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Mutagenicity of Experimental Inhalational Anesthetic Agents: Sevoflurane, Synthane, Dioxychlorane, and Dioxyflurane

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A modification of the Ames bacterial assay system employing two histidine-dependent strains of *Salmonella typhimurium*, TA1535 and TA100, was used to test the mutagenicity of four experimental, inhalational anesthetic agents: sevoflurane, synthane, dioxychlorane, and dioxyflurane. None of the anesthetics was mutagenic. Increased activity was seen only with vinylidene chloride, the positive control. (Key words: Anesthetics, volatile: sevoflurane; synthane; dioxychlorane; dioxyflurane. Bacteria: mutagenicity. Toxicity: mutagenicity.)

EPIDEMIOLOGIC AND LABORATORY DATA suggest that inhalational anesthetics may have carcinogenic potential. The Ames[1] Salmonella assay provides a simple test system for the detection of chemical carcinogens as mutagens. Approximately 90 per cent of known animal and human carcinogens examined in this system are mutagenic, and many of the mutagens are carcinogenic. We have previously used this assay to test the mutagenicity of the inhalational agents: halothane, enflurane, methoxyflurane, isoflurane, nitrous oxide, fluroxene, trichloroethylene, divinyl ether, and cyclopropane. Of this group, only the double-bonded compounds, fluroxene, trichloroethylene, and divinyl ether, had mutagenic activity. We have also tested the mutagenicity of oxygen, nitrous oxide at pressures up to six atmospheres, and the urine of operating room personnel working in scavenged suites; with the exception of a marginally positive response in tests of oxygen at high concentrations, these studies have been negative. References to these studies, as well as to approximately 100 related papers are published in our recent review of the mutagenic effects of anesthetic agents.[2] Since publication of that article we have tested four, experimental, inhalational anesthetic agents using the Salmonella assay system. That material is presented here.

Materials and Methods

Two histidine-dependent strains of *Salmonella typhimurium*, TA1535 and TA100, were employed using the method described by Ames *et al.*[1] as modified for volatile agents by Baden *et al.*[3] Anesthetic agents tested were: two ethers, sevoflurane, $CH_2F\text{-}O\text{-}CH(CF_3)_2$ and synthane, $CHF_2\text{-}O\text{-}CHFCF_2CF_2$; and two dioxolanes, dioxychlorane, $C_3H_2Cl_2F_2O_2$, and dioxyflurane, $C_3H_2F_4O_2$. Vinylidene chloride, 3 per cent, was the positive control. Two assay procedures were used. In the first bacteria on petri plates were exposed to test anesthetic vapor (0.1–30.0 per cent) for 8 hours in desiccators. A direct plate assay was also performed in which liquid anesthetic was added to soft agar and bacteria, and the mixture was spread on histidine-deficient culture medium. Tests were run in the presence or absence of a metabolic activation system prepared from the livers of enzyme-induced rats.

Results and Discussion

Sevoflurane and synthane were not mutagenic at concentrations ranging from 0.1–30 per cent in the desiccator assay (table 1). All four agents were negative in direct plate assays. In some assays in desiccators, the higher vapor concentrations of anesthetic produced a decrease in the number of revertants per plate due to cell toxicity. Vinylidene chloride was mutagenic in all assays.

The results of this study are in agreement with previous reports of the mutagenic activity of inhalational anesthetic agents. Since none of the agents tested were double-bonded, it was unlikely that they would have mutagenic activity. This was found to be the case. The Salmonella assay for mutagenicity is of value in the development of new pharmaceuticals as it is a good predictor of the carcinogenicity of chemicals in humans and animals. It is inexpensive to perform and results are available within a few days of beginning the assay. This

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TABLE 1. Number of Revertants per Plate (±SD)*

	Strain	Control Air	Vapor Per Cent (v/v) (Desiccator) 0.1	1	2	10	20	µl/Plate (Direct Plate) 30	1	10	30	Vinylidene Chloride 3 Per Cent
Sevoflurane	TA1535	23 ± 3	27 ± 3	26 ± 7	20 ± 2	23 ± 3	16 ± 1	15 ± 2	15 ± 3	13 ± 4	11 ± 3	69 ± 5
(n = 6)	TA100	128 ± 7	141 ± 9	145 ± 11	124 ± 3	124 ± 23	130 ± 33	150 ± 12	100 ± 17	121 ± 15	100 ± 10	415 ± 25
Synthane	TA1535	18 ± 2	18 ± 3	16 ± 3	16 ± 2	18 ± 2	8 ± 1	6 ± 0	25 ± 6	13 ± 3	15 ± 3	56 ± 7
(n = 6)	TA100	107 ± 3	98 ± 4	97 ± 11	116 ± 17	128 ± 3	18 ± 0	1 ± 19	122 ± 17	100 ± 13	129 ± 13	380 ± 31
Dioxychlorane	TA1535	45 ± 9							41 ± 5	41 ± 6	33 ± 5	408 ± 48
(n = 3)	TA100	141 ± 7							143 ± 17	134 ± 13	146 ± 7	985 ± 35
Dioxyflurane	TA1535	20 ± 5							16 ± 5	26 ± 3	15 ± 3	155 ± 5
(n = 3)	TA100	97 ± 15							114 ± 10	117 ± 13	101 ± 1	611 ± 7

* With liver metabolic activation system (S9).

is in marked contrast to the $500,000 (or more) cost and the two to three years required to perform a lifetime carcinogenicity study in experimental animals. It is unlikely that a pharmaceutical manufacturer would, in the future, develop an anesthetic agent without thought to its carcinogenic potential. The four experimental agents tested in this study are not mutagenic and, thus, probably not carcinogenic.

References

1. Ames BN, Lee FD, Durston WE: An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc Natl Acad Sci USA 70:782–786, 1973
2. Baden JM, Simmon VF: Mutagenic effects of inhalational anesthetics. Mutation Res 75:169–189, 1980
3. Baden JM, Brinkenhoff M, Wharton RS, et al: Mutagenicity of anesthetics: Halothane. ANESTHESIOLOGY 45:311–318, 1976