Anesthesiology
50:306–309, 1979

Chronic Exposure to Low Concentrations of Halothane–Nitrous Oxide:

Lack of Carcinogenic Effect in the Rat

W. B. Coate, Ph.D., Borge M. Ulland, D.V.M.,† Trent R. Lewis, Ph.D.‡*

The effects of prolonged exposure to low-concentration combinations of halothane and nitrous oxide on tumor incidence, especially with regard to the reticuloendothelial system, were studied. Three groups of 50 male and 50 female Fischer 344 rats each were studied. For seven hours/day, five days/week, for 104 weeks, Group I was exposed to filtered air (control); Group II, to halothane, 1 ppm, and nitrous oxide (N_2O), 50 ppm; Group III, to halothane, 10 ppm, and N_2O, 500 ppm. No evidence of exposure-related effects on body weight, appearance, behavior, survival, or hematologic findings was found. Histologic evaluation of the reticuloendothelial system and of other major organs revealed neither enhancement of the spontaneous tumor rate nor any unusual neoplasm. Thus, this study did not lend support to the hypothesis that these anesthetic agents in low concentrations are responsible for the reportedly higher than average incidence of reticuloendothelial malignancies in operating room personnel. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, volatile: halothane. Cancer. Blood: lymphatic vessels, noditis.)

CORBETT AND CO-WORKERS[1] supplemented the findings of Bruce *et al.*[2] purporting to demonstrate a trend toward a higher than average incidence of reticuloendothelial malignancies among anesthesiologists. The suspected agent was trace-level anesthetic vapors and gases. However, a prospective study by Bruce *et al.*[3] failed to reveal any unusually high incidence of malignancies among anesthesiologists. The purpose of the present study was to assess the effects of chronic inhalational exposure to low concentrations of halothane plus nitrous oxide (N_2O) on the incidence of reticuloendothelial malignancies in a rodent model. The Fischer 344 rat was selected, inasmuch as the spontaneous incidence of such malignancies was known to be relatively low in this strain. Halothane and nitrous oxide were tested because they are the inhalational anesthetics most widely used.[4] The concentrations tested represent levels commonly found in operating rooms without scavenging devices.[5]

* Director, Inhalation Toxicology, Hazleton Laboratories America, Inc.

† Pathologist, Hazleton Laboratories America, Inc.

‡ Chief, Experimental Toxicology Branch, National Institute for Occupational Safety and Health, Cincinnati, Ohio.

Received from the Hazleton Laboratories America, Inc., Vienna, Virginia. Accepted for publication July 14, 1978. Supported by Contract No. CDC-99-74-46 with the National Institute for Occupational Safety and Health.

Address reprint requests to Dr. Coate: Inhalation Toxicology Department, Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia 22180.

Materials and Methods

Three hundred Fischer 344 strain rats, 150 males and 150 females, cesarean section-derived and barrier-sustained, were obtained from Charles River Breeding Laboratories, Inc. When approximately 30 days old, they were evenly divided into three groups of 50 of each sex by random selection. Group I was exposed throughout the study to filtered air only. Group II was exposed to halothane, 1.1 ± 0.4 ppm; plus N_2O, 49.0 ± 5.0 ppm, Group III was exposed to halothane, 9.8 ± 2.6 ppm, plus N_2O, 501.5 ± 49.4 ppm.

The animals in the three treatment groups were exposed in glass and stainless steel chambers 6 cu m in size operated with 1.2 cu m/min airflow. Exposures were conducted for seven hours/day, five days/week, for 104 weeks. Control male and female rats (Group I) were exposed to filtered room air in a similar chamber with flow characteristics identical to those of the chambers housing the treatment groups. The animals were individually numbered with ear tags and housed in groups of five/sex in stainless steel mesh cages with stainless steel top-loading feeders and automatic drinking valves. The cages were arranged at the midline of the chamber on one layer throughout the study. Water and basal laboratory diets (Purina Rat Chow) were available *ad libitum*. The cages were changed and washed weekly. The exposure and concentration monitoring procedures have been described.[6]

Individual body weights were recorded before exposure and weekly for the first 24 weeks, biweekly for the succeeding 28 weeks, once every four weeks for the next 36 weeks, and biweekly thereafter. Daily observations were made for morbidity and mortality. Gross signs of systemic toxicity and incidences, sizes, and locations of tissue masses were recorded at the times body weights were recorded. Complete blood counts were determined for eight male and eight female animals from each group at 13, 26, 52, 78, and 104 weeks.

