A COMPARISON BETWEEN THE EFFECTS OF HALOTHANE AND ENFLURANE ON LYMPHOCYTE FUNCTIONS

(A PROSPECTIVE STUDY DONE AT THE KENYATTA NATIONAL HOSPITAL)

BY

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A DISSERTATION SUBMITTED IN PART FULFILMENT FOR THE DEGREE OF

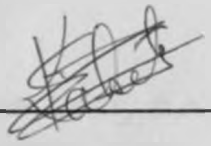
MASTER OF MEDICINE (ANAESTHESIA)

IN THE UNIVERSITY OF NAIROBI

(1987)

DECLARATION

This dissertation is my own original work and has not, to my knowledge, been presented for a degree in any other University.



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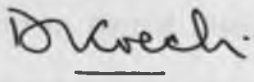

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ACKNOWLEDGEMENTS

I wish to express my sincere thanks and gratitude to the following:

Dr. S.K. Kahuho, MBChB (NBI), D.A.(Lond.), FFARCS(Ireland) Head of the Department of Anaesthesia, University of Nairobi for his guidance and advice during the study and preparation of this work.

Dr. D.K. Koech, BSc, MS, PhD, CBiol, MIBiol, FInstPM, SS Specialist Immunologist, Principal Research Officer and Director, Biomedical Sciences Research Centre, Kenya Medical Research Insitute, for his supervision and guidance during the study.

All fellow doctors and clinical officers in anaesthesia, and all other theatre staff for being co-operative during the study.

All the staff of the Immunology Laboratory at the Biomedical Sciences Research Centre, Kenya Medical Research Institute, without whom I could not have been able to carry out the study.

My wife Doris Njeri and members of my family who have been co-operative and encouraging during the period of the study.

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SUMMARY.

A comparative study on the effect of lymphocyte function after exposure to enflurance and halothane was done between April and November 1986 at Kenyatta National Hospital. Fourty-six patients who qualified as ASA Grades I and II with ages ranging from fourteen years to seventy seven years were included in the study. All were premedicated with atropine and pethidine. Thirty-four of them were induced with thiopentone, nine induced with ketamine and diazepam, two induced with diazepam alone while one was induced with halothane alone. Three samples of venous blood were taken from each patient. Sample A taken before induction, sample B after induction and sample D about twenty four hours after induction. Seperation and stimulation of lymphocytes by mitogens (PHA, Con A) and antigens (PPD and SKSD), incubation with radioactive thymidine and measuring of radioactivity uptake by the lymphocytes from a counter was done at the immunology laboratory of the Biomedical Sciences Research Centre laboratories of the Kenya Medical Research Institute. Thirty seven patients were exposed to halothane and nine were exposed to enflurane. Eight of the patients studied had malignant tumours, 10 had non malignant tumours and 28 had no tumours. The duration of anaesthesia ranged from 9 minutes to 3.5 hours with a mean of 1.25 hours. Data comparison was done using Student's "t" test.

Both halothane and enflurane were found to have positive response on lymphocyte transformation following stimulation with both mitogens and antigens in patients with malignant tumours as well as those with non malignant tumours and enflurane caused more significant response than halothane. The present data reveal that low serum levels of both halothane and enflurane cause prolonged and increased *in vitro* lymphocyte responses to mitogens and antigens.

AIMS AND OBJECTIVES

The main aims and objectives of this study were:

1. To assess the effects of halothane and enflurane on in vitro lymphocyte functions, and relate these to the surgical conditions of patients with malignant and non-malignant tumours.

2. To relate the findings of the study with contemporary clinical anaesthetic practice.

INTRODUCTION AND LITERATURE REVIEW

Exposure to anaesthetic agents have been shown to alter both specific and non specific mechanisms of the immune system either directly or indirectly through hormones (1, 2).

