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A/J MOUSE AS A MODEL FOR LUNG TUMORIGENESIS CAUSED BY TOBACCO SMOKE: STRENGTHS AND WEAKNESSES

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□ *Strain A/J mice have successfully been used to develop an animal model for tobacco smoke carcinogenesis. In 18 individual studies, reported by 4 different laboratories, a significant increase in lung tumor multiplicities following exposure from 50 to 170 mg/m^3 of total suspended tobacco smoke particulates was found in 15 studies (83%) and a significant increase in lung tumor incidence in 10 studies (56%). However, tumor multiplicities are comparatively low (from an average of 1.1 to 2.8 tumors per lung). From a toxicological standpoint, this indicates that cigarette smoke is a weak animal carcinogen. Although the assay allowed one to detect substantial chemopreventive activity of a mixture of myo-inositol and dexamethasone, it was less successful in showing efficacy for several other agents.*

Keywords *A/J mouse, mice, lung tumors, tobacco smoke, chemoprevention*

EXPOSURE OF STRAIN A MICE TO TOBACCO SMOKE

The first experiment in which strain A mice were exposed to tobacco smoke appears to have been done as early as 1943 [1]. The smoke concentration seemed to be inordinately high—1000 mg/m^3 of smoke particulates—and yet, despite a 250-day exposure, no increase in tumors was found. About 10 years later, Essenberg observed an increased lung tumor incidence in strain A mice exposed for 1 year: 91% in the smoke-exposed animals versus 59% in the controls [2]. The findings were confirmed in one later study by the same author [3], but not in another one [4]. Although these early experiments were suggestive of the possibility that strain A mice might serve as a model to mimic tobacco smoke carcinogenesis in experimental animals, the results were not really conclusive.

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Renewed interest in tobacco smoke toxicity and carcinogenesis was prompted by the raising concern about the effects of environmental tobacco smoke (ETS) [5]. This concern led to a newer series of studies beginning in 1990. Initially, no positive results were obtained. In 1995 we reported that a 6-month exposure to a low concentration of ETS (4 mg/m^3 of total suspended particulate matter [TSP]) did not increase lung tumor multiplicities or incidence in male strain A/J mice [6]. Exposure of the same strain to much higher concentrations of tobacco smoke (248 mg/m^3) for 26 weeks, followed by a 5-week recovery period, failed to provide evidence for increased carcinogenic potential or for lung tumor promotion in mice treated with the tobacco smoke–specific nitrosamine NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) [7]. It was a modification of the conventional exposure protocol that eventually allowed us to demonstrate an unequivocal effect of ETS on lung tumorigenesis [8]. Strain A mice were exposed first to a comparatively high concentration of ETS, generated from the sidestream (89%) and mainstream (11%) smoke from burning Kentucky 1R4F cigarettes, as described before in detail [9]. After a 5-month exposure, the animals were allowed to recover in air for another 4 months before evaluation of the lung tumor response. The same protocol was eventually adopted by three other laboratories [10–12]. Table 1 summarizes the available data from experiments that had used this protocol. It can be seen that in 6 experiments, where strain A mice were exposed to up to 99 mg/m^3 of TSP, lung tumor multiplicities were significantly higher in 4 out of 6 experiments and incidences in 2 out of 6. At higher concentrations of TSP (between 100 and 176 mg/m^3 of TSP), 11 out of 12 experiments showed a significant difference in lung tumor multiplicity between smoke-exposed and controls animals and incidences were significantly higher in 8 of the 12 experiments.

The accumulated data show some interesting dose-effect relationships. If plotted in absolute terms, i.e., tumor multiplicities as a function of average concentrations of TSP, the data show a comparatively shallow dose-response curve that nevertheless deviates significantly from 0 ($P < .05$) (Figure 1). The graph also shows some considerable variation in background (zero exposure) tumor multiplicities. If the response is calculated for each experiment as being proportionally increased over background, the dose response is no longer apparent and the curve runs parallel to the x axis (Figure 2). A third possibility would be to calculate a dose-response by subtracting in each experiment the background multiplicity found in the control group from the tumor multiplicity seen in the experimental group. In this case, a dose-response becomes apparent again and the slope of the curve deviates significantly from 0 (Figure 3).

