the standard control phospholipids were dissolved in the mixture of n-hexane: isopropanol (6 : 8, V/V). The concentration was 2g/L.

HPLC analytical method

ISUZU LC-6A liquid chromatograph, ISUZU Shim-Pack CLC-SIC column (6 mm × 15 cm), Guarc PAKTM prepared column were used. The detection was performed at 206nm. After reaction of low concentration and high concentration of halothane or sevoflurane with mitochondrial membrane phospholipids, 10ml reaction solution was taken out for repeated injection[3]. Each sample was repeated for 8 times, and linear velocity (mm/min) was recorded. Qualitative analysis was made by identification of the retention time with standard control samples. The eluting sequence referred to Patton sequence[4]. Quantitative ana lysis was made by calculating the peak area and the relative content of phospholipids was expressed by the ratio between peak area and albumin.

RESULTS

Qualitative analysis

By comparing the HPLC chromatograph peak of phospholipids affected by halothane at low and high concentration with that of the normal liver mitochondria phospholipids, it could be seen that the main phospholipid peak decreased to some degree and LPC peak increased, especially when at high concentration. Sevoflurane at low concentration had no influence on phospholipid peak, but at high concentration it could decrease the main phospholipid peak and increase the LPC peak. However, the effect was not so obvious as that caused by halothane.

Quantitative analysis

The change of liver mitochondrial phospholipids caused by halothane and sevoflur ance is shown in Table 1. Halothane at both high and low concentration could decrease the main liver mitochondrial phospholipids and increase LPC significantly. The change of phospholipids had no significant difference between sevofurane at low concentration and the control while at high concentration the difference was marked. At high concentration, the change of phospholipids in liver mitochondria caused by halothane was much more obvious than that caused by sevoflurane.

Time-phase change

The effect on the liver mitochondrial phospholipid principle started and went up rapidly as soon as halothane contacted with mitochondria and reached the peak at 4h. At low concentration it could recover to the level of the control group at 6h-8h while at high concentration it could not even within 24h. In each phase there had no significant difference between low concentration of sevoflurane and the control. At high concentration the effect caused by sevoflurane reached the peak at 4h and recovered to the control level at 8 h - 12 h. The time-phase change on LPC by halothane and sevoflurane at high concentration is demonstrated in Figure 1.

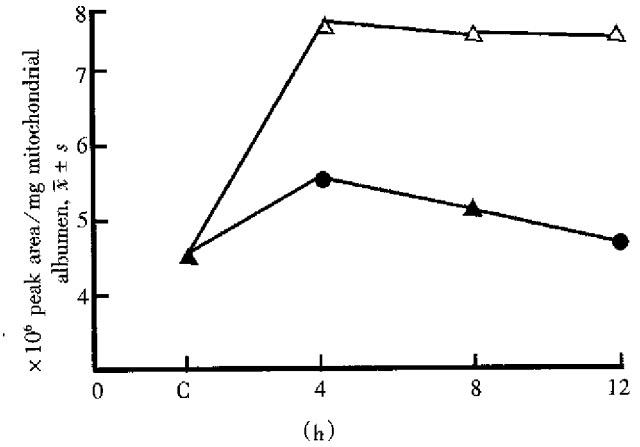

Figure 1 The time phase change on LPC affected by ha lothane and sevoflurane in high concentration.

Table 1 The variation of phospholipid in liver mitochondrial membrane affected by halothane and sevoflurane (phospholipid unit: $\times 10^9$ peak area/g mitochondrial albumin $\bar{x} \pm s$)