The non specific mechanisms of the immune system that are affected include the ciliated epithelium of the respiratory tract whose movement in such a way as to direct foreign materials upwards so as to be expelled. The cilia movement has been shown to be depressed by anaesthetic agents (3). Pulmonary bactericidal activity is affected by exposure to cyclopropane and methoxyflurane (4). In addition phagocytosis by macrophages is directly depressed by halothane and diethyl ether (5, 6). Anaesthetic agents have been reported to affect the chemotaxic attraction of phagocytes and lymphocytes to the site of infection (7).

Anaesthetic agents may also affect those cells involved in specific immune reactions. These cells are T and B lymphocytes. Briefly T lymphocytes are processed in the thymus and the B lymphocytes in "bursa equivalents" (such as the bone marrow, payers patches in mammals). Both cell types are from precursors that migrate from haemopoietic tissues or stem cells to the processing sites. When the lymphocytes are exposed to antigens, they proliferate and give rise to specific clones of cells that play an important role in the specific immune responses.

These cells may be regulatory, effector, cytotoxic, suppressors, inducers, or antibody producing. T lymphocytes participate primarily in cell-mediated immune responses while B lymphocytes participate in antibody-mediated immune responses. There is, however, a cooperation between the two cells. Sub-populations of these cells do occur especially T lymphocytes. There are helper T lymphocytes which besides their role in cell-mediated immunity also assist B lymphocytes in antibody production. There are also suppressor T lymphocytes whose primary role is regulatory. Cytotoxic T lymphocytes are capable of destroying foreign target cells in grafts or virus infected cells.

The proliferation of the lymphocytes after they are stimulated by antigens involves the transformation of small resting non-dividing cells into proliferative cells that synthesize macromolecules and proteins. They subsequently enlarge morphologically and then divide into daugther cells that are specifically sensitized to the stimulating antigen and they will participate in the destruction of the antigen either directly (T lymphocytes) or via the production of specific antibodies (B lymphocytes). The dividing cells go through the following sequential steps: the **M** phase or mitosis phase, the **G** phase or post mitotic phase, the **S** phase or DNA Synthesis phase resulting in the doubling of the nuclear DNA), and the the **G** phase or a brief resting phase before dividing starts once again (8, 9, 10, 11).

Lymphocyte transformation in vitro can be induced by mitogens and antigens. Phytohaemogglutinin (PHA) and concanavalin A (Con A) are the most commonly used non specific mitogens (13). Thymidine is a specific precursor of DNA. Radioactive thymidine, if incubated in vitro with lymhocytes, will be taken up into the DNA of the dividing lymphocytes. The degree of mitogen stimulation can be measured by way of radioactivity following radioactive thymidine uptake.

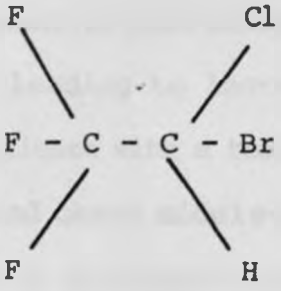
Depression of lymphocyte transformation has been observed following exposure to various anaesthetic agents such as halothane which has been shown to depress lymphocyte transformation by inhibiting DNA synthesis (8, 9), lignocaine and ketamine which in high doses, been shown to depress lymphocyte transformation (10, 12).

Halothane and enflurane are inhalational anaesthetic agents commonly used at the Kenyatta National Hospital theatres. Both have similar pharmacologic functions. They depress the central nervous system, cardiovascular system and respiratory system. This activity leads to reduced cardiac output, reduced blood pressure, and reduced pharyngeal and laryngeal reflexes. They differ in their rate of metabolism, effect on the liver and cells.

Halothane

Halothane is the most widely used inhalational anaesthetic agent in the world. It was synthesized in 1951 and introduced into clinical practice in the United Kingdom in 1956 and in the United States in 1958 (13, 14).

Halothane is a halogenated hydrocarbon whose chemical name is 2-bromo 2-chloro 1,1,1-trifluoroethane, and the structure pictorially represented as:



It is a colourless, volatile agent, with a characteristic odour, and a molecular weight of 197. It has a saturated vapour pressure at 20° C of 243 mm Hg and a minimal aveolar concentration of 0.75 volume per cent.

