PATHOLOGY ONCOLOGY RESEARCH
Vol 3, No 1, 1997

Science Press Publishing Ltd. TUDOMÁNY KIADÓ

ARTICLE

Carcinogenicity of Sulfuric Acid in Rats and Mice

Saulé ULECKIENÉ and Laima GRICIUTÉ

Lithuanian Oncology Center, Vilnius, Lithuania

An International Agency for Research on Cancer (IARC) committee recognized aerosol of sulphuric acid as a human carcinogen on the basis of epidemiological studies. No experimental studies on the carcinogenicity, either of sulfuric acid aerosol or of sulfuric acid itself was available. Our aim was to determine whether sulfuric acid is a causal or modifying factor in carcinogenesis, especially in the respiratory tract. We used two species of laboratory animals (both sexes) – 315 Wistar rats and 219 CBAxC57Bl mice in a long term experimental study. The rats were treated with sulfuric acid (maximal tolerated doses, by chronic intratracheal instillations or by gastric intubations) and/or benzo(a)pyrene (by intratracheal instillations). The mice were treated with sulfuric acid (by chronic gastric intubations) and/or urethane (by intraperitoneal injections). We observed the animals throughout their lives and performed gross and microscopic examination of all organs. The results of the first year of study did not provide clear evidence either for sulfuric acid carcinogenicity or for co-carcinogenicity. However, in the second year tumors appeared in those organs where sulfuric acid acted directly. A modifying (stimulating) effect of sulfuric acid on carcinogenesis induced with benzo(a)pyren was observed in rats. Sulfuric acid did not influence lung carcinogenesis induced with urethane in mice. (Pathology Oncology Research Vol 3, No 1, 38–43, 1997)

Key words: sulfuric acid, carcinogenicity, rats, mice

Introduction

Strong inorganic acids may be present in the occupational environment as mists, vapors or gases. The most prevalent is sulfuric acid which occurres in a wide variety of industries.

In 1992, occupational exposure to strong inorganic-acid mists containing sulfuric acid was recognized as carcinogenic to humans.[3] Oncoepidemiological studies showed that sulfuric acid aerosol may increase the risk of laryngeal and lung cancer.[8] Increased lung cancer risk also was observed in the central part of Lithuania near fertilizer industries with major emissions of sulfuric acid aerosol.[2] However, there have been no experimental studies of laboratory animals on the carcinogenicity of either sulfuric acid aerosol or of sulfuric acid itself. Also no data was available on genetic and related effects of exposure to acid mist in experimental systems, and only some in vitro investigations have been performed. Significant increases in the incidence of sister chromatid exchange, micronucleus formation and chromosomal aberrations in peripheral lymphocytes were observed in a single study on workers engaged in the manufacture of sulfuric acid.[3]

The aim of our study was to determine whether sulfuric acid – the main acting component of mist – is a causal or pathogenetic modifying factor in carcinogenesis (especially in respiratory tract carcinogenesis) due to sulfuric acid never acting alone as it is a single component of the mixture of environmental pollutants.

Materials and Methods

The long term experimental study on carcinogenicity of sulfuric acid was performed on 315 Wistar rats and 219 CBAxC57Bl mice of both sexes. The animals were two months of age at the beginning of the experiment and were exposed to maximal tolerated doses. Two routes of exposure were used in rats: per oral and intratracheal, and only per oral in mice. Also, on rats the known chemical carcinogenic

Received: Sept 16, 1996, accepted: Nov 8, 1996

Correspondence: Saule ULECKIENÉ, MD; Lithuanian Oncology Center, Santariskiu 1, 2600 Vilnius, Lithuania, Fax: (370)-2-750164, Tel.: (370)-2-614155

