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Laboratory Report

Failure of Enflurane and Halothane Anesthesia to Inhibit Lymphocyte Transformation in Volunteers

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Changes in the peripheral blood leukocyte count and in the ability of lymphocytes to transform in response to phytohemagglutinin were studied in healthy volunteers undergoing prolonged enflurane or halothane anesthesia without coincident surgical operation. Anesthesia was associated with a modest leukocytosis that persisted into the first postanesthetic day, primarily due to an influx of neutrophils into the circulation. There was no significant alteration, either during or following anesthesia, in the ability of the volunteers' lymphocytes to transform in response to phytohemagglutinin when compared with either preanesthetic values or unanesthetized controls. Depression of lymphocyte transformation does not appear to follow prolonged enflurane or halothane anesthesia in the absence of a surgical procedure. (Key words: Immune response, volatile anesthetics; Blood, leukocytes, immune response: Anesthetics, volatile, enflurane; Anesthetics, volatile, halothane.)

THE *IN-VITRO* DETERMINATION of the ability of lymphocytes to respond to specific antigens or nonspecific mitogens is commonly accepted as a method for assessing immunologic competency.[1] Since the lymphocyte plays a central role in specific immunity to foreign antigens, a deficiency in this phase of the immune response suggests a predisposition to malignancy or infection.

Depression of lymphocyte transformation has been reported to occur in man after anesthesia and operation. It is demonstrable within two hours of induction of anesthesia,[2] maximal in the immediate postoperative period,[3] and persists for as long as three weeks.[4] This reduced lymphocyte responsivenesss is closely correlated to the extent of surgical trauma,[5-7] the amount of blood lost,[2] and the presence of debilitating disease.[3,8] It has also been suggested that anesthetic agents and techniques do not affect lymphocyte transformation[5,10]; however, the influence of general anesthesia in the absence of coincidental operation has not been determined. In this report we describe the effects of general anesthesia with enflurane or halothane, administered to healthy volunteers, on lymphocyte transformation and the peripheral blood leukocyte count.

Methods and Materials

Enflurane or halothane anesthesia was administered to unpremedicated human volunteers in San Diego. The protocol for these studies was approved by the Human Research Committees of the Veterans Adminis-

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TABLE 1. Effects of Enflurane in Human Volunteers (Mean ± SE)

	Before Anesthesia	Mid-anesthesia	End of Anesthesia	One Day after Anesthesia	Five Days after Anesthesia
Lymphocyte transformation (per cent of pre-anesthetic value)					
Anesthesia (Group I)	100	184 ± 34.6	162 ± 54.1	236 ± 19.8	205 ± 51.0
No anesthesia (Group II)	100	116 ± 6.5	124 ± 9.7	162 ± 56.4†	140 ± 76.8†
Mitogenic index					
Anesthesia (Group I)	4.4 ± 0.6	6.8 ± 0.9 *	8.4 ± 1.4	11.2 ± 1.7	10.1 ± 2.9
No anesthesia (Group II)	4.5 ± 1.0	4.2 ± 0.5	4.6 ± 0.9	5.9 ± 2.9†	11.7 ± 7.5†
Leukocyte count ($\times 1{,}000$ mm^3)					
Anesthesia (Group I)	7.3 ± 0.4	10.0 ± 0.4	12.0 ± 0.7*	9.4 ± 0.5	8.3 ± 0.9
No anesthesia (Group II)	8.3 ± 0.7	8.7 ± 0.9	8.1 ± 0.9	7.3 ± 0.0†	9.5 ± 1.6†
Lymphocytes ($\times 1{,}000$ mm^3)					
Anesthesia (Group I)	2.8 ± 0.2	3.2 ± 0.3	3.3 ± 0.2*	3.1 ± 0.2	3.4 ± 0.4
No anesthesia (Group II)	2.4 ± 0.1	2.4 ± 0.2	2.4 ± 0.2	2.5 ± 0.5†	3.1 ± 0.4†
Hours until into culture					
Anesthesia (Group I)	51.5 ± 0.6*	50.1 ± 3.0*	43.4 ± 0.5	37.0 ± 4.6	43.5 ± 4.5
No anesthesia (Group II)	42.6 ± 4.3	38.3 ± 4.3	44.2 ± 0.3	39.0 ± 8.9†	41.0 ± 11.9†

* Significantly different from corresponding unanesthetized control value ($P \leq .05$).
† n = 2.

tration Hospital and University of California, San Diego. A detailed informed consent was obtained from each subject.

