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Carcinogen Bioassay of Isoflurane in Mice

Jeffrey M. Baden, M.D., Yashdev R. Kundomal, Ph.D.,† Richard I. Mazze, M.D.,‡ Jon C. Kosek, M.D.§*

A carcinogen bioassay of isoflurane was performed in groups of Swiss/Webster mice exposed to either air (n = 181), 0.1% isoflurane (n = 167), or 0.4% isoflurane (n = 165), for 4 h per day, 5 days per week. After 78 weeks of exposure, mice were left untreated for 3 weeks and were then killed. Mice killed at this time when they were 86 weeks of age, and those killed or dying at other times during the study were subjected to complete gross and microscopic examination. Throughout most of the study, mean body weights of mice exposed to 0.1% isoflurane and 0.4% isoflurane were less by 1–5% and 5–8%, respectively, than that of mice exposed to air alone. Otherwise, no gross toxic treatment effects were noted. The first neoplastic lesion was detected 23 weeks after starting treatment and, by the end of the study, 190 tumors had been detected in 179 mice. However, there were no statistical differences among the groups in the number of mice with a particular tumor at a specific site, the ratio of benign to malignant tumors, or the time to tumor appearance. It was concluded that isoflurane is unlikely to have carcinogenic potential and is a remarkably non-toxic anesthetic in mice. (Key words: Anesthetics, volatile: isoflurane. Toxicity: carcinogenicity.)

THE 1970s WILL BE REMEMBERED as a decade in which there was great concern regarding the potential carcinogenicity of naturally occurring and man-made chemicals. Inhaled anesthetics did not escape scrutiny. In 1977, the Food and Drug Administration (FDA) considered initiating a program of mandatory testing of inhaled anesthetics for carcinogenicity and teratogenicity.¶ At the same time, we[1] began a program in our laboratory of carcinogenicity testing of rodents using a protocol similar to that recommended by the National Cancer Institute (NCI), but using one instead of two species.** In previous years, we have reported the results of our studies with halothane, enflurane, and nitrous oxide.[2–4] With this report, which examines the carcinogenic potential of isoflurane ($CF_3CHClOCHF_2$) in mice, we conclude this program.

Materials and Methods

Two hundred and fifty-five male and 258 female 3-week-old Swiss/Webster mice were kept in quarantine for 2 weeks after receipt from the breeder.†† They were individually ear tagged and randomly assigned to three groups. The numbers in each group and the percentage survival at several times throughout the study are shown in table 1. Mice were housed not more than four to a cage in polycarbonate plastic cages with stainless-steel lids and were bedded on ground corn cob.‡‡ They were fed small animal chow§§ and were allowed to drink tap water *ad libitum* except during the daily treatment period when food and water were removed. There was a fixed diurnal cycle of 12 h light and 12 h darkness, with isoflurane exposure during the light phase. All mice were inspected daily for disease and were weighed at least every 4 weeks.

Group I (control group) mice were exposed to compressed air, group II mice to 0.1% isoflurane, and group III mice to 0.4% isoflurane for 4 h per day, 5 days per week, for 78 weeks. Exposures were performed in air-tight, 1000 l capacity, Plexiglas chambers operating at a negative pressure of 1 cm H_2O. Groups of mice were exposed at the same time each day in three separate chambers. In preliminary, subchronic studies it was established that a concentration of 0.4% for 4 h per day was the maximum tolerated dose (MTD) of isoflurane that could be administered in a life-time study, *i.e.*, a dose sufficiently high that it would cause close to, but not greater than, a 10% weight differential compared with control mice and still not cause early death.

Animal cages were randomly placed in the chambers and isoflurane was vaporized with medical grade compressed air delivered to the chambers through latex rubber tubing. An initial flow rate of 20 l/min was used until the desired concentration was achieved (about 10 min), at which time maintenance flows of 5 l/min were used. Isoflurane concentrations were continuously measured with a Miran 1A-IF infrared gas analyzer and were maintained within 5% of the desired value. Chamber temper-

* Associate Professor of Anesthesia (SUSM); Chief, Anesthesiology Service (VAMC).

† Postdoctoral Fellow in Anesthesia/Toxicology (SUSM/VAMC).

‡ Professor of Anesthesia (SUSM); Staff Anesthesiologist (VAMC).