Figure 1. Papilloma of forestomach, sulfuric acid by gavage; HE, x36

substance – benzo(a)pyrene (BP) (Fluka), and on mice – urethane ("purum", Kiev, Ukraine) were administered to determine the synergism of sulfuric acid (purity 98%) with known carcinogens. Urethane induces pulmonary adenomas – grossly visible nodules which can be identified on the surface of the lung within 3-4 months. The multiplicity of tumors, directly related to the dose of the carcinogen, is an important quantitative dividend.[6,7] Also, induced lung adenomas are a good model to see whether modifying agents (in our case, sulfuric acid) influence carcinogenesis.[1]

The animals were kept in plastic cages under normal laboratory conditions and maintained on a conventional diet. Water was given *ad libitum*. The maximal tolerated dose (maximum dose compatible with survival comparable to that of control animals) of sulfuric acid, established by us according to methodical recommendations,[4] was 0.3-0.5 ml 0.6% sulfuric acid in distillated water for rats (given by intratracheal instillations or by gastric intubations) and 0.2 ml 0.2% sulfuric acid for mice (given by gastric intubations).

Figure 2. Squamous cell carcinoma of forestomach, sulfuric acid by gavage; HE, x80

Design of experiments

There were 6 groups of rats: I. (male: 30; female: 30) given 0.5 ml 0.6% sulfuric acid water solution was by gastric intubations once a week for life; II. (m: 30; f: 30) 0.3 ml 0.6% sulfuric acid were given by intratracheal instillations twice a month for 12 months; III. (m: 30; f: 30) BP 5 mg/rat mixed with India black-ink powder* in saline was given by intratracheal instillations twice a month for 2 months (total dose – 20 mg) and 0.3 ml 0.6% sulfuric acid by intratracheal instillations twice a month for 12 months; IV. (m: 30; f: 30) – positive control – BP 5 mg/rat mixed with India ink powder in saline was given by intratracheal instillations twice a month for 2 months (total dose – 20 mg); V. (m: 30; f: 30) – untreated control. VI. (f: 15) – Ii control – 5 mg/rat of India ink powder in saline was given by intratracheal instillations twice a month for 12 months. (India ink powder is not carcinogenic but absorbs and retains the carcinogen, at least for a time.)[5]

There were 4 groups of mice: I. (m: 30; f: 27) 0.2 ml 0.2% sulfuric acid water solution was given by gastric intubations once a week for life; II. (m: 30; f: 22) urethane 10 mg/mouse was given by intraperitoneal injections twice a week (total – 10 injections – 100 mg) and 0.2 ml 0.2% sulfuric acid by gastric intubations once a week for life; III. (m: 30; f: 23) – positive control – were given urethane 10 mg/mouse by intraperitoneal injections twice a week (total – 10 injections – 100 mg). IV. (m: 30; f: 27) untreated control.

The animals were observed for their entire life. Necropsy was performed on all animals which were killed when moribund or found dead. Gross examination was performed on all organs. The following tissues were examined histologically: trachea, lungs, esophagus, stomach, spleen, liver, kidney and all other organs in which pathological changes were identified. The tissues were fixed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin. In some cases mucicarmin and other dyes were also used. Survival time, time of apparition of first tumor, overall tumor morbidity, as well as types of tumor, were considered.

The Students t-test was used to compare experimental and control data.

Results

Rats

Survival was shortest in the BP+0.6% sulfuric acid group (96.1±5.4 weeks); longest in the untreated control group (111.4±3.7 weeks, $p<0.05$). The survival of female rats given sulfuric acid intratracheally or by gavage, as well as of those given BP only, was longer than the survival of males.