Anesthesia was induced by inhalation, with end-tidal anesthetic gas concentrations maintained between 1.0 and 2.0 MAC for each anesthetic agent. Total anesthesia time was 5 to 7 hours. Seven volunteers were anesthetized with halothane in oxygen. They received no additional medication and were maintained eucapnic by controlled ventilation. Nine volunteers were anesthetized with enflurane in oxygen except for two periods when the carrier gas was changed to 70 per cent nitrous oxide in oxygen. Three of the nine enflurane volunteers each received a single dose of succinylcholine to facilitate endotracheal intubation. While controlled ventilation was used throughout most of the study to maintain Pa_{CO_2} at normal levels, each enflurane volunteer was allowed to breathe spontaneously for two periods, early and late during the anesthesia, to assess the effects of the anesthetic on respiratory function.

Heparinized blood was obtained from the volunteers and simultaneously from a comparable group of unanesthetized control subjects immediately before, midway through, at the end of, one day after, and five days after the procedure. The samples were airmailed at room temperature to Seattle, where they were put immediately into culture.

Lymphocyte transformation in response to phytohemagglutinin (PHA) was assessed by the whole-blood technique of Pauly *et al.*,[11] as outlined in detail previously.[5] Briefly, the leukocyte count was determined electronically, the percentage lymphocytes counted, and a sample of whole blood diluted in unsupplemented RPMI-1640 culture medium (Grand Island Biological Co.) to a concentration of 10^5 lymphocytes per ml. Three-ml volumes of the suspension were then pipetted into polypropylene tubes (Falcon plastics #2063) and 10 μg of PHA (Burroughs Wellcome) were added to each of seven of the tenth tubes prepared for each blood sample. Lymphocytes were incubated for five days at 37 C in an atmosphere of 5 per cent CO_2 in air. Tritiated thymidine (1 μCi) was added to each culture vessel 24 hours prior to the end of the incubation period. The extent of lymphocyte tranformation was quantitated in a liquid scintillation counter and expressed

either as a percentage of the preanesthetic value for each individual or as a mitogenic index where:

$$\text{Mitogenic index} = \frac{\text{DPM of stimulated cultures (PHA added)}}{\text{DPM of unstimulated cultures (no PHA)}}$$

The lymphocytic response of halothane volunteers was also assessed independently by one of us (R.B.) in San Diego, using the method of Park and Good.[12] These specimens were cultured immediately, avoiding any influence of delays associated with transport of cells to Seattle.

Data were analyzed statistically by Student's t test for paired data, comparing the responses during and after anesthesia with preanesthetic values, and by Student's t test for unpaired data, comparing the response of volunteers with that of simultaneously-sampled unanesthetized controls. The level of significance was accepted as $P < 0.05$.

Results

Due to mailing difficulties, there was variation in the intervals from drawing the specimens to establishing the cultures. In general, delays were similar for enflurane-anesthetized volunteers and awake controls, but they differed at the initial and mid-anesthesia sampling periods (table 1). In addition, only two specimens were received from unanesthetized controls on days 1 and 3 of the enflurane study, making these results of little value. Similar time-related variability was not found in the halothane study (table 2).

General anesthesia was associated with a slight leukocytosis that persisted into the first postanesthetic day. Although this was not significant for the halothane volunteers, it was valid for those receiving enflurane compared with both preanesthetic values and values for unanesthetized controls. The increased leukocyte count was mainly due to an influx of neutrophils, although lymphocytosis was also apparent in the enflurane volunteers.

Lymphocyte transformation, expressed as percentage of preanesthetic levels, increased during enflurane anesthesia, but was not significant. (table 1) Because awake subjects demonstrated a similar change in transformation, a normal diurnal variation is suggested. When expressed as a mitogenic index, there was a similar increased transformation during enflurane anesthesia that was not as obvious in unanesthetized controls. Indeed, the mitogenic index was significantly greater in anes-

TABLE 2. Effects of Halothane in Human Volunteers (Mean ± SE)

