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Effects of Halothane Anesthesia on the Fetal Rabbit in Utero

**Seung Hoon Choi[1], M.D., Seok Joo Han[1], M.D., Myungsoo Kim[2], M.D.
Jongsei Park[2], M.D., Jin Su Kim[3], M.D., Duk Sun Ahn[4], M.D.
Chul Lee[5], M.D., Yong Won Park[6], M.D., Chan Ho Song[6], M.D.
and Eui Ho Hwang[1], M.D.**

Department of Surgery[1], Anesthesiology[3], Physiology[4], Pediatrics[5], Obstetrics & Gynecology[6], Yonsei University College of Medicine, Seoul, Korea and Doping Control Center[2], KIST, Seoul, Korea

=국문초록=

전신마취제 할로탄이 자궁내의 가토에 미치는 영향

연세대학교 의과대학 외과[1], 마취과[3], 생리학[4], 소아과[5], 산부인과[6]
한국과학기술연구원 도핑콘트롤센터[2]

**최승훈[1] · 한석주[1] · 김명수[2] · 박종세[2]
김 진 수[3] · 안 덕 선[4] · 이 철[5]
박 용 원[6] · 송 찬 호[6] · 황 의 호[1]**

가토에서 산모에게 할로탄으로 마취를 하였을 때 태아수술중 태아가토의 혈중에 미치는 영향을 검사하였다. 18마리의 임신중인 뉴질란드 가토를 세 군으로 나누어 60분간 각각 0.25%, 0.5%, 0.75%의 할로탄으로 마취하였다. 마취중 호기와 흡기의 할로탄농도 호기중의 이산화탄소농도를 SARAcap 기계를 사용하여 측정하였다. 산모가토는 대퇴동맥에 넣은 도관을 통하여 10분마다 혈액을 채취하였고 태아가토의 혈액은 제대정맥에서 10분마다 채취하였다. 채취한 혈액은 원심분리후 mass spectrometer를 이용하여 혈중 할로탄농도를 측정하였다. 산모가토의 혈중 할로탄농도는 마취시의 할로탄농도와 비례하였으나 태아와 산모의 혈중 할로탄농도의 비율은 통계적으로 차이를 보이지 않았다. 이것은 태아수술시 이용한 할로탄이 태반을 통하여 직접 태아에게 전달됨을 의미하여 태아에서도 마취를 일으키는 농도에 이른다는 것을 보여주었다. 할로탄은 현재 태아수술시 가장 많이 사용하는 마취제로 자궁이완과 사용이 쉽다는 이점이 있으나 태아에서도 높은 혈중농도를 나타내기 때문에 사용에 있어서 주의가 필요함을 알 수 있었다.

Key Words: Halothane, Fetal surgery

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INTRODUCTION

Halothane is one of the most popular anesthetic agents for fetal surgery because it can easily be administered to the mother and it improves surgical exposure by relaxing the uterus. It also has a tocolysis effect on the uterus during surgery. But recent reports[1,3,8,10] showed maternal halothane anesthesia has detrimental effects on the fetus by reducing fetal cerebral blood blow, fetal cardiac output and shunting of blood away from the placenta. The effect of maternal halothane administration during fetal surgery on fetal blood has not been rigorously studied. Maternal safety is an overriding issue for fetal surgery. We tried to demonstrate the maternal fetal correlation of halothane in the rabbit.

The aim of the this study was to assess the effect of maternal halothane administration on fetal blood and to determine the optimum concentration of halothane during fetal surgery.

MATERIALS AND METHODS

1) Animal preparation

Eighteen New Zealand White rabbits with time-dated gestations of 23 to 25 days were fasted 24 hours. Anesthesia was induced in all animals with intravenous administration of ketamine 10 to 20 mg/kg. The tracheostomy was preformed. And the animal was ventilated with halothane and oxygen to maintain their $PaCO_2$ in the physiologic range of 30 to 40 mmHg. Right femoral arterial catheter was placed to collect blood during the operation. The animals were divided into three groups, each group consisted of 6 rabbits and they were received 0.25%, 0.5% or 0.75% halothane respectively. SARAcap A.G. was placed between endotracheal tube and anesthetic tube line, inspired and expired halothane and expired PCO_2 were monitored every 10 minutes.