Halothane causes cardiac depression, vasodilatation, sympathetic ganglion blockade, and central vasomotor centre depression. All

these result in a fall in cardiac output and therefore a fall in blood pressure. At the level of central nervous system, it is a potent anaesthetic but a weak analgesic.

Halothane is also a respiratory depressant. It causes bronchodilatation by blocking reflex bronchoconstrictive pathways. In addition, it abolishes pharyngeal and laryngeal reflexes with no stimulation of secretions, and can be a good muscle relaxant.

Hepatcellular hepatitis has been associated with halothane exposure. This hepatitis is histologically similar to that caused by hepatitis A virus. Factors thought to contribute to the development of post halothane exposure hepatitis are repeated exposures (leading to increased incidence of post operative jaudice), patients with a tendency to develop specific autoimmune diseases, and obese middle-aged patients.Several theories of mechanisms of halothane-induced hepatitis includes oxidised metabolites of halothane (which may alter the antigenicity of the hepatocyte membrane thus resulting in increased vulnerability to antibodies against the membrane components), and genetic factors (which may affect the production of antibodies that act on the hepatocytes membrane). In addition, products of halothane reductive metabolism result in direct toxicity to the hepatocytes through covalent binding (13, 14). So far, there is no report on post exposure hepatitis due to enflurane.

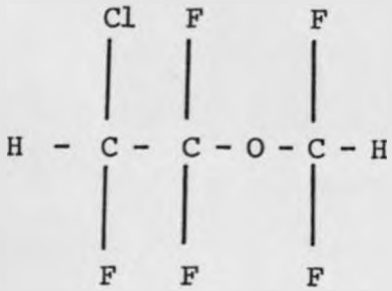
Halothane has been shown to arrest diving cells of root tip of broad bean at metaphase. It has also been shown to depress DNA synthesis, lymphocyte transformation, and bone marrow division (8, 9, 15, 16).

Twenty per cent of halothane is metabolised in the liver into bromides, chlorides, trifluoroethanolamine, chlorobromofluoroethylene and trifluoroacetic acid (14). Rate of metabolism is increase by concomittant use of drugs (such as barbiturates) that induce hepatic enzyme production.

Due to the cardiovascular depressive action of halothane, and its toxic effects on to the liver resulting from high metabolism another inhalational agent, enflurane was introduced in 1963 and used clinically in 1966 in the United States (17, 18).

Enflurane

The chemical name of enflurane is 2-chloro 1,1,2-trifluoro ethyl-difluoroether, and the structure is pictorially represented:



Unlike halothane which is a halogenated hydrocarbon enflurane is a halogenated ether. It is a colourless, non inflammable compound with a molecular weight of 184. It has a vapour pressure of 23.2 kpa at 20^{o} C and minimal alveolar concentration of 1.68 in oxygen and 0.56 in 70% nitrous oxide.

Enflurane causes epileptiform changes in the electoencephalogram. It causes concentration dependent myocardial depression. No serious arrythmias have been reported with enflurane use, and no hepatocellular changes have been associated with its use.
About 2% of enflurane is metabolised into inorganic fluorides that do not reach the nephrotoxic levels of 50 umol/l(17). No work has been done on its effects on body cells.

Enflurane appears to have some advantages over halothane such as cardiovascular stability (minimal hypotension,no serious arrythmias), absence of liver toxicity, and very low metabolism.

Many studies done have shown depression of cellular activity following halothane exposure.

