

## Failure of Halothane Anesthesia to Alter Growth of Sarcoma in Mice

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The effects of halothane anesthesia on growth of sarcoma I tumor in syngeneic (A/Jax) and allogeneic (C57/Black/6) mice were determined. Eleven of 22 A/Jax mice, inoculated IP with  $5 \times 10^3$  cells, were anesthetized for 7 hours with 0.8 per cent halothane in air on the day of inoculation. Twelve of 21 A/Jax mice, inoculated IP with  $5 \times 10^3$  cells, were anesthetized for 7 hours with 0.5–1.0 per cent halothane in air on day 1 and again on day 7. All animals developed ascites tumor and all died. There was no significant difference between mean survival times in anesthetized mice and controls. Nineteen of 35 A/Jax mice, inoculated with  $1 \times 10^3$  cells SC, were anesthetized with 0.7 per cent halothane in oxygen for 5 hours on day 1 and again on day 7. There was no significant difference between anesthetized mice and air-breathing controls with respect to time of tumor appearance, tumor growth rate, or survival time. Twenty-four of 47 C57/Black/6 mice, inoculated with  $5 \times 10^4$  cells SC, were anesthetized with 0.5–0.7 per cent halothane for 5 hours on day 1 and day 7. There was no significant effect of halothane anesthesia with respect to time of tumor appearance, tumor growth rate, or time of tumor regression. (Key words: Cancer; halothane; Anesthetics, volatile; halothane; Immune response; halothane.)

PATIENTS WITH CANCER occasionally have exacerbation of their disease following surgical treatment.<sup>1</sup> Since competency of the immune response is considered fundamental to resistance against cancer,<sup>2</sup> and since anesthesia may inhibit certain aspects of the immune response,<sup>3</sup> it is reasonable to inquire whether anesthesia may contribute to the spread or growth of malignant disease. To assess this possibility, we have observed the

effect of halothane anesthesia on the growth of freshly implanted sarcoma I (SAI) tumors in mice. Studies were performed with syngeneic (A/Jax) mice, which have minimal immunity to the SAI tumor, and with allogeneic (C57/Black/6) mice, which have the immunologic capacity to cause tumor regression.

### Methods

#### STUDIES IN SYNGENEIC MICE

Young, male A/Jax mice (Jackson Laboratories, Bar Harbor, Maine), weighing approximately 20 g, were inoculated with SAI tumor cells obtained from ascitic fluid of tumor-bearing mice. A cell suspension was prepared by dilution of tumor cells in Hank's balanced salt solution (HBSS). The viability of these tumor cells was in excess of 95 per cent, as determined by exclusion of trypan blue dye. Inocula were prepared such that the predetermined number of tumor cells (counted with an electronic cell counter) was contained in 0.1 ml of HBSS. Seventy-eight animals were divided into three groups as follows.

*Group I.* Twenty-two animals were inoculated intraperitoneally with  $5 \times 10^3$  cells. Immediately following the inoculation, 11 animals were anesthetized in an airtight chamber for 7 hours with 0.8 per cent halothane in air. Gases flowed into the 11,000 cu cm-sized chamber at approximately 3 l/min. Halothane concentrations in the chamber were monitored with an infrared gas analyzer. Eleven fasting control mice did not receive anesthesia. All animals were weighed daily to monitor tumor growth, and the date of death was recorded.

*Group II.* Twenty-one animals were inoculated intraperitoneally with  $5 \times 10^3$  cells. Twelve animals were anesthetized for 7 hours with 0.5–1.0 per cent halothane in air on the day of tumor inoculation and again for

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Received from the University of Washington School of Medicine, Seattle, Washington 98195. Accepted for publication April 5, 1974.