	Before Anesthesia	Mid-anesthesia	End of Anesthesia	One Day after Anesthesia	Five Days after Anesthesia
Lymphocyte transformation (per cent of preanesthetic value)					
Anesthesia (Group I)	100	111.9 ± 18.0	87.9 ± 10.1	87.3 ± 14.5	98.1 ± 23.1
No anesthesia (Group II)	100	114.0 ± 18.6	126.2 ± 19.6	99.6 ± 10.9	97.8 ± 18.1
Mitogenic index					
Anesthesia (Group I)	3.1 ± 0.5	3.5 ± 0.7	2.8 ± 0.4	2.7 ± 0.4	2.2 ± 0.3
No anesthesia (Group II)	3.8 ± 0.9	4.5 ± 1.0	4.8 ± 0.9	3.8 ± 0.8	3.0 ± 0.5
Leukocyte count (×1,000/mm³)					
Anesthesia (Group I)	6.8 ± 1.0	8.1 ± 0.8	9.0 ± 0.6	8.9 ± 0.9	7.7 ± 2.0
No anesthesia (Group II)	7.5 ± 0.4	7.5 ± 0.3	8.1 ± 0.4	7.5 ± 0.4	8.2 ± 1.1
Lymphocytes (×1,000/mm³)					
Anesthesia (Group I)	2.1 ± 0.2	2.4 ± 0.1	2.2 ± 0.1	2.7 ± 0.2	2.0 ± 0.2
No anesthesia (Group II)	2.4 ± 0.2	2.5 ± 0.1	2.9 ± 0.3	2.6 ± 0.2	2.5 ± 0.2
Hours until into culture					
Anesthesia (Group I)	38.6 ± 4.5	43.7 ± 3.7	42.6 ± 5.2	39.4 ± 9.5	44.7 ± 7.8
No anesthesia (Group II)	38.4 ± 4.5	46.9 ± 5.3	39.3 ± 3.6	39.4 ± 9.5	44.7 ± 7.8

No value was significantly different from the corresponding unanesthetized control value ($P \leq 0.05$).

thetized than in control subjects at the mid-anesthesia sampling period. This single statistically valid difference may be due, in part, to the large variation in times between sampling and establishing the cultures for the mid-anesthesia specimens.

Halothane anesthesia was associated with no significant change in lymphocyte transformation, expressed either as percentage of pre-anesthetic value or as a mitogenic index (table 2). At the end of anesthesia there was a tendency toward slight depression of transformation, which returned to control values by day 5. A normal diurnal variation was again seen in control subjects but was not demonstrable in volunteers given halothane. There was, however, no statistically significant difference between the responses of anesthetized and unanesthetized individuals at any time.

Similar results for halothane volunteers were obtained in our San Diego laboratories using the method of Park and Good. There was no significant alteration in lymphocyte transformation during or after halothane anesthesia when cells were cultured without the delay associated with mailing to Seattle.

Discussion

Anesthetic agents are capable of many cellular effects, and it is reasonable to implicate them in postoperative immune deficiency states. While lymphocyte transformation is inhibited by halothane *in vitro*, the duration of exposure to clinically relevant concentrations must exceed 24 hours.[13,14] The present study demonstrates that halothane or enflurane anesthesia in man is not associated with alterations in lymphocyte transformation in response to PHA. These results corroborate similar observations by Kanto[15] and Cullen,[5] who observed no abnormality of lymphocyte function when patients underwent minor operations with brief anesthesias.

Although the response of volunteers anesthetized with either agent was not associated with any significant difference from the response of unanesthetized controls, the data suggest that enflurane tends to augment transformation and halothane to reduce transformation. Assuming that inhibition of lymphocyte transformation is harmful, these differences suggest that enflurane may be more beneficial than halothane. However, the data do not allow valid comparisons of this type since the volunteers in the two studies were not the same and the experimental protocols differed slightly. First, total anesthetic exposure was greater for the halothane volunteers (mean ± SEM 13.7 ± 0.8 MAC-hours) than for the enflurane volunteers (9.6 ± 0.4 MAC-hours). Second, the enflurane volunteers were subjected to periods of inhalation of nitrous oxide with a reduced inspired oxygen tension. Nitrous oxide has been associated with reduced lymphocyte transformation in human volunteers[16] but not *in vitro*.[17] However, the reduction *in vivo* correlated with the adrenal cortical response to light anesthesia rather than a direct effect of nitrous oxide. Third, volunteers receiving enflurane were subjected to two periods during which their Pa_{CO_2}'s rose to more than 70 torr. This stress was not given to the subjects receiving halothane. Finally, the time from blood collection to placement of cells into culture was more varied in the enflurane study. Although lymphocytes remain viable for at least 57 hours at room temperature[13,18] with little effect on T-cell responsiveness,[19] subtle differences in lymphocyte reactivity at the extremes of acceptable time intervals may, in part, account for the different trends with different drugs. However, the similarity of results when cells cultured in Seattle were compared with those cultured in San Diego suggests that the delay in mailing samples was not a significant factor.

In conclusion, we feel that no significant immunosuppression, as assessed by leukocyte count and by PHA-induced lymphocyte transformation, has been demonstrated in volunteers anesthetized with either halothane or enflurane in the absence of operation. The impaired lymphocytic responsiveness associated with operation must therefore be secondary to other aspects of the procedure, most probably neurohumoral responses to surgical stress.[20,21] The anesthetic drugs appear to affect immune competence more by modifying the response to this surgical stress than by any direct effect on immunocompetent lymphocytes.

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