§ Professor of Pathology (SUSM); Assistant Chief, Pathology Service (VAMC).

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Address reprint requests to Dr. Baden: Anesthesiology Service (112A), VA Medical Center, Palo Alto, California 94304.

¶ Requirements for inhalation anesthetic drug products: Studies for carcinogenic and teratogenic potential. Federal Register 42:37538–37543, July 22, 1977.

** Sontag JM, Page NT, Saffioti U: Guidelines of Carcinogen Bioassay in Small Rodents. NCI-CG-TR-1, Technical Report Series No. 1 DHEW Pub No. (NIH) 76-801. National Cancer Institute, National Institutes of Health, Bethesda, Maryland, February 1976.

†† Hilltop Labs, Chatsworth, California.

‡‡ San I-Cell, Paxton Processing Company, Paxton, Illinois.

§§ Wayne Lab Blox, Allied Mills, Inc., Chicago, Illinois.

TABLE 1. Number of Mice and Percentage Survival at Various Stages of the study.

	Sex	Start (5 Weeks of Age)	52 Weeks of Age	Scheduled Killing (86 Weeks of Age)
Control (air)	M	89	81 (91)*	43 (48)*
	F	92	90 (98)	75 (82)
0.1% isoflurane	M	84	77 (92)*	47 (56)*
	F	83	82 (99)	68 (82)
0.4% isoflurane	M	82	72 (88)*	38 (46)*
	F	83	83 (100)	73 (88)

* $P < 0.05$ *vs.* female.

ature was maintained at 25 ± 2° C, humidity at 50 ± 10%, oxygen concentration at 21–23%, and carbon dioxide concentration at less than 0.2%; these conditions were the same in all three chambers. At the end of each exposure, chambers were evacuated within 10 min *via* a wall exhaust system to the outside of the building.

After 78 weeks of exposure, a period without treatment was allowed to insure that examination for carcinogenicity would not be confounded by acute toxic changes. After 3 weeks without treatment, a decision was made to kill all the surviving mice by carbon dioxide overdose, because the number of mice in some groups had fallen close to the minimum required for adequate statistical analysis.

All mice killed at the scheduled time (86 weeks of age) or dying *in extremis* at other times were subjected to complete autopsy examinations. The only exceptions were ten mice in which cannibalism or advanced autolysis precluded examination. The procedures used for the gross autopsy have been described.[1–3] Briefly, more than 40 tissues (table 2) were examined *in situ*, then dissected from the carcass, incised, reexamined, and fixed in 10% neutral buffered formalin. Liver, spleen, kidneys, and testes were weighed fresh. After fixation, tissues were again examined grossly, sectioned, and processed for microscopic examinations. Tissue sections were cut at 4–6 μm and stained with hematoxylin-eosin. Microscopic examinations were performed by a pathologist (J.C.K.) experienced in mouse histology who was unaware of the treatment groups at the time of examination. Consultation was obtained from other pathologists when a histopathologic diagnosis was in doubt.

Intergroup statistical comparisons were made using analysis of variance with post-hoc tests, or Chi-square test; $P < 0.05$ was considered statistically significant.

Results

The mean body weights of mice in group 2 (0.1% isoflurane) and in group 3 (0.4% isoflurane) were less by 1–5% and 5–8%, respectively, than in group 1 (air) throughout much of the study (fig. 1). Otherwise, no gross

TABLE 2. Tissues Examined Grossly and Microscopically

Gross lesions	Colon
Tissue masses or suspect tumors and regional lymph nodes	Rectum
	Mesenteric lymph nodes
Skin	Liver
Mammary gland	Gallbladder
Salivary Gland	Pancreas
Thigh muscle	Spleen
Sciatic nerve	Kidneys
Vertebrae and femur (plus marrow)	Adrenals
	Bladder
Thymus	Seminal vesicles
Larynx	Prostate
Trachea	Testes
Lungs and bronchi	Ovaries
Heart	Uterus
Thyroids	Nasal cavity
Parathyroids	Brain
Esophagus	Pituitary
Stomach	Eyes
Duodenum	Spinal Cord
Jejunum	
Ileum	
Cecum	

toxic treatment effects were noted. Organ weights were not statistically different among the groups (table 3). There were no differences in survival rate among the groups, but, as in our previous studies,[2,3] male mice had a significantly lower survival rate at 52 and 86 weeks of age than female mice (table 1). The reason for this difference was not readily apparent. We speculated previously[3] that fighting and subsequent infection led to the lower survival rate in male mice. In this study, however, fighting males were separated early and did not suffer an inordinately excessive death rate. Presumable, unknown genetic factors are the real cause of the difference.