TABLE 1. Mean ( $\pm$  SD) Time of Tumor Appearance, Tumor Growth Rate, and Survival Time in Syngeneic (A/Jax) Mice\*

	Group I		Group II		Group III	
	Control	Anesthetized	Control	Anesthetized	Control	Anesthetized
Tumor dose (cells)	$5 \times 10^6$		$5 \times 10^6$		$1 \times 10^6$	
Tumor location	Intraperitoneal		Intraperitoneal		Subcutaneous	
Number of mice	11	11	12	9	16	19
Anesthesia	Air	Halothane/air	Air	Halothane/air	Air	Halothane/O <sub>2</sub>
Tumor appearance (days)					14.4 $\pm$ 5.9	15.1 $\pm$ 2.3
Tumor growth rate (mm/day)					0.9 $\pm$ 0.3	0.9 $\pm$ 0.2
Survival time (days)	19.8 $\pm$ 0.9	19.6 $\pm$ 1.5	21.2 $\pm$ 7.6	19.6 $\pm$ 5.9	36.9 $\pm$ 7.1	35.9 $\pm$ 4.4

\* No statistically significant difference ( $P < .05$ ) was observed.

the same period 7 days later. Nine control animals did not receive anesthesia but were fasting on the days when experimental animals received halothane. All animals were observed daily and the date of death was noted.

**Group III.** Thirty-five animals were included in this group. Two experiments with an identical protocol were performed and nonanesthetized control animals were included on each occasion. All animals had  $1 \times 10^6$  SAI cells injected subcutaneously in the back. Nineteen animals received 7 hours of 0.7 per cent halothane in oxygen on the day of tumor implantation and 5 hours of 0.6 per cent halothane in oxygen again one week later. Sixteen control animals breathing room air fasted on day 1 and day 7. All animals were observed for the day on which tumor appeared and also the day on which the animal died. Rate of tumor growth was determined by measuring the mean diameter of the readily identifiable subcutaneous nodule. Measurement was discontinued if the tumor became ulcerated or if the margins were not distinctly visible.

STUDIES IN ALLOGENEIC MICE

Forty-seven male C57/Black/6 mice (Jackson Laboratories, Bar Harbor, Maine) were inoculated with  $10^6$  SAI tumor cells, subcutaneously, on the back. Twenty-four mice were anesthetized with 0.5–0.7 per cent halothane in air for 5 hours on the day of tumor inoculation, and again for 5 hours one week later. Twenty-three air-breathing con-

trol mice fasted on day 1 and again on day 7. The mice were observed for time of tumor appearance, rate of tumor growth, and time of complete tumor regression.

Results

STUDIES IN SYNGENEIC MICE

The minimum lethal dose of SAI tumor cells in the A/Jax mouse has not been determined. Subcutaneous inoculation of  $10^6$  tumor cells, however, causes progressive tumor growth in more than 85 per cent of normal A/Jax recipients, while more than  $10^5$  cells causes a 100 per cent incidence of tumor growth. All animals bearing SAI tumor die from generalized tumor metastasis, and this occurs more rapidly and with greater frequency when the tumor is implanted intraperitoneally. In the present study, an inoculum of as few as 500 cells was sufficient to initiate tumor growth which progressed to kill the recipient animals. Only 2/78 A/Jax mice survived the challenge with SAI tumor cells, and these two mice were exposed to halothane anesthesia. The data for all A/Jax mice are summarized in table 1.

**Group I.** All mice developed ascites tumors and died. There was no significant difference between mean survival times in control and halothane-anesthetized animals. There was also no significant difference in tumor growth rate as reflected in total body weight gain. As a separate control, five mice were anesthetized for 7 hours with 1 per cent halothane in air but were not inoculated with

TABLE 2. Mean ( $\pm$  SD) Time of Tumor Appearance, Tumor Growth Rate, and Time of Complete Tumor Regression in Allogeneic (C57/Black/6) Mice\*

Tumor dose (cells) Tumor location	Control	Anesthetized
	5 $\times$ 10 <sup>6</sup> Subcutaneous	
Number of mice	23	24
Anesthesia	Air	Halothane/air
Tumor appearance (days)	2 $\pm$ 0.0	2 $\pm$ 0.0
Tumor growth rate (mm/day)	1.6 $\pm$ 0.7	1.5 $\pm$ 0.6
Tumor regression (days)	20.3 $\pm$ 5.5	22.4 $\pm$ 6.1

\* No statistically significant difference ( $P < .05$ ) was observed.

tumor. None of the five mice developed tumor or died during the two months following anesthesia.