2) Procedure

After a midline laparotomy the fetus was exposed through a longitudinal hysterotomy incision. Maternal blood was collected via femoral arterial catheter and the fetal blood was collected by sacrificing a fetus every 10 minutes. Fetal blood was obtained in the umbilical vein. During the procedure, maternal inspired and expired halothane and expired PCO_2 were monitored continuously by SARAcap A.G. When both maternal and fetal blood were aspirated, tubes were covered with parafilm which was punctured to obtain serum. Maternal and fetal blood were centrifuged for 10 minutes at 3,000 rpm in the air tight tubes. Minimum requirement of the serum volume was 0.2 ml in each specimen. The tubes of the serum were capped, and stored in the deep freezer.

3) Serum halothane measurement

(1) Chemicals: Halothane (100% of halothane containing 0.01% of thymol, Il sung Pharmaceutical Company, Seoul, Korea), Toluene-d8 (99+ atom %D, Sigma Chemical Co., St. Louis, MO), and Methanol (J.T. Baker, Philipsburg, NJ) were used. All other chemicals and solvents used were of the highest grade purity available and were used without further purification.

(2) Preparation of standard solutions: Stock solution (10 ug/ml, 10 ppm, w/v) of halothane and toluene-d8 were prepared by dissoluitng weighed quantities of each compound in methanol. Working solutions were prepared immediately prior to use by serial dilution of the

stock solutions with methanol.

(3) Preparation of samples: Frozen serum samples were thawed at room temperature. Then, 200 ul of the serum sample was transferred to 20 ml volume of head space vial (23 ×75 mm) where 100 ul of toluene-d8 was added afterwards.

The total volume of 1 ml was in the head space vial and the vial was sealed with teflon coated seal using hand crimper. The sealed headspace vial was loaded to magazine in headspace vial sampler for quantitation.

(4) Construction of calibration curve: Calibration curve of halothane was constructed accordingly as in the sample preparation except adding the stock solution of halothane to be the concentrations of 50ppb, 100ppb, 200ppb and 500ppb. Calibration curve was set up by the integration ratio of halothane and internal standard.

(5) Instrument: Mass spectrometery was carried out on a HP 5988A mass spectrometer (Hewlett Packard, Palo Alto, CA) interfaced directly to a HP 5890A gas chromatograph (Hewlett Packard, Palo Alto, CA) connected directly to a HP 19395A head space Sampler (Hewlett Packard, Italy). A fused silica capillary column, Ultra-2, (50 m×0.2 mmi.d., 0.33 um film thickness, Hewlett Packard, Palo Alto, CA) was used with helium (0.45 ml/miu) as the carrier gas. The temperature of column oven was held at 35℃ for 3 minutes and then increased to 200℃ at the rate of 10℃/min. Injector and transfer line temperature were maintained at 280℃. The mass spectrometer was operated on an electron energy of 70eV for the electrone impact (EI) mode. Ion source temperature was 200℃. The headspace sampler was operated at the condition of Table 1. From the electron impact mass spectra of halothane and toluene-d8, the ions in Selected Ion Mouitoring (SIM) mode were selected m/z 117 and 198 for halothane and m/z 98 and 100 for toluene-d8 for the quantitation of halothane. The retention times were 4.90 minutes for halothane and 9.14 minutes for toluene-d8 as shown in Fig. 1. The calculation of halothane was automatically done by the program of the HP 59970 ChemSta-tion (Hewlett Packerd, Palo Alto, CA).