A total of 190 tumors in 179 mice were detected

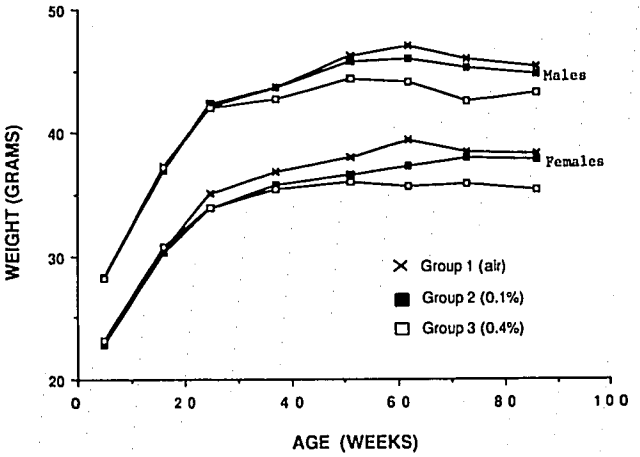

FIG. 1. Mean body weights of male and female mice throughout the study. From 25 weeks of age, mice exposed to 0.1% isoflurane and 0.4% isoflurane weighed less by 1–5% and 5–8%, respectively, than mice exposed to air alone.

TABLE 3. Mean ± SD of Selected Organ Weights for Mice Killed at 86 Weeks of Age (g)

	Control		0.1% Isoflurane		0.4% Isoflurane	
	M	F	M	F	M	F
n	43	71	47	63	38	68
Liver	2.33 ± 0.41	1.77 ± 0.34	2.33 ± 0.53	1.79 ± 0.26	2.35 ± 0.49	1.72 ± 0.21
Spleen	0.14 ± 0.06	0.17 ± 0.09	0.13 ± 0.09	0.16 ± 0.10	0.13 ± 0.01	0.14 ± 0.02
Right kidney	0.46 ± 0.06	0.31 ± 0.12	0.44 ± 0.08	0.29 ± 0.04	0.45 ± 0.08	0.27 ± 0.05
Left kidney	0.46 ± 0.06	0.31 ± 0.02	0.43 ± 0.06	0.28 ± 0.04	0.46 ± 0.07	0.27 ± 0.05
Right testis	0.08 ± 0.02		0.08 ± 0.02		0.07 ± 0.02	
Left testis	0.08 ± 0.02		0.08 ± 0.01		0.07 ± 0.02	

throughout the study (table 4). Fifty-one of these (26.8%) were malignant. No mouse had more than two tumors and there was no association between the occurrence of two tumors and isoflurane exposure. Alveolar cell adenomas of the lung were the tumors most frequently detected, followed by basophilic adenomas of the liver. The high incidence of alveolar cell adenomas of the lung in the control group has been a consistent finding in all our carcinogenicity studies with this strain of mice. Other tumors detected, such as lymphoma, adenoma of the kidney, carcinoma of the breast, carcinoma of the thyroid, and myosarcoma, never occurred in more than two mice per group. Analysis of the number of mice with a particular tumor at a specific site revealed no statistical differences among the groups either for mice dying or killed before 86 weeks of age or those killed at 86 weeks of age. The first neoplastic lesion was detected 23 weeks after starting treatment and, as expected, the frequency of such lesions increased as the mice aged. However, there were no differences among the groups at any time during the study in the number of mice with a particular tumor. Only 11 tumors (6% of the total of 190) were detected solely on histological examination. Detailed gross and microscopic examination of tissues also revealed a variety of degenerative and inflammatory lesions that were unrelated to treatment.

Discussion

Isoflurane was approved for clinical use in 1979 and is now the most widely used volatile anesthetic in the United States. Of the approximately 17.5 million surgical patients who have general anesthesia in the U. S. each year, at least 10 million receive isoflurane alone or in combination with nitrous oxide.¶¶ In addition, approximately 250,000 operating room personnel are chronically exposed to trace concentrations of waste anesthetics.*** Thus, it is important to know whether isoflurane has carcinogenic potential.