MATERIALS AND METHODS

Patients

Fourty six patients who qualified as ASA 1 and 2 were studied. Their ages ranged from 14 years to 77 years with a mean age of 38 years. Males were 27 (58.7%) while females were 19 (41.3%). All patients were premedicated with pethidine (50 mg) and atropine (0.6 mg) before coming to the theatre. Thirty four of the patients were induced with thiopentone (250 mg), 9 patients induced with ketamine (2 mg/kg) and diazepam (10 mg) 2 patients induced with diazepam (15 mg) alone while one patient was induced with halothane (3.5%) alone. A total of 29 patients (69%) were maintained on spontaneous ventilation while 70 patients (31%) were maintained on intermittent positive pressure ventilation. Thirth seven (7.4%) of the patients were exposed to halothane while 9 patients (12.6%) were exposed to enflurane. Eight patients (17.4%) had malignant tumours, 10 (21.7%) had non malignant tumours while 28 (60.9%) of the patients had no tumours. Duration of anaesthesia ranged from 9 minutes to 3.5 hours with a mean duration of 1.25 hours (Tables 1-6).

Ten millilitres of blood was taken through venepuncture, placed in a sterile heparinized tube and taken to the Kenya Medical Research Institute, Biomedical Sciences Research Centre laboratories for immunological analysis. This was done on each patient before induction of anaesthesia, 30 minutes to 1 hour after induction and 24 hours later.

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TABLE 1

Age and Sex Distribution

AGE (YRS)	MALES	FEMALES	TOTAL
10-15	1	0	1
16-20	2	3	5
21-25	3	2	5
26-30	3	2	5
31-35	2	4	6
36-40	3	3	6
41-45	3	2	5
46-50	1	0	1
51-55	2	1	3
56-60	1	0	1
61-65	2	0	2
66-70	3	2	1
>70	1	0	1
TOTAL	**27**	**19**	**46**

TABLE 2

Induction Agents

INDUCTION AGENTS	NUMBER OF PATIENTS
Thiopentone	34
Ketamine and Diazepam	9
Diazepam alone	2
Halothane alone	1

TABLE 3

Maintenance of Anaesthesia and Inhalational Agents

	HALOTHANE	ENFLURANE	TOTAL
Spontaneous Ventilation	26 patients	3 patients	29
Intermitent Positive Pressure Ventilation	11 patients	6 patients	17
TOTAL	**37**	**9**	**46**

TABLE 4

Clinical Conditions of the Patients

	NUMBER	%
Malignant Tumours	8	17.4
Non Malignant Tumours	10	21.7
Others (Non-Tumours)	28	60.9
TOTAL	**46**	**100.0**

TABLE 5

Duration of Exposure to Anaesthesia

TIME IN MINUTES	NUMBER OF PATIENTS
0–20	6
21–40	12
41–60	6
61–80	6
81–100	6
101–120	6
121–140	0
141–160	1
161–180	2
181–200	0
201–220	1
TOTAL	46

Range of Duration of anaesthesia = 9 minutes to 218 minutes

Mean duration of anaesthesia = 72.9 minutes

TABLE 6a

Stimulation with Mitogens and Antigens Following Holothane Inhalation.

	NO. OF PATIENTS
Malignant Tumours	5
Non Malignant Tumours	10
Non Tumours	22
TOTAL	37

TABLE 6b

Stimulation with Mitogens and Antigens Following Enflurane Inhalation

	NO. OF PATIENTS
Malignant Tumours	3
Non Malignant Tumours	0
Non Tumours	6
TOTAL	**9**

Immunological analysis

Stimulation of lymphocytes by mitogens and antigens was performed according to the method described by Koech et al (19). Briefly for each patient, lymphocytes were prepared at a concentration of 4 x 10 cells/ml. 100ul of the cell suspension and 10ul of stimulant (mitogen or antigen) were placed in quadruplicate in micro-test plate cells. Parallel control wells (without stimulants) were also prepared in quadruplicate. Mitogens used were phytohaemogglyttin (PHA) and concavalin A (ConA) and the antigens used were purified protein dirivative (PPD) and streptokinaire-streptodornase (SKSD). All cultures were incubated at 37^{o} C in humidified 5% Co2 incubator for either 3 days (in case of mitogens) or 5 days (in case of antigens). At the end of incubation period, each well was pulsed with 0.5 uCi of (methyl ^{3}H) thymidine and incubated for further 18 hours. Cells were harvested onto glass fibre mat using a titertek cell harvester. The glass fibre mat discs were cut out and transfered to scintillation vials to which 1ml of scintillation liquid was added Triated thymidine uptake by the transforming lymhocytes was measured in a beta scintillation counter and interpreted as disintergrations per minute (dpm).