4) Statistical methods

Statistical analysis were performed using the one-way analysis of variance and multiple

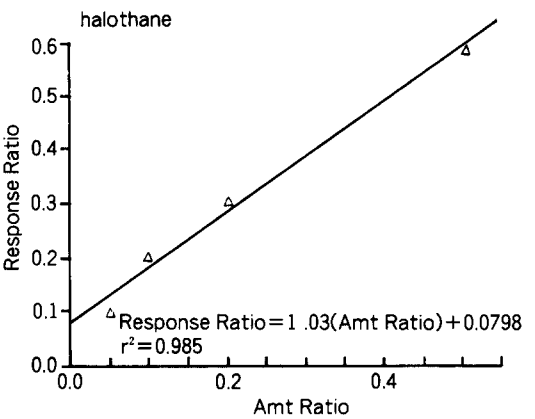

Fig. 1. Calibration curve for halothane.

Table 1. Headspace method and condition

Equilibration time	: 1 min
Method	: 1
Bath temperature	: 50℃
Valve/Loop temperature	: 55℃
Sample interval time	: 27 min
Sequence run time	: 20 min
Probe	: 1 sec
Pressure	: 3′
Vent/Fill loop	: 6′
Inject	: 6′
Carrier gas (He)	: 1.5 bar
Servo air	: 3~4 bar
Auxillary gas	: 1.5~2 bar

comparison tests using one-way ANOVA with the statistical computer program, SPSS-PC Ver. 3.0. Statistical significance was declared at F probability <0.05.

RESULTS

Pregnant rabbits' weight averaged 3.79 +/− 0.35 kg in 0.25 % halothane group, 3.84 +/− 0.46 kg in 0.5% halothane group, and 3.51 +/− 0.32 kg in 0.75% halothane group. There were no statistical differences between the groups. Fig. 2 illustrates the relationship of maternal inspired and expired halothane concentration in each group.

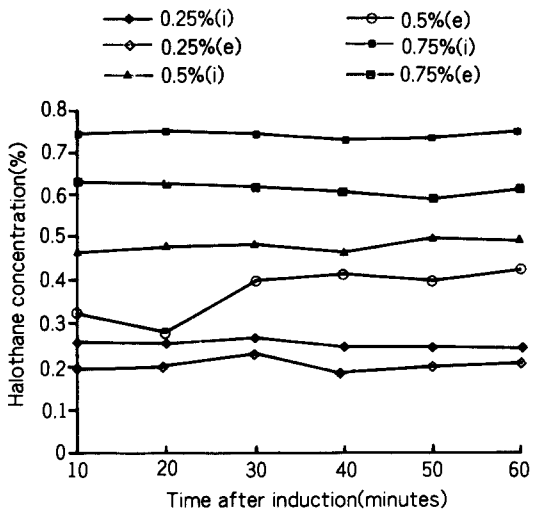

Fig. 2. Changing patterns of maternal halothane concentration in various halothane anesthesia. i; inspired gas e; expired gas.

Inspiratory halothane concentrations were maintained well in each group. Expired halothane concentrations were between 0.78 and 0.83 of inspired halothane, and these findings were observed in all studied animals. Expired PCO_2 levels were within physiologic range of 30 to 40 mmHg. PCO_2 tended to decrease as the anesthesia prolonged, but it could be maintained within normal range by adjusting the respiratory rate and ventilation volume.

Fig. 1. illustrated the calibration curve obtained for halothane using toluene-d8 as an internal standard, and SIM mode head-space-gas chromatography/mass spectrometer (GC/MS) as a tool. The calibration curve showed good linearity over the range from 50 ppb to 500 ppb and the correlation coefficient r^2 was 0.985. The limit of detection of halothane using head space GC/MS in Selected Ion Monitoring (SIM) mode was 1 ppb (1 ng/ml) for quantitation.