At the termination of the study, all surviving rats were sacrificed and complete gross necropsies were performed. Necropsies were also done on those animals dying or killed *in extremis* during the study, with the following exceptions among animals that died: Group I, one male and one female; Group II, one

0003-3022/79/0400/0306 $00.70 © The American Society of Anesthesiologists, Inc.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/50/4/306/300189/0000542-197904000-00005.pdf by guest on 01 October 2023

male and three females; Group III, two males and two females. Of these exceptions, two were rats that died early in the study—one Group I female and one Group II female and were inadvertently not necropsied. Advanced autolysis precluded meaningful necropsy in the remaining instances. Heart, liver, spleen, kidneys, testes with epididymides, thymus, hepatic lymph nodes, mesenteric lymph nodes, thoracic lymph nodes, thymic lymph nodes, cervical lymph nodes, axillary lymph nodes, inguinal lymph nodes, bone marrow, unusual lesions, and any tissue masses from each necropsied animal were excised and preserved in 10 per cent neutral buffered formalin. Organ weights were recorded for the heart, liver, spleen, kidneys, and testes with epididymis. These were used to calculate organ weight/body weight ratios. Following fixation in formalin, the tissues§ from each necropsied animal (except for one Group I male that died early in the study and was necropsied, but for which the tissues were not subsequently located) were embedded in Paraplast®, sectioned at 5 μm, slide-mounted, and stained with the hematoxylin and eosin. Bone marrow smears were stained with the Wright–Giemsa stain. All slides were examined under a light microscope by a veterinary pathologist who knew the treatment history of each animal.

Although necropsies were performed on most rats that died, autolysis was too far advanced for diagnostic observations to be made on any lymphatic tissues in one or two animals in each group, and on one or more tissues from a few other animals in each group. Because many regional lymph nodes were undetectable at necropsy, this tissue survey was incomplete for the majority of animals. However, the large majority of animals yielded at least three regional lymph nodes for microscopic evaluation. Femoral bone marrow plugs were usually examined, but when this was not feasible, decalcified sections of femur and/or sternebrae were examined.

Data for terminal body weight, organ weights, and organ weight/body weight ratios were analyzed by a single-classification analysis of variance. For all analyses, the level of probability chosen for rejecting the null hypothesis was ≤ 0.05.

Results

Throughout the study, there was no evidence of any anesthetic exposure-related effect with regard to the physical appearance or behavior of the animals. Incidental occasional findings commonly seen in laboratory rats of this strain were observed at comparable rates in the control and exposed animals of both sexes and increased in frequency as the animals aged. These signs included sores on the body (particularly on the tail), nasal or ocular discharges, staining of the fur, occasional soft feces, and alopecia. Tissue masses and wartlike lesions consistently observed throughout the study were located in the ears, nose, axilla, back, and legs, and were present at comparable incidences in the control and exposed groups.

Survival rates at 104 weeks among the males were 74 per cent (37/50) for Group I, 76 per cent (38/50) for Group II, and 72 per cent (36/50) for Group III. Survival rates among the females were 86 per cent (43/50) for Group I, 78 per cent (39/50) for Group II, and 84 per cent (42/50) for Group III. Of the non-surviving animals, five males and six females in Group I, three males and five females in Group II, and five males and six females in Group III were sacrificed in a moribund condition. Hematologic evaluations showed large variations in percentages of monocytes among the groups in both males and females at weeks 78 and 104. However, no consistent trend was found. All other hematologic variables were within normal limits for this species, and no significant difference among groups was found.

Heart weight, kidney weight, and the kidney weight/body weight ratio for the Group III males were slightly but significantly lower than the corresponding values obtained for the control males, but the values for females in the two groups were comparable. Spleen weights and spleen weight/body weight ratios for Group II and Group III females were appreciably and significantly lower than the corresponding values for control females. The values for males in the three groups were nearly identical. No other significant difference was found among the control and exposed animals.

No exposure-related lesion was observed at necropsy.

On microscopic examination, a variety of nonneoplastic lesions were observed in all groups, without relationship to the experimental regimen. Most commonly observed were chronic renal disease, chronic lymphadenopathy, cholangiofibrosis, and biliary hyperplasia. Lymphadenopathy was characterized by lymphoid atrophy, pigmentation, and plasmacytosis of medullary cords.

No exposure-related pattern of malignancy was evident for any organ system or any of the three groups (table 1). A slight disparity in incidences of mammary fibroadenoma in the control *vs.* exposed

§ The testes were not prepared for histologic examination since they were expected to have and did have a high incidence of benign Leydig-cell tumors typical of this strain of rat.[7]

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/50/4/306/300189/0000542-197904000-00005.pdf by guest on 01 October 2023

TABLE 1. Numbers of Rats with Tumors in Specific Organ Systems (Excluding Leydig Cells)