For statistical convenience, the individuals examined were divided into two groups: those with malignancies and those without any malignancies.

The change in response before and after anaesthesia induction was represented as $\alpha = (B - A)/A \times 100$ and the change in response 24 hours after induction of anesthesia being $\beta = (C - A)/A \times 100$ where:

A = response before induction of anaesthesia

B = response following induction of anaesthesia

C = response twenty four hours following induction of anaesthesia.

These values for both halothane and enflurane were compared using Student's "t" significance test.

RESULTS

Out of the 46 stimulated samples, lymphocytes from 8 patients did not respond to both the antigens and the mitogens, 4 did not respond to antigens and 2 did not respond to mitogens.

Exposure to Halothane

Response of lymphocytes to PHA following halothane exposure showed a significant increase before induction for both malignant tumours and non malignant tumours. Malignant tumours showed significantly less response than non tumours (Table 7). The same was seen for Con A after exposure to halothane (Table 8). A significant negative response was observed on stimulation with PPD following halothane exposure in patients with malignant tumours with a positive response 24 hours after induction. There was a positive response following halothane exposure in non malignant tumour patients with a more positive change 24 hours after the exposure Patients with malignant tumours were less stimulated than those with non malignant tumours (Table 9). Similarly, a negative response to SKSD following halothane exposure was observed in malignant tumour patients but response after 24 hours became positive. Lymphocytes from patients with malignant tumours were stimulated less than those with non malignant tumours (Table 10).

TABLE 7

In Vitro Response to PHA of Peripheral Blood Lymphocytes from Patients Exposed to Halothane

Clinical Condition	Per cent change (± 1 s.d.) in response	
	α	β
Malignancies (n=3)	10± 6.4 (P<0.0005)	N.D.
Non malignancies (n=28)	54± 17 (P<0.0005)	42± 12 (P<0.0005)
	P* <0.0005	N.D.

P = Significant level in relation to response before drug Exposure

P* =Significant level in relation to malignancies and non malignancies

N.D.= not done

α = change in response following drug induction (B-A)/A x 100.

β =change in response 24 hours following drug induction (C-A)/A x 100

1 s.d. = one standard deviation

TABLE 8

In Vitro Response to Con A of Peripheral Blood Lymphocytes from Patients Exposed to Halothane

Clinical condition	Per cent change (± 1 s.d.) in response	
	∝	β
Malignancies (n=3)	116± 15 (P<0.0005)	56± 19 (P<0.0005)
Non malignancies (n=9)	74± 28 (P<0.0005)	32± 14 (P<0.0005)
	P* <0.0005	**P* <0.005**

Notations as in Table 7

TABLE 9

In Vitro Response to PPD of Peripheral Blood Lymphocytes from Patients Exposed to Halothane

Clinical condition	Per cent change (± 1 s.d.) in response	
	α	β
Malignancies (n=3)	-7.9± 6.1 (P<0.025)	10.9 ± 2.5 (P<0.0005)
Non malignancies (n=15)	32±14 (P<0.0005)	45±20 (P<0.0005)
	P* <0.0005	**P* 0.0005**

Notations as in Table 7

TABLE 10

In Vitro Response to SKSD of Peripheral Blood Lymphocytes from Patients Exposed to Halthane

Clinical condition	Per cent change (± 1 s.d.) in response	
	α	β
Malignancies (n=3)	-26± 5.5 (P<0.0005)	17± 3.5 (P<0.0005)
Non malignancies (n=7)	41± 7.4 (P<0.0005)	63± 10 (P<0.0005)
	P* <0.0005	**P* <0.025**

Notations as in Table 7

Exposure to Enflurane

A significantly positive response to PHA following enflurane exposure was seen in lymphocytes from patients with malignant tumours with a decline although positive response 24 hours after drug exposure. There was a negative response in non malignant tumour patients with a positive response 24 hours after exposure. Lymphocytes from malignant tumour were stimulated more than those from non malignant tumour patients (Table 11). A significantly positive response to con A for lymphocytes from both malignant and non malignant tumour patients was observed following enflurane induction with a decline although positive response following exposure 24 hours later. Lymphocytes from patients with malignant tumours were less stimulated than from those with no malignant tumours (Table 12).