Table 1. **Tumor morbidity in rats**

Group/ Treatment	No of animals		No of animals with tumors		No of animals with multiple tumors%	Respiratory tract		Oesophagus forestomach		Lymph-omas	Other	
	Original	Effective*	(%)	M%		B	M	B	M		B	M
I. Sulfuric acid (by gavage)												
	30	27	9 (33.3)	2 (7.4)	1 (3.7)	–	1	8	–	1	–	–
	30	27	12 (44.4)	2 (7.4)	2 (7.4)	–	–	8	–	–	3	2
Total	60	54	21 (38.8)[a]	4 (7.4)	3 (5.6)	–	1	16	1	1	3	2
II. Sulfuric acid (by intratracheal instillation)												
	30	28	14 (50.0)	9 (32.1)	4 (14.3)	–	1	6	4	3	2	3
	30	28	14 (50.0)	10 (35.7)	6 (21.4)	1	2	6	2	5	2	2
Total	60	56	28 (50.0)[c]	19 (33.9)[d]	10 (17.9)[a]	1	3	12	6	8	4	5
III. BP + Sulfuric acid												
	30	24	8 (33.3)	4 (16.7)	2 (8.3)	–	2	7	–	1	–	–
	30	28	14 (50.0)	12 (42.9)	6 (21.4)	–	4	4	1	5	2	4
Total	60	52	22 (42.3)[b]	16 (30.8)[c]	8 (15.4)[a]	–	6	11	1	6	2	4
IV. BP												
	30	24	8 (33.3)	4 (16.7)	2 (8.3)	–	1	6	–	2	1	1
	30	25	7 (28.0)	7 (28.0)	3 (12.0)	–	2	3	–	3	–	3
Total	60	49	15 (30.6)[a]	11 (22.4)[b]	5 (10.2)[a]	–	3	9	–	5	1	4
V. Untreated control												
	30	27	4 (14.8)	1 (3.7)	1 (3.7)	–	–	6	1	1	1	–
	30	30	9 (30.0)	3 (10.0)	2 (6.7)	–	–	3	–	2	2	1
Total	60	57	13 (22.8)	4 (7.0)	3 (5.3)	–	–	9	1	3	3	1
VI. Ii control	15	14	4 (28.6)	2 (14.3)	2 (14.3)	–	–	1	–	–	1	3

* No. of animals for which complete histological investigation could be performed; B – benign, M – malignant; (a) $p>0.05$ (b) $p<0.05$, (c) $p<0.01$, (d) $p<0.01$ (in comparison with untreated control group, Yates corrected p–values; Ii – India ink

During the first year of study, the main pathological changes detected in dead or moribund animals were bronchitis and chronic pneumonias with abscesses.

The first malignant tumor, a generalised lymphosarcoma with expansion to lungs, was noticed at the 34th week in a female rat given BP+0.6% sulfuric acid intratracheally. There were only a few tumors in the various experimental groups during the first year of study: lymphosarcoma, mammary gland adenocarcinoma, preputial gland carcinoma, forestomach micropapilloma and adrenal gland adenoma. There were no tumors in any of the control groups.

During the second and third year, various tumors appeared, both benign and malignant, mainly of the respiratory tract, forestomach and lymphomas.

Tumor morbidity in rats is shown in *Table 1*. The rats exposed to gavage of 0.6% sulfuric acid had an increased incidence of benign forestomach tumors (papillomas or micropapillomas; *Fig.1*): 29.6% versus 15.8% in untreated control groups ($p<0.1$). Two malignant tumors of forestomach (squamous cell carcinoma; *Fig.2*) occurred: one in the treated and one in the untreated control animals (1/54, 1/57). Hyperplasia of the epithelium of the forestomach, hyperkeratosis and acanthosis were frequently observed in the group receiving 0.6% sulfuric acid. In addition, in a few cases, atypical proliferation of the epithelium was observed. One malignant lung tumor – a poorly differentiated adenocarcinoma – was noticed in a male rat of this group.

Following intratracheal instillations of 0.6% sulfuric acid or sulfuric acid and/or BP, various tumors occurred, however, the frequency of them in the treated groups varied. The animals treated with 0.6% sulfuric acid and/or BP had a higher incidence of and multiplicities of malignant tumors than the untreated control. The first respiratory tract tumor, squamous cell carcinoma of the lung, was noted at 69th week in a female rat given the combined treatment – BP and 0.6% sulfuric acid. In the groups which received only BP or 0.6% sulfuric acid, the first respiratory tumors appeared 17 and 26 weeks later, respectively. The average periods before lung tumors observed in rats given intratracheal instillations of BP + 0.6% sulfuric acid, BP or 0.6% sulfuric acid, were 88, 105 and 118 weeks, respectively. The incidence of malignant lung tumor was highest (11.5%) in the group which received BP + 0.6% sulfuric acid. The histological types of respira-