Two factors originally led to the FDA proposed rule that all inhaled anesthetics undergo carcinogenicity testing and to the start of our own program of carcinogenicity testing. First was Corbett's finding that exposure to isoflurane increased the incidence of hepatic tumors in Swiss/ICR mice.[4] Second was the body of epidemiologic data available at that time which indicated that waste anesthetic gases may pose a carcinogenic hazard to operating room personnel.*** We initially tested halothane, administering the MTD for 18 months to mice of the same strain used by Corbett.[1] In subsequent years, we studied enflurane and nitrous oxide using similar exposure regimens, all with negative results.[1-3] The results of the present study are also negative, completing the carcinogenicity testing of the commonly used inhaled anesthetics.

In addition to Corbett's study and our study, the carcinogenic potential of isoflurane has been examined in one other animal study. Using the same general protocol

¶¶ Paul Thomas, Anaquest: Personal communication.

*** US Department of Health, Education and Welfare: NIOSH criteria for a recommended standard: Occupation exposure to waste anesthetic gases and vapors. DHEW (NIOSH) pub no. 77-140. Public health service center for disease control, (NIOSH), March 1977.

TABLE 4. Number of Mice with Tumors at Specific Sites

	Dead or Killed Before 86 Weeks of Age						Killed at 86 Weeks of Age					
	Control		0.1% Isoflurane		0.4% Isoflurane		Control		0.1% Isoflurane		0.4% Isoflurane	
	M	F	M	F	M	F	M	F	M	F	M	F
n	46	21	37	20	44	15	43	71	47	63	38	68
Lung	2	1	2	0	2	2	16	14	21	14	10	17
Liver	0	0	1	0	0	1	4	1	6	2	2	0
Other	14	10	7	8	6	6	3	2	4	3	3	6

as Corbett, Eger *et al.*[5] examined the carcinogenic potential of isoflurane, halothane, enflurane, and nitrous oxide. Isoflurane was delivered at concentrations of 0.04%, 0.15%, or 0.6% for 2-h periods every other day both *in utero* during the second half of pregnancy (four exposures) and after delivery (24 exposures). Mice treated in this fashion showed no carcinogenic activity. In their paper,[5] Eger *et al.* discussed the many flaws in study design that could have led to Corbett's positive results. Chief among them were the small group sizes, contamination of the feed by polybrominated biphenyls, the use of historical controls, and failure to perform histologic examinations without knowledge of treatment. Polybrominated biphenyls have subsequently been shown to induce hepatocellular carcinomas in mice.††† Our results agree with those of Eger *et al.*[5] Because we administered isoflurane to the mice for 18 months, a treatment regimen believed to be necessary for adequate carcinogenicity testing,[6] our negative results strengthen the conclusion that isoflurane is not a chemical carcinogen in this species.‡‡‡ Although it is always difficult to extrapolate results from animals to humans, numerous studies over the last two decades have indicated that results of studies in rodents predict the carcinogenic potential of a drug in humans.[7]

Another result from our study is worth emphasizing: no organ damage was detected on histologic examination after 390 administrations of 0.4% isoflurane for 4 h, attesting to the remarkable lack of acute and chronic toxicity of the agent. Similar comments could be made regarding halothane, enflurane, and nitrous oxide, as organ damage was not observed with these agents either. However, there was a large difference in the MTD among the anesthetics. Halothane was by far the least well tolerated anesthetic, with a total of only 25 MAC hours administered in the course of the study compared with 180 MAC hours for enflurane, 230 MAC hours for nitrous oxide, and 460 MAC hours for isoflurane. Our finding that chronic administration of halothane resulted in a smaller weight gain at equipotent doses than the other commonly used inhaled anesthetics is consistent with the results of a study by Stevens *et al.*[8]

Finally, it is of interest that of the total of 190 tumors, all but 11 were identified at gross autopsy. This raises the question of the necessity for performing extensive histological examinations in studies of this type.

††† National Toxicology Program, Technical Report Series, No. 244, Carcinogenicity studies of polybrominated biphenyl mixture (Firemaster FF-1) in F344/N rats and B6C3F$_1$ mice (gavage studies).

‡‡‡ The conclusions would have been strengthened further if we had extended the exposure period to the whole of the life span of the mice as presently recommended by the NCI.

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