Response of lymphocytes from patients with malignant tumours to PPD following induction with enflurane was noted which increased 24 hours later (Table 13). Data from patients with non malignant tumour was not analysable. A significantly positive response to SKSD in lymphocytes from both patients with malignant and non malignant tumours after induction with enflurane. Patients with malignant tumours had negative response 24 hours after exposure while those with non malignant tumours had a positive response 24 hours after exposure. Lymphocytes from patients with non malignancies were

more stimulated much than those from patients with malignant tumours (Table 14)

A comparison of the two inhalational agents showed that the response of lymphocytes to both mitogens and antigens was more in both malignant tumours and non malignant tumours following exposure to enflurane than after exposure to halothane.

TABLE 11

In Vitro Response to PHA of Peripheral Blood Lymhocytes from Patients Exposed to Enflurane

Clinical condition	Per cent change (± 1 s.d.) in response	
	∝	β
Malignancies (n=3)	222± 36 P<0.0005	30± 14 P<0.01
Non malignancies (n=4)	-25± 6.2 (P<0.0005)	11± 4.1 (P<0.005)
	P* <0.005	**P* <0.025**

Notations as in Table 7

TABLE 12

In Vitro Response to Con A of Peripheral Blood Lymphocytes from Patients Exposed to Enflurane

Clinical conditions	Per cent change (± 1 s.d.) in response	
	α	β
Malignancies (n=3)	229±58 (P<0.0005)	68±31 (P<0.01)
Non malignancies (n=5)	499± 129 (P<0.0005)	165± 68 (P<0.005)
	P* <0.005	**P* <0.025**

Notations as in Table 7

TABLE 12

In Vitro Response to Con A of Peripheral Blood Lymphocytes from Patients Exposed to Enflurane

Clinical conditions	Per cent change (± 1 s.d.) in response	
	α	β
Malignancies (n=3)	229±58 (P<0.0005)	68±31 (P<0.01)
Non malignancies (n=5)	499± 129 (P<0.0005)	165± 68 (P<0.005)
	P* <0.005	P* <0.025

Notations as in Table 7

TABLE 13

In Vitro Response to PPD of Peripheral Blood Lymphocytes from Patients Exposed to Enflurane

Clinical conditions	Per cent change (± 1 s.d.) in response	
	α	β
Malignancies (n=3)	49±15 (P<0.0025)	56± 48 (P<0.10)
Non malignancies (n=0)	N.D.	N.D.
	P* N.D.	P* N.D.

Notations as in Table 7

TABLE 14

In Vtro Response to SKSD of Peripheral Blood Lymphocytes from Patients Exposed to Enflurane

Clinical condition	Per cent change (± 1 s.d.) in response	
	α	β
Malignancies (n=3)	348± 100 (P<0.0005)	-3.2± 4.3 (P<0.15)
Non malgnancies (n=3)	282± 64 (P<0.0005)	319± 24 (P<0.0005)
	P* <0.15	**P* <0.0005**

Notations as in Table 7

DISCUSSION

The findings of this study have revealed increased lymphocyte response to both mitogens (PHA and ConA) and antigens (PPD and SKSD) after patients with malignant and non-malignant tumours have been exposed to halothane and enflurane. These findings are a little divergent from those of earlier studies. These are similar to those of Koech et al. (20). Their study, on the effect of sodium stibogluconate (Pentosam^{R}) at very low blood levels, revealed the drug to be lymphostimulatory. At high levels, the drug is inhibitory. (Pentosam^{R} is the current drug of choice in treatment of vesceral leishmaniasis).