	Group I		Group II		Group III	
	M (n = 48)	F (n = 49)	M (n = 50)	F (n = 49)	M (n = 48)	F (n = 48)
Hematopoietic						
Monocytic leukemia	6	7	7	4	6	3
Lymphoreticular						
Histiocytic lymphoma						1
Lymphocytic lymphoma		1				
Liver						
Neoplastic nodule	1		3		2	
Biliary carcinoma			1			
Gastrointestinal						
Squamous-cell carcinoma, stomach					1	
Respiratory						
Pulmonary adenoma		1	1	1	1	
Reproductive						
Fibroadenoma, mammary	1	2		5	5	5
Adenoma, mammary				1		1
Arrhenoblastoma, ovary		1				
Papillary adenocarcinoma, uterus				1		
Endometrial stromal sarcoma, uterus		1				
Carcinoma, preputial gland			1			
Endocrine						
Pheochromocytoma, adrenal		1		1		
Islet cell adenoma, pancreas	1					
Islet cell carcinoma, pancreas			1			
Skeletal						
Osteoma, calvarium						1
Osteogenic sarcoma, calvarium			1			
Integumentary						
Fibroma, subcutaneous	3		3		3	2
Fibrosarcoma, subcutaneous		1	2	2	1	
Papilloma, skin		1				
Malignant hemangiopericytoma, skin		1				
Trichofollicular adenoma, skin		1				
Squamous-cell carcinoma, skin		1				
Miscellaneous						
Mesothelioma, coelomic	2	1		2	1	
Osteogeneic sarcoma, coelomic	1					

groups was found, but was considered to reflect a lower than expected incidence in control animals.

Discussion

Under the conditions of this experiment, long-term administration of trace concentrations of nitrous oxide and halothane was not associated with an increase in the incidence of neoplasia in general, and specifically did not increase the incidence of reticuloendothelial tumors. The wide variety of tumors observed in the control and exposed animals has been observed previously in this strain of rat. The overall incidence of monocytic leukemia in this study (14 per cent of males and 10 per cent of females) was considerably lower than the 25 per cent incidence found by Jacobs and Huseby[7] in older Fischer 344 rats. A slightly higher than average incidence of mammary fibroadenoma was found in the exposed groups, but in our opinion was an equivocal finding. Fibroadenoma is not typical of the responses to known mammary carcinogens such as 9,10-dimethyl 1,2-benzanthracene.[8] Furthermore, in our experience with the Fischer 344 rat, the incidences of mammary fibroadenoma in some historical female control groups have, in fact, been higher than that observed in any of the present groups.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/50/4/306/300189/0000542-197904000-00005.pdf by guest on 01 October 2023

The design of this experiment admittedly did not permit detection of a small exposure-related increase in the incidence of neoplastic disease, since the background (control group) incidence was high (32 per cent of the animals had tumors). A 46 per cent incidence in a test group of 100 rats would have been necessary for significance at the 0.05 level of probability. However, the observed incidences of 32 and 31 per cent (not including Leydig-cell tumors), in the low- and high-anesthetic-concentration test groups, respectively, did not even suggest the possibility of a tumorigenic effect.

The body weight growth rates of the males and females in Groups II and III were similar to the growth rates of control males and females. No evidence of an exposure-related effect with regard to physical appearance or behavior was found among the animals exposed to the mixtures of N_2O and halothane. Routine hematologic evaluation (complete blood counts) after 13, 26, 52, 78, and 104 weeks of exposure did not indicate any exposure-related effect. Survival rates were high and were similar for all groups. Thus, there was no indication of any adverse effect of the exposure regimens.

The significantly lower spleen weight (and spleen/body weight ratio) seen in the females exposed to N_2O and halothane at both levels was due entirely to a number of leukemic control rats that had greatly increased spleen weights.

In conclusion, this study lends support to results of the prospective study of Bruce *et al.*,[3] which showed no difference between the incidences of cancer in a sample of anesthesiologists and a control sample. Inasmuch as the total duration of exposure and the segment of the lifespace involved in our study were sufficient to allow for expression of a carcinogenic effect, even with a long latency period, this negative finding should provide some reassurance to those whose occupations have exposed them to traces of these anesthetics for appreciable periods.

References

1. Corbett TH, Cornell RG, Lieding K, et al: Incidence of cancer among Michigan nurse-anesthetists. ANESTHESIOLOGY 38: 260–263, 1973
2. Bruce DL, Eide KA, Linde HW, et al: Causes of death among anesthesiologists: A 20-year survey. ANESTHESIOLOGY 29: 565–569, 1968
3. Bruce DL, Eide KA, Smith NJ, et al: A prospective study of anesthesiologist mortality, 1967–1971. ANESTHESIOLOGY 41:71–74, 1974
4. Linde HW, Bruce DL: Occupational exposure of anesthesiologists to halothane, N_2O, and radiation. ANESTHESIOLOGY 30:363–368, 1969
5. Whitcher CE, Cohen EN, Trudell JR: Chronic exposure to anesthetic gases in the operating room. ANESTHESIOLOGY 35:348–353, 1971
6. Coate WB, Kapp RW Jr, Lewis RT: Chronic low-level halothane–nitrous oxide exposure: Reproductive and cytogenetic effects in the rat. ANESTHESIOLOGY 50:30–38, 1979
7. Jacobs BB, Huseby RA: Neoplasms occurring in aged Fischer rats, with special reference to testicular, uterine, and thyroid tumors. J Natl Cancer Inst 39:303–309, 1967
8. Young S, Cowen DM, Sutherland LE: The histology of induced mammary tumors in rats. J Pathol 85:331–340, 1963

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/50/4/306/300189/0000542-197904000-00005.pdf by guest on 01 October 2023