Group	PE	PI	PS	CL	PC	SPH	LPC
Control	1.48±0.26	1.16±0.19	0.84±0.09	1.02±0.11	2.93±0.28	3.98±0.59	4.54±0.42
At low concentration							
Sevoflurane	1.37±0.19	1.08±0.09	0.78±0.07	0.95±0.11	2.72±0.17	3.76±0.33	4.89±0.33
	(-7.62%)	(-7.12%)	(-6.93%)	(-6.55%)	(-7.19%)	(-5.52%)	(+7.81%)
Halothane	1.19±0.12	0.96±0.06	0.70±0.80	0.85±0.16	2.50±0.26	3.37±0.29	5.90±0.12
	(19.60%)[a,b]	(-17.24%)[a,b]	(-16.94%)[a,b]	(-16.25%)	(-14.38%)[a,b]	(15.43%)[a,b]	(+30.1%)[a,b]
At high concentration							
Sevoflurane	1.30±0.12[a]	1.02±0.04a	0.63±0.04[a]	0.89±0.70[a]	0.64±0.16[a]	3.57±0.22[a]	5.69±0.32[c]
	(11.89%)	(12.02%)	(-11.26%)	(-12.55%)	(-10.36%)	(-10.40%)	(+25.35%)
Halothane	1.06±0.09[c]	0.81±0.07[c]	0.78±0.09[c]	0.75±0.09[c]	2.09±0.16[c]	2.95±0.24[c]	7.81±0.67[c]
	(-28.38%)[b]	(-2.41%)[b]	(-25.24%)[b]	(-26.59%)[b]	(-2867%)[b]	(-26.61%)[b]	(+41.87%)[b]

[a]$P<0.05$, [c]$P<0.01$ *vs* control; [b]$P<0.05$ *vs* sevoflurane.

DISCUSSION

This study indicated that halothane at low concentration could degrade mitochondrial membrane major phospholipids and increase LPC, at high concentration it could damage mitochondrial membrane irreversibly. Although sevothane had action on mitochondria, the effect was reversible. Probably due to its molecular stru cture halothane soluble in liver mitochondria easily and destroy phospholipids obviously. Halothane had the similar result in the study on the inhalational anesthetic effect on liver mitochondria intimal fluidity[5].

Phospholipase A (PLA1, PLA2) is universal in liver membrane. Characterized by intramembranous mode of action, PLA2 has a high activity in mitochondria and the highest catalytic speed toward PE (twice that of PC, ten times of CL). PLA2 could be excited by Ca equilibration of liver cell caused by poison *in vivo* and *in vitro*. The phospholipid structure variation greatly influences biomembrane function and physical property including membrane congugase and receptor kinetics[6]. Some other studies showed that lipid variation such as mitochondrial phospholipids degradation and lipid peroxidation is an important original cause of liver cell damage. Destruction of the integration of mitochondria is the result of mutual function of the above-mentioned two mechanisms while degradation of membrane phospholipid caused by activation of mitochondria probably plays a more important role in the early damage of overall function of liver cells[7].

Besides hypoxia and low volume of blood flow the study also showed that mitochondrial phospholipids variation in the unorganized test is the main factor of halothane hepatotoxicity. Inhabition of PLA_2 activity and antilipid peroxidation may be the important measure of antihalothane hepatotoxicity[8].

REFERENCES

1 Estabrook IH. Oxidative and phosphorylation. Methods in enzymology. Vol 10. New York: Academic Press Inc. 1997:45;10003-10012
2 Higgins JA. Separation analysis of membrane lipid components, In: Findlay JBC, ed: Biological membrane: a practical approach. Washington DC: IRC Press, 1987:103-107
3 Yu WF, Liao MY. Effect of sevoflurane, anflurane, isoflurane and halothane on mice isolated liver mitochondrial respiration function.*Chin J Anesthesiol*, 1986;16:121-123
4 Patton G, Fasulo J, Robins S. Separation of phospholipids and individual molecular species of phospholipids by high performance liquid chromatography. *J Lipid Res*,1982;23:190-194
5 Sui B, Yu WF, Liao MY. The effect of inhalational anesthetic on fluxion property of liver mitochondria. *Chin J Anesthesiol*, 1995;15:561-562
6 Liu MS, Karg GF. Activation of phospholipase A1 and A2 in heart, liver and blood during endotoxin shock. *J Surgical Res*, 1988;45:472-477
7 Yeagle PL. Lipid regulation of cell membrane structure and function. *FASEB J*,1989;3:1833-1838
8 Yu WF, Wang JY, Liu SY. The hepatotoxicity of halothane and sevoflurane on primary culture mice liver cell. *Chin J Anesthesiol*, 1993;13:243-246

Edited by MA Jing-Yun