Tabel 2. **The histological types of respiratory tumors appearing in rats after intratracheal exposure to 0.6% sulfuric acid and/or BP**

Group / *Treatment*	*The histological types of respiratory tumors*	
	*Benign**	*Malignant*
0.6% sulfuric acid	Angiopericitoma of lungs	Chondrosarcoma of trachea *(Fig.3)* Bronchial adenocarcinoma Histiocytoma of lungs *(Fig.4.)*
BP+0.6% sulfuric acid	–	Anaplastic carcinoma of lungs Chondrosarcoma of trachea Bronchoalveologenic carcinoma Spinocellular carcinoma of lungs Fibrosarcoma of lungs
BP	–	Spinocellular carcinoma of lungs Haemangiopericitoma of lungs Anaplastic carcinoma of lungs

* Bronchial polyps (micropolyps) were not included in this table

tory tumors are shown in *Table 2*. Note that no lung tumors were observed in control rats.

Both lymphomas and malignant tumors at various sites (6 forestomach tumors among them) were seen more frequently in groups exposed to intratracheal instillations of the tested chemical substance when compared to the untreated control. The tumors occurred both in males and females. The small numbers which do not allow speculation on prevalence by sex. 22.8% (13/57) and 28.6% (4/14) of animals with spontaneous tumors were identified in the untreated and Ii control groups, respectively. These rats mostly developed benign tumors, mainly micropapillomas of forestomach. The number of animals with malignant tumors in the untreated control was still lower.

Mice

The shortest survival was observed in the group which received urethane + 0.2% sulfuric acid (87.7±5.7 weeks) and longest in the untreated control group (110.1±7.4 weeks) ($p<0.05$).

Pulmonary tumors, as well as lymphomas, were the most frequent tumors in all exprimental groups. Pulmonary adenomas were induced in all mice in urethane or urethane + 0.2% sulfuric acid groups (*Table 3*). Only a few adenomas became malignant and expanded into all lobes of the lungs. There was no significant difference in tumor number per mouse or in the size of lung adenomas (usually 1 mm in diameter).

The number of mice with pulmonary adenomas in the group which received only 0.2% sulfuric acid by gastric intubations and in control group was 19% and 27%, respectively. Tumors were observed only during the second year. Lymphomas were found in all experimental groups both in males and females (*Fig.5*). It is known that they occur spontaneously in CBAxC57Bl mice during the second half of life. The lower rate of mice with lymphomas in groups which received urethane or urethane + 0.2% sulfuric acid is certainly related to the shorter survival of these animals.

Apart from lung adenomas and lymphomas, several forestomach tumors were observed in the experimental groups, both in males and females. The incidence of forestomach papillomas (micropapillomas) was higher in groups which received only 0.2% sulfuric acid (4/47) or urethane + 0.2% sulfuric acid (5/46) as compared with the untreated control (2/45) or urethane group (2/47). The difference, however, was not significant ($p<0.1$). There were no malignant tumors of the forestomach in any group.

Hyperplasia of the epithelium, hyperkeratosis, as well as acanthosis were frequently observed in the forestomachs