The flat dose-response suggests that tobacco smoke is a comparatively weak carcinogen. A previous study in which a dose-response was conducted in one single experiment came to the same conclusion [13]. It may to some

TABLE 1 Summary of Inhalation Studies in A/J mice With Tobacco Smoke Generated From Kentucky 1R4F Cigarettes

TSP[a]	Controls[b]	Smoke[b]	TS/Air[c]	Reference
53	0.7 ± 0.2 (23) 13/23 (57%)	1.1 ± 0.2 (21) 15/21 (71%)	1.6	8
83	0.22 ± 0.15 (9) 2/9 (77%)	1.11 ± 0.26 (9)[d] 7/9 (78%)	5.1	10
87	0.5 ± 0.2 (24) 9/24 (38%)	1.4 ± 0.2 (24)[d] 20/24 (83%)[d]	2.8	8
79	0.5 ± 0.1 (24) 10/24 (42%)	1.3 ± 0.3 (26)[d] 15/26 (58%)	2.6	59
83	0.9 ± 0.2 (29) 20/29 (69%)	1.3 ± 0.1 (33)[d] 24/33 (73%)	1.4	46
99	0.7 ± 0.2 10/25 (40%)	1.1 ± 0.2 18/25 (72%)[d]	1.6	13
100	0.5 ± 0.08 (38%)	1.07 ± 0.1[d] (65%)	2.1	11
120	0.7 ± 0.2 10/25 (40%)	2.2 ± 0.3[d] 23/25 (92%)[d]	3.1	13
120	0.25 ± 0.1 (20) 5/20 (25%)	1.05 ± 0.17 (20)[d] 15/20 (75%)[d]	4.4	10
132	1.2 ± 0.2 (25) 15/25 (60%)	2.3 ± 0.3 (26)[d] 23/26 (88%)[d]	1.9	50
132	0.6 ± 0.1 (30) 15/30 (50%)	2.1 ± 0.3 (35)[d] 30/35 (85%)[d]	3.5	41
137	1.0 ± 0.1 (54) 35/54 (65%)	2.4 ± 0.3 (28)[d] 25/28 (89%)[d]	2.4	42
137	0.9 ± 0.2 (30) 18/30 (60%)	2.8 ± 0.2 (38)[d] 38/38 (100%)[d]	3.1	42
147	1.6 ± 0.2 (34) 25/34 (74%)	2.4 ± 0.3 (29)[d] 27/29 (93%)	1.5	58
156	1.0 ± 0.2 (24) 18/24 (75%)	1.9 ± 0.3 (25)[d] 22/25 (88%)	1.8	50
161	1.1 ± 0.3 (15) 11/15 (73%)	1.6 ± 0.3 (22) 17/22 (77%)	1.5	44
161	0.9 ± 0.2 (47) 26/47 (55%)	2.1 ± 0.2 (52)[d] 48/52 (92%)[d]	2.3	44
176	0.7 ± 0.2 (25) 10/25 (40%)	1.9 ± 0.3 (22)[d] 18/22 (82%)[d]	2.7	13

[a]Average concentration of TSP in inhalation chambers for most of the experiment (in some instances, this concentration was only reached after a 5-week acclimatization period in which TSP concentrations were gradually increased [27, 41, 42, 58].

[b]Data for lung tumor multiplicity are given as mean ± SE with number of animals in parenthesis; incidence data are given as number of animals with tumors/total number of animals at risk (%).

[c]Ratio of lung tumor multiplicity in tobacco smoke–exposed animals/controls.

[d]Significantly different ($P<.05$) from controls with Student's t test for multiplicity and Fisher's exact test for incidence.

