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HALOTHANE INDUCED CHROMOSOME ABERRATION OF HUMAN LYMPHOCYTE

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Abstract

Human lymphocytes obtained from healthy volunteer were cultured in Eagle's minimal essential medium equilibrated with various concentrations of halothane balanced with air for 48 hours and changes of their chromosomes were observed. Aberration rate of chromosomes of the lymphocytes exposed to 1 % halothane was increased significantly (<0.01). Aberration rate with 0.5 % halothane exposure tended to be slightly higher than that with air exposure while rates with other lower concentrations of halothane were quite identical with that in air exposure.

Secondarily four gas mixtures were tested to observe additive effect of nitrous oxide. Namely (1) 1 % halothane + 74 % nitrous oxide + 25 % oxygen, (2) 75 % nitrous oxide + 25 % oxygen, (3) 1 % halothane + air, (4) air alone were tested. Consequently no aggravating effect of nitrous oxide on the aberration rate was demonstrated.

Thirdly to determine the phase of cell cycle in which the aberrations would be induced, lymphocytes were divided into two groups. One of them was exposed to 1 % halothane for 24 hours at first and then to air for the second 24 hours. The other group was exposed to air first and then to 1 % halothane. Aberrations of the both groups were mostly identical and 87.5 % of these was classified into chromatid type and only 12.5 % into chromosomal type. It was indicated that halothane could cause changes in chromosomes mostly in the S phase, namely DNA synthesis period.

INTRODUCTION

Halothane is used most practically as a major inhalation anesthetic agent in clinical anesthesia. However accumulated data[1,2,3] suggested that incidence of spontaneous abortion and congenital anomalies were greater in nurses or anesthesiologists who were exposed to various anesthetic agents in very low concentration in operating room than other personnel who worked out of

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operating room. Smith *et al*[4] found that halothane of 2 % concentration in inspiratory gas decreased significantly fertility rate of chick embryos. Bussard and his coworker[5] observed that fetal death occurred more frequently in hamsters anesthetized with nitrous oxide and halothane. However Bruce[6] reported that fertility rate would not be affected by halothane anesthesia in mice. Therefore effect of anesthetic agents on fertility of either human or animal is still obscure. On the other hand, it has been well known that spontaneous abortion, congenital anomalies and neoplastic diseases are often associated with chromosome aberrations. Therefore it was presumed that aberration of chromosomes might be occurred in a long term of exposure to of very low concentration and consequently spontaneous abortion might be occurred halothane at higher rate in nurses who worked in operating room.

In this study it was intended to observe effect of halothane and/or nitrous oxide on chromosomes of human lymphocytes cultured in vitro.

MATERIALS AND METHODS

Lymphocytes were prepared from venous blood obtained from healthy adults who have not been recently exposed to any anesthetic gas and have not been medicated with any drug. The blood was withdrawn into a sterile heparinized syringe and then all procedure was carried out with sterile technique. The erythrocytes were sedimentated by placing the syringe upright for one hour and leucocyte rich plasma was obtained in the supernant. The leucocytes were diluted with Eagle's minimal essential medium (EMEM) to result in suspension of 10^6 counts/ml of lymphocyte. Then one ml of the cell suspension was added 10 ml EMEM supplemented with 20 % calf serum and 1 % phytohemaglutinin (PHA) in a 110 ml glass culture bottle. Mixtures of air and halothane of designed concentrations (1.0, 0.5, 0.1, 0.01, 0.001, and 0.000 %) were prepared in a fluorinated resin bag with 60 liters capacity (Experiment 1). The gas mixture was delivered from each bag into the culture bottle which was shaked gently for 20 minutes and was allowed halothane to equilibrate quite well with the medium. Then the culture bottle was capped quickly with a silicone rubber stopper and kept at 37 °C in an incubator for 48 hours. The concentration of halothane in the bottle was checked at every 12 hours in other experimental series and it was corroborated to be kept mostly constant for 72 hours. Concentration of the anesthetic gases was analyzed by gas chromatography (Yanaco G-80 equipped with flame ionized detetor). In order to observe additive effect of nitrous oxide on the lymphocytes were oxposed to four different gas mixtures in Experiment 2, namely 1 % halothane plus 74 % nitrous oxide and 25 % oxygen (GOF group), 75 % nitrous oxide and 25 % oxygen (GO group), 1 % halothane and room air (F group) and room

air alone (control group). In Experiment 3, a half of the lymphocytes was exposed to 1 % halothane for 24 hours at first and to air for the second 24 hours (A group). The other half of the lymphocytes was exposed to air for 24 hours at first and then to 1 % halothane balanced with air for the second 24 hours. Colchtin (1×10^{-5} M) was added to arrest mitosis at 2 hours before the end of experiment. The lymphocytes collected by centrifugation were treated to spead the chromosomes with 0.2 % NaCl solution for 15 minutes and fixed in methanol/acetic acid (3 : 1 v/v). The cells were transferred on a placedon slide glass[7] and were countered number of aberration of chromosome in 100 cells for each treatment by a single observer. The same experimental procedure was carried out 3 times for each in the above series. X^2 test was used for statistical analysis of the data.