Results from the present studies deviate somewhat from those of earlier studies. One of the main reasons is that most of the earlier studies have been on laboratory animals (3, 4, 5, 11) while the present one was on humans. The concentration of inhalational anaesthetics were also different. This difference in concentration may affect lymphocytes differently as has been observed in other systems (19, 20). Aldhoete et al. (21) did a study on the effets of prolonged hydocarbon gases exposure on hemopoietic system in rats and demonstrated a depression of the system. Later, Nun et al, using halothane, demonstrated a dose dependent murine bone marrow depression (15). In addition, in some of the earlier studies, lymphocytes were exposed to the inhalational agents in vitro. This leads to the fact that the concentration of an inhalational agent directly from a vapourizer

(inspired concentration) to a lymphocyte in vitro is different from that concentration attained in blood after uptake of the drug from alveolar. The lymphocyte in vitro will be exposed to a much higher concentration of the drug than that in vivo. At the same time, the drug concentration in blood would also include drug metabolites. The effects of metabolites may be different from those of the parent drugs. Halothane and enflurane undergo metabolism to the extent of 20 per cent and 2 per cent respectively (13, 14, 17, 18).

In previous studies, lymphocytes were exposed to high concentrations of inhalational agents unlike in present study where lymphocytes were exposed to therapeutic concentrations in vivo. Direct exposure of lymphocytes to high concentrations of the drug might explain the findings obtained from earlier studies where only PHA was used as a stimulant (21).

There is evidence from this study that lymphocyte responses to halothane and enflurane are different. The reason for observed differences may be multifactorial, such as drug metabolism and metabolites blood levels of the drugs and the number of samples analysed. About 20 per cent of inhaled halothane is metabolised in the body into bromides, chlorides, trifluoroethyl ethanolamine, chlorobromofluoroethylene, and trifluoroaecetic acid which might be less lymphocyte stimulating as opposed to enflurane which is only metabolised to the extent of 2% and therefore lymphocyte exposure to its metabolites is minimal and

stimulates lymphocytes more than does halothane.

Thirty seven (87.4%) of the patients were exposed to halothane while only nine (12.6%) were exposed to enflurane. Six of the patients exposed to enflurane were maintained on intermittent positive pressure ventilation (IPPV). The concentration of inhalational drug used during IPPV was low (0.6% for enflurane and 0.5% for halothane) because the patients were already paralysed and just required a maintenance dose to prevent awareness. Low exposure of drugs to lymphocytes in vivo would cause a more positive response. Majority of the patients exposed to halothane were maintained on spontaneous ventilation. The concentration of the inhalational agent required was usually high so that it could abolish all movements and relaxed the patient following surgical stimuli. Exposure to high concentrations of an inhalational agent would, therefore, inhibit lymphocyte trnsformation. Thus, an overall more positive response was observed in those patients who were exposed to enflurane.

After both enflurane and halothane exposure lymphocytes from patients with no malignancies responded more positively than those with malgnancies. Patients with malgnancies are immunosuppressed and therefore the observations made in this study are in conformity to the immunological stata of these patients.

CONCLUDING REMARKS

Exposure to both halothane and enflurane in vivo in therapeutic concentrations was shown to cause positive responses in lymphocyte transformation following stimulation with both mitogens (PHA, Con A) and antigens (PPD and SKSD) in both patients with malignancies and those without malignancies.

There is evidence to suggest that the use of enflurane does not cause as much immunosuppression as would halothane since the lymphocytes from patients who had been exposed to enflurance responded more to both mitogens and antigens. No follow-up was carried out to assess post-operative relative susceptibility to infections and spread of malignancies. Further comparative studies to cover follow-ups are required in order to include some of the parameters not considered in the present study. Such parameters may include age, sex, surgical conditions, premedications and duration of anaesthetic exposure.

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