**Group II.** All mice in Group II developed ascites tumors and died. The mean survival time in the control group was not significantly different from that observed in mice given two exposures to halothane.

**Group III.** When tumor cells were injected into subcutaneous tissue, there was no significant difference between halothane/oxygen-anesthetized mice and air-breathing control mice with respect to mean time to tumor appearance, mean survival time, or mean rate of tumor growth. All 16 control and 17/19 of the anesthetized animals developed tumor by Day 28. Two mice in the anesthetized group had no gross indication of tumor and were sacrificed 55 days after tumor inoculation. The original number of mice in each group was larger, but three anesthetized and seven control mice died from unknown causes within one week of tumor inoculation. No tumor was apparent on these animals. It is unlikely that this early death resulted from accidental inoculation with an overdose of tumor cells, since death following subcutaneous implantation of as many as 10<sup>9</sup> cells will not occur in less than 16 days.

#### STUDIES IN ALLOGENEIC MICE

All C57/Black/6 mice successfully rejected the tumor when SAI cells were inoculated subcutaneously. There was no significant difference between halothane/air-anesthetized mice and air-breathing controls with

respect to time to tumor appearance, rate of tumor growth, or time of complete tumor regression (table 2). There was no death among anesthetized or control mice.

#### Discussion

This study was undertaken in an attempt to clarify confusion in the literature regarding the effect of anesthesia on cancer. In 1916, Gaylord and Simpson<sup>1</sup> inoculated mice with mammary carcinoma and observed that 30 minutes of chloroform or ether anesthesia on ten successive days significantly increased the incidence of tumor growth. The concentrations of chloroform and ether were not measured. Four decades later, Trevino<sup>2</sup> reported that a single intraperitoneal injection of pentobarbital, or one hour of ether anesthesia, significantly increased the number of tumor "takes" in rats following subcutaneous injection of Walker 256 carcinoma. Agostino<sup>3</sup> inoculated rats intravenously with Walker 256 carcinoma and observed that the number of pulmonary tumor nodules was increased following a 30-minute exposure to chloroform or ether. There was no significant effect from a single intraperitoneal injection of pentobarbital. It was suggested that the increased incidence of pulmonary metastases in anesthetized animals may have been secondary to an alteration in the coagulation status of blood.

In contrast to the above, other reports have suggested that anesthesia does not influence tumor growth. Schatten,<sup>7</sup> for example, observed that anesthesia, operation, and cortisone administration did not affect the growth of S-91 melanoma pulmonary metastases in mice. Mice were inoculated with tumor intravenously and sacrificed 28 days later. All animals developed pulmonary metastases, but the number of lesions per mouse was not significantly increased following "intraperitoneal" anesthesia. The anesthetic agent and dose were not specified. A similar lack of anesthetic effect on tumor growth was noted by Fischer.<sup>8</sup> He injected Walker 256 carcinoma into the portal vein of the rat and noted no increase in hepatic metastases following a short period of ether anesthetic.

Our data substantiate the findings of Schatzen and Fischer. We observed that halothane anesthesia in mice fails to influence the growth rate of SAI tumor, mortality, or the incidence of tumor rejection. This is true whether the tumor is implanted intraperitoneally, or in subcutaneous tissue. It is also true regardless of whether tumor immunity is relatively weak, as in syngeneic (A/Jax) mice, or strong, as in allogeneic (C57/Black/6) mice. The duration of anesthesia was short, and comparable to that utilized in ordinary clinical care of patients. A/Jax mice in Group I received 7 hours of halothane anesthesia on the day of tumor implantation. A/Jax mice in Groups II and III, and C57/Black/6 mice, were given a second 5-7-hour exposure to halothane again one week after tumor implantation. Despite reports that oxygen may be immunosuppressive,<sup>9</sup> we observed no difference in mean survival time when mice anesthetized with halothane in oxygen were compared with non-anesthetized air-breathing controls.