RESULTS

Experiment 1. Aberration rate was tabulated as mathematical mean in the table 1. Following the exposure to 1 % halothane aberration rate of

TABLE 1.
Aberration rates of chromosomes of human lymphocytes after 48 hours exposure to halothane with various concentrations

	control	concentration of halothane				
		1%	0.5%	0.1%	0.01%	0.001%
Aberration (%)	7	23*	17	10	12	8
chromatid gap	4	14	10	7	7	4
chromosomal gap	0	0	1	0	1	1
chromatic break	3	2	3	3	4	2
chromosomal break	0	1	3	0	0	0
dicentric chromosome	0	2	0	9	0	1
acentric fragment	0	4	0	0	0	0
Normal (%)	93	77	83	90	88	92

*$p<0.01$

chromosomes was increased definitely compared with that in the control ($p<0.01$). The rate with 0.5 % halothane tended to be higher than that with the control while the rates with the other lower concentrations of halothane were quite identical with that in the control. Chromosomal aberration observed were typed in chromatid gap and break, chromosomal gap and break, dicentric chromosome and acentric fragment, as shown in the table 1. Namely 83 % of them was classified into chromatid type and the remained 17 % was classified into other types.

Experiment 2. Aberration rates in the four groups were shown in the

table 2. The aberration rate in the GOF group was significantly higher ($p<0.01$) than that in the GO group. However there was no significant difference either between the aberration rate in the GOF group and in the F group or that in the GO group and in the control.

TABLE 2.
Aberration rates of chromosomes after 48 hours exposure to halothane and/or nitrous oxide.

treatment	chromosomes of human lymphocyte	
	normal	aberration
GOF	76%	24%
GO	92	8
OF	77	23
control	93	7

Legend for Table 2.
Abbreviation
G : N_2O 74~75%
O : O_2 20~21%
F : halothane 1%
Control : room air
also see the text

Experiment 3. Aberration rate of chrosomes in the group A in which lymphocytes were exposed to 1 % halothane for the second 24 hours of their cell cycle was 18 %, namely 14 % of chromatid gaps, 2 % of chromosomal gaps and 2 % of chromosomal breaks. On the other hand, aberration rate in the B group in which lymphocytes were exposed to 1 % halothane in the first 24 hours was 14 %, namely 12 % of chromatid gaps and 2 % of chromosomal gaps. No statistically significant change was noted among these aberration rates.

DISCCUSION

This study demonstrated that halothane could induce definitely irreversible, cytogenetical changes in cultured human lymphocytes. American Society of Anesthesiologists reported that incidences of abortion and cancer were higher in females who worked in operating room than females who worked out of operating room[1]. This finding has been advocated by additional studies in the Soviet Union[8] and the United Kingdom[9]. Therefore it was anticipated that cytogenetical changes might be caused by long term exposure to halothane of low concentration as similarly as in the operating room. To corroborate this it was carried out that human lymphocytes cultured in vitro were exposed to halothane of various concentrations. No significant difference, however, was noted in incidence of chromosome aberrations between the groups in which

the concentration were prepared most identically with that in operating room and the control group. Nevertheless a significantly higher incidence compared with the control was noted in the group exposed to 1 % halothane. It was indicated that halothane should be avoided from anesthesia for pregnant woman. Recently Coate *et al*[10] reported that combination of halothane and nitrous oxide could produce apparent changes on chromosomes of bone marrow and spermatogonical cells. In our study, however, it was not able to demonstrate that nitrous oxide could deal much with halothane on the chromosomes additively or synergistically.

It has been reported that cell growth is suppressed at various stages of cell cycle by halothane. Nunn *et al*[11] observed that mitosis in M phase was ceased by halothane. Bruse[12] observed that halothane decreased DNA content in nuclei and suppressed RNA synthesis in S phase. It is documentated that S phase of human lymphocyte will commence within 24–28 hours after blast-formation by PHA and subsequent G1 phase and will last approximately for 12 hours[13]. Then the subsequent G2 phase will last approximatley for 3 hours and M phase also for 3 hours. Therefore it is supposed that chromosomes under the first mitosis would be examined in the third experimental series. Evans and Scott[14] clarified that chemical agents and x-ray could produce chromosomal aberrations in G1 phase and chromatid aberrations in S and G2 phase. However various types of aberration would be occurred when these agents would affect on chromosomes at M phase. In the experiment 3, the chromosomes in the group A were exposed to the halothane in G1 ~ S phase and these in the B group were exposed in S ~ M phase. However there was no difference in incidence of aberration between these two groups. And also chromosomal types were found only in 12.5 % of all aberrations. Therefore it seemed likely that halothane could affect on chromosomes mostly in S phase. Usubiaga and Smith[15] observed that human lymphocyte would not be aberrated by 2 hours exposure to 1.0 - 2.5 % halothane after addition of colcemide. Their data seem not to be conflict with our present results since they exposed the cells in M and G2 phase with very short duration.

It is most acceptable that chromatid or chromosomal gaps will not induce death of cell since these abnormalities are not crucial to viability of cell but also are inherited to daughter cells. Therefore it is presumed that these changes in germ cells will induce merely congenital anomalies in individual.

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