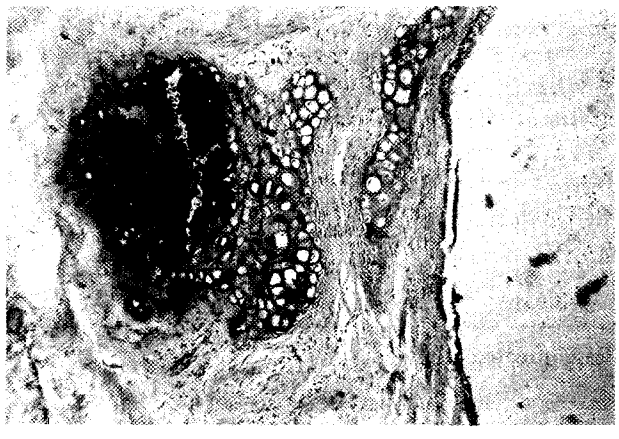

Figure 3. *Chondrosarcoma of trachea, sulfuric acid, intratracheal; HE, x36*

Figure 4. Histiocytoma of lung, sulfuric acid intratrach., HE, x80

of mice exposed to 0.2% sulfuric acid. There were only a few tumors at other sites in the various groups: 1/47 (I) 5/46 (II), 1/47 (III) and 3/45 (IV).

Discussion

From to the literature, there are some hypothesis on the role of sulfuric acid acrosol in carcinogenesis.[8] One proposes that sulfuric acid may be a tumor promoter through the mechanism of chronic tissue irritation. Other suggests that it may be that sulfuric acid, instead of acting as a direct carcinogen or as a promoter itself, can work only together with other agents. Therefore, it was not known whether sulfuric acid is a carcinogenic or a modifying factor.

The aim of this study was to answer three questions: 1. Is sulfuric acid carcinogenic to laboratory animals? (Group I of rats and mice); 2. Is sulfuric acid carcinogenic in the respiratory tract? (Group II of rats); 3. Does sulfuric acid influence the carcinogenicity of benzo(a)pyrene and urethane in the respiratory tract? (Groups III-IV of rats and Groups II-III of mice).

The results of our expriments demonstrated that sulfuric acid is carcinogenic to experimental animals because

- sulfuric acid increased overall tumor morbidity, as well as the number of malignant tumors in different localizations, both in rats and mice;
- sulfuric acid given to rats intratracheally induced several malignant tumors of the trachea and lungs – sites where spontaneous lung tumors occur extremely rarely in these animals;
- in the groups of rats and mice given sulfuric acid by gavage, the morbidity of papilloma of the forestomach was higher than in controls, though not significant statistically.

The fact that the majority of tumors, both malignant and benign, appeared in the organs on which sulfuric acid worked directly – in the trachea and lungs after intratracheal instillations and in the forestomach when given by gavage – showed that it is a locally acting carcinogen. Notably, sulfuric acid aerosols act locally in humans as well: laryngeal

Table 3. **Lung adenomas in mice CBAxC57BL**

Group	*Treatment*	*No. of mice*		*With lung adenomas (%)*	*Average number of lung adenomas per mice (M±m)*
		Original	*Effective*		
I. 0.2% sulfuric acid (by gavage)					
		30	23	5 (22%)	0.3 ± 0.1
		27	24	4 (17%)	0.4 ± 0.2
	Total	57	47	9 (19%)	0.4 ± 0.1
II.Urethane (i.p.)* + 0.2% sulfuric acid (by gavage)					
		30	27	27 (100%)	6.1 ± 0.7
		22	19	19 (100%)	7.2 ± 0.9
	Total	52	46	46 (100%)	6.7 ± 0.7
III. Urethane (i.p.)					
		30	24	24 (100%)	5.9 ± 0.7
		23	23	23 (100%)	5.2 ± 0.5
	Total	53	47	47 (100%)	5.6 ± 0.4
IV. Untreated control					
		30	22	8 (36%)	0.6 ± 0.2
		27	23	4 (17%)	0.5 ± 0.2
	Total	57	45	12 (27%)	0.6 ± 0.1