Maternal serum concentration of halothane directly correlated with the inhaled halothane concentration. Differences are statistically significant between the groups (Table 2). These data suggest that the gas chromatography detect the actual level of aqueous halothane. The present methodology aids the rapid attainment of stabe in vitro concentration of volatile anesthetics. Table 3 presents the relative ratio of fetal/maternal serum halothane

Table 2. Changes in halothane concentration of maternal blood

	10 min (ng/ml)	20 min (ng/ml)	30 min (ng/ml)	40 min (ng/ml)	50 min (ng/ml)	60 min (ng/ml)
0.25%*	$224^{+,\#}$	$203^{+,\#}$	$269^{+,\#}$	356^{+}	291^{+}	$256^{+,\#}$
0.5%	$414^{++,\#}$	$506^{++,\#}$	$447^{++,\#}$	368^{++}	376^{++}	$468^{++,\#}$
0.75%	$699^{+,++}$	$879^{+,++}$	$999^{+,++}$	$952^{+,++}$	949^{+++}	$909^{+,++}$

*Anesthetic concentration of helothane, N=6 each group
+,++,#Denote pairs of groups significantly different at the 0.050 level.

Table 3. Relative ratio of retus/mother halothane concentration

	10 min	20 min	30 min	40 min	50 min	60 min
0.25%	0.137	0.145	0.331	0.216	0.580	0.625
0.5%	0.299	0.348	0.525	0.868	0.723	0.682
0.75%	0.343	0.452	0.482	0.480	0.389	0.402

No two groups are significantly different at the 0.050 level.
N=6 each group

concentration. In these three groups, halothane level of the fetus had a tendency to increase significantly during the first 30 minutes after the induction of anesthesia and remained stable thereafter. Relative ratio of fetal/maternal serum halothane concentration is higher in 0.5% group than the other groups. However, it was not statistically different from the others. Average ratio of fetal/maternal halothane concentration are between 0.339 and 0.574, which means halothane cross placenta readily and the fetus can be adversely affected by the high level of maternal halothane level.

DISCUSSION

The technical development in diagnostic ultrasound has made it possible to diagnose many malformations in utero[2,9]. Trials of prenatal correction of malformation in several life threatening but potentially correctable diseases have documented the effect of in utero repair[4,5]. Halothane is one of the most commonly used anesthetics in mordern practice, because it is safe, economic and stable at room temperature. This agent has become the preferred anesthetic agent during fetal surgery because of its familiarity and uterine relaxation effect[4~6]. Premature labor remains a serious and major complication of fetal surgery. Development of better tocolytics are recommended for the progress. Halothane also has a benificial effect on the tocolysis because it makes uterine relaxes during surgery. It is natural to assume that this volatile agent crosses the placenta and achieve a certain anesthetic level in the fetus. However, fetal level of inhalation agents during fetal surgery has not been documented. We undertook this study to evaluate the transplacental crossing of this volatile agent and to determine the optimum anesthetic level of halothane. Because volatile anesthetics partition between aqueous and gaseous media, their concentration in in vitro assay media is influenced by various factors: media volume, chamber size ratio, temperature, and duration of agitation[7]. Meticulous sealing and freezing of the serum after centrifuge prevented underestimation of this volatile agent in this study. The relationship between the volume of undiluted halothane added to the assay buffer and the aqueous halothane concentration was linear.

Recent reports showed maternal halothane anesthesia had detrimental effects on the fetus by reducing fetal cerebral blood blow, fetal cardiac output and shunting of blood away from the placenta in ewes, lambs and sheeps[1,3,8,10]. Changes of fetal blood volume were directly related to changes in uterine blood flow, and these changes lead to marked changes in fetal renal function and lung liq-

uid flow[11]. This study clearly demonstrates that maternal halothane readily cross the pacenta and the concentrations of halothane in the fetus are from 33.9% to 57.4% of mother's level in various halothane anesthesia. Halothane is a good anesthetic agent for fetal surgery, but it is desirable to maintain lowest possible halothane concentration during fetal surgery to prevent its adverse effect on the fetus.

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