The SAI tumor system was chosen for study because of its accepted use in tumor immunology, its ready availability, and its relatively rapid growth. The tumor was first induced chemically in 1949, and since that time it has been used extensively as a model for both allograft rejection<sup>10</sup> and tumor enhancement.<sup>11</sup> Immunity to the tumor in syngeneic (A/Jax) mice, as evidenced by a decreased rate of tumor growth and prolonged survival time, has been achieved by pretreatment of mice with irradiated tumor cells.<sup>12</sup> Recent studies have also demonstrated that *Mycobacterium bovis*, strain BCG, is effective in preventing tumor growth in A/Jax mice.<sup>13</sup> In allogeneic (C57/Black/KS) mice, enhanced tumor growth has been produced by repeated immunization with tumor cells, and this predisposition to enhanced growth has been passively transferred to normal recipients with hyperimmune serum.<sup>14</sup> Immunity to the SAI tumor is not humoral, *i.e.*, antibody-mediated; rather, it is cell-mediated. In the allogeneic host, immunity has been attributed to the effector activity of specifically immune macrophages capable of killing tumor cells *in vitro*.<sup>13,16,17</sup>

In the present study mice were exposed to

less than 7 hours of halothane anesthesia, on each occasion, in an attempt to mimic the clinical situation. We also timed the anesthetic exposure to coincide with those periods when immunologic defense mechanisms against tumor are the most critical. One such important period, that of tumor recognition, occurs immediately following tumor implantation. This process is thought to require phagocytosis of tumor by mono-nuclear cells. Bruce<sup>18</sup> observed inhibition of bacterial phagocytosis in mice anesthetized with halothane, but halothane does not inhibit phagocytosis *in vitro*.<sup>19</sup> The second critical period for development of an immune response to tumors is thought to occur about one week after tumor inoculation. The effect of anesthesia on this phase of the immune response, involving both humoral and cell-mediated events, has not been fully evaluated. It has been shown, however, that halothane can cause a decrease in production of antibody-producing cells,<sup>20</sup> and that halothane will inhibit phytohemagglutinin-stimulated transformation of lymphocytes.<sup>21,22</sup>

In conclusion, this study has demonstrated that halothane anesthesia does not influence tumor growth, mortality from tumor, or tumor rejection. It is important to stress, however, that the data pertain only to one tumor type administered to two strains of mice. The failure of halothane to have any demonstrable protective or detrimental effects on mouse resistance to SAI does not necessarily imply that all forms of cancer will be unaffected by anesthesia in the surgical patient. The patient incurs considerably greater physiologic stress and usually receives other medications in addition to the inhalation anesthetic. Immunity to all human cancers is also not likely to be identical to the immune response of mice to SAI tumor. The data from this study also do not prove that all inhalation anesthetics are free of effects on tumor immunity. Nevertheless, it would be surpris-

1 Rosenbaum K, Orkin F: The effect of halothane on *in vitro* phagocytosis. Abstracts, Annual Meeting of the American Society of Anesthesiologists, San Francisco, CA, 1973, pp 71-72; Cullen BF: Effect of halothane and nitrous oxide on phagocytosis and human leukocyte metabolism. Presented at the meeting of the International Anesthesia Research Society, San Francisco, 1974.

ing if the effects of other anesthetics on tumor immunity would differ from that of halothane, since the effects of most anesthetics on many immunologic phenomena and cellular processes are, in general, quite uniform.

The authors greatly appreciate the technical assistance of Mr. Scott Mattern. Halothane was donated by Ayerst Laboratories.

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