* i.p. – intraperitoneally

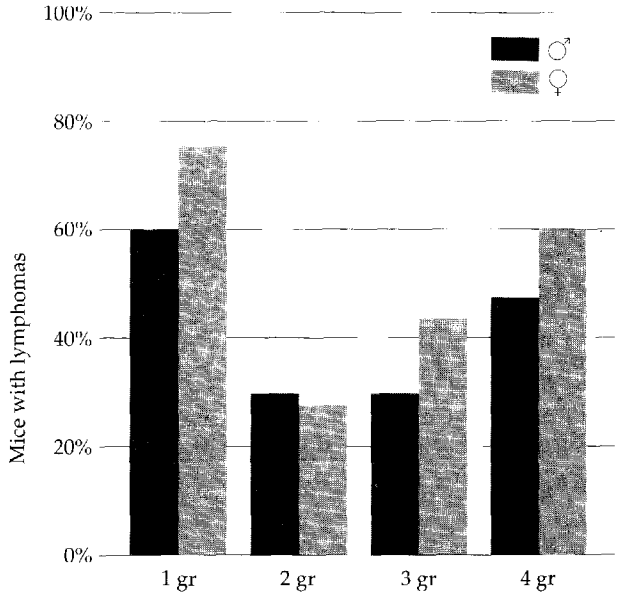

Figure 5. The percentage of mice (CBAxC57BL) with lymphomas: 1 group – 0.2% sulfuric acid (by gavage); 2 group – urethane (i.p.) + 2% sulfuric acid (by gavage); 3 group – urethane (i.p.); 4 gr.-untreated control

and pulmonary tumors were observed in persons exposed to the air polluted with sulfuric acid mists.[3]

Sulfuric acid must be considered to be a weak chemical carcinogen, due to the fact that:

- the tumors that appeared in respiratory tract were not numerous;
- the forestomach tumors were mostly benign and the morbidity differences in control groups were statistically insignificant;
- during the first year of experiment, the first half of animals life, there were almost no malignant tumors.

Also the synergistic action of sulfuric acid and BP was observed:

- in the group of rats given both sulfuric acid and BP by intratracheal instillations, there were more malignant tumors than in those that were given BP alone?
- the time of manifestation of the first malignant tumor, as well as the first malignant lung tumor, was shortest in the group of rats which were given both compounds.

We have no explanation for the slight sex differences in tumor morbidity for animals which were treated with sulfuric acid.

In conclusion, long term experimental study has shown that sulfuric acid itself is a weak, locally acting chemical carcinogen which may work synergistically with other locally acting carcinogenic substances (there was no synergism with the organotropic carcinogen – urethane). Though no sulfuric acid aerosol was used in the study, our feeling is that the main acting factor of the mist is the sulfuric acid itself.

Therefore, we consider that the establishment of carcinogenicity of this acid is an adequate experimental confirmation of epidemiological data.

Acknowledgements

The study received financial support from the International Agency for Research on Cancer. The authors express their appreciation to Dr. Mrs. I. R. Didziapetris for statistical evaluation of the results and to Mrs I Genienc for editing the manuscript.

References

1. *Barauskaite S:* Modifying effect of some chemical factors on carcinogenesis, induced with urethane or N-nitrosodiethylamine in mice: M.D. thesis (In Russ.), Vilnius, 1987.
2. *Gurevicius R:* Methological aspects of descriptive epidemiology of lung cancer (according to the material of Lithuania): M.D. thesis (In Russ.), Vilnius, 1987.
3. IARC Monographs on the Evaluation of carcinogenic risks to humans. Vol. 54, Lyon, France, 41-119, 1992,
4. Methodological recommendations for the investigation of carcinogenic effects of chemical substances and biological products in long term studies on animals (In Russ.), Moscow-Leningrad, 1981.
5. *Shabad LM, Pylev LN, Kolesnichenko TS:* Importance of the deposition of carcinogens for cancer induction in lung tissue. J Nat Cancer Inst 33:135-141, 1964.
6. *Shimkin MB, Stoner GD:* Lung tumors in mice: application to carcinogenesis bioassay. Advan Cancer Res 21:1-58, 1975.
7. *Shimkin MB, Weisburger JH, Weisburger EK, et al:* Bioassay of 29 alkylating chemicals by the pulmonary-tumour responses in strain A mice. J Nat Cancer Inst 36:915-935, 1966.
8. *Soskolne CL, Zeighami EA, Hanis NM, et al:* Laryngeal cancer and occupational exposure to sulfuric acid. Am J Epidemiol 120:358-369, 1984.