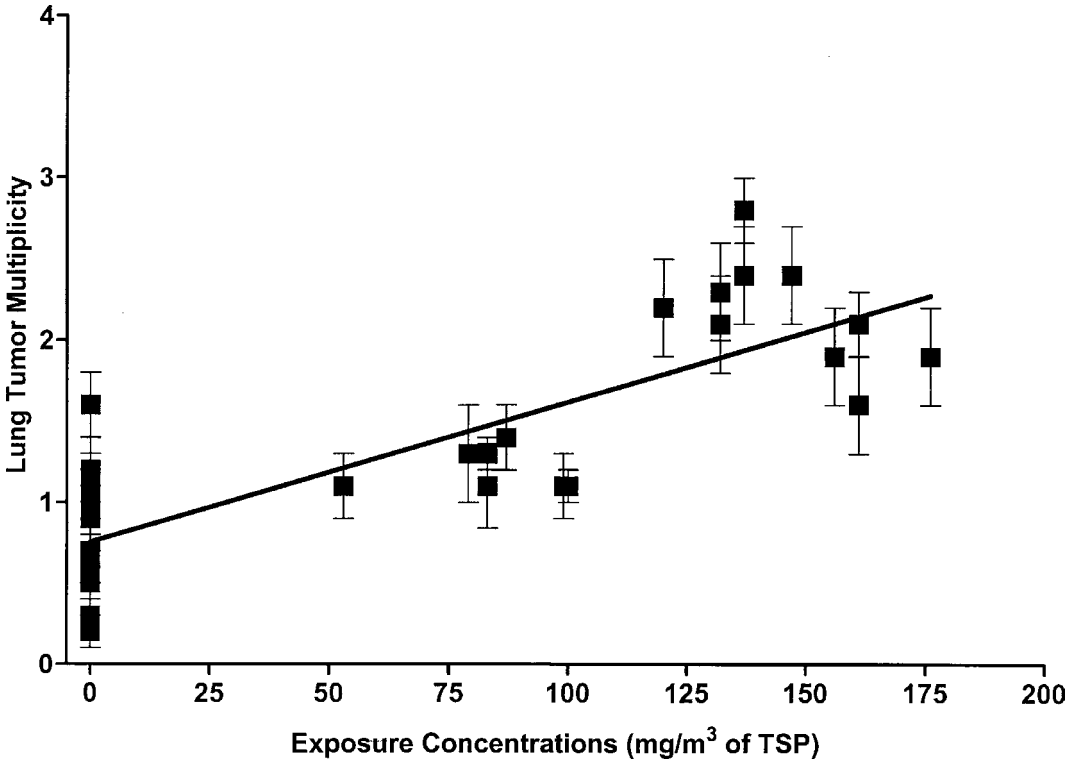

FIGURE 1 Lung tumor multiplicities in strain A/J mice exposed to tobacco smoke plotted as a function of average concentration of smoke (mg total suspended particulates/m^3). Slope of the regression line significantly different from 0 ($P<.05$). For references, see Table 1.

extent explain why most inhalation studies done with tobacco smoke in mice failed to give a positive tumor response [14, 15]. The fact that "only" 10% to 25% of all smokers develop lung cancer [16] might also be construed to indicate that tobacco smoke is not a very potent carcinogen in man. This of course is meant only in a toxicological sense and the observations should by no means be construed to imply that tobacco smoke is not an important, if not the most important, human carcinogen. Its devastating effects on human health have been amply documented by multiple epidemiological studies. The smoking of cigarettes, through the sheer magnitude of its widespread consumption, makes tobacco smoke a major current and future public health problem [17, 18].

In summary, strain A mice may provide an experimental model for the study of tobacco smoke carcinogenesis. This could make it a convenient tool for hazard assessment and, possibly, even risk assessment. It is interesting to note that when data from this model were used for potency estimation and human risk assessment, the calculated range of risk overlapped the risk implied in humans derived from case control studies showing increased lung cancer risk in lifelong nonsmokers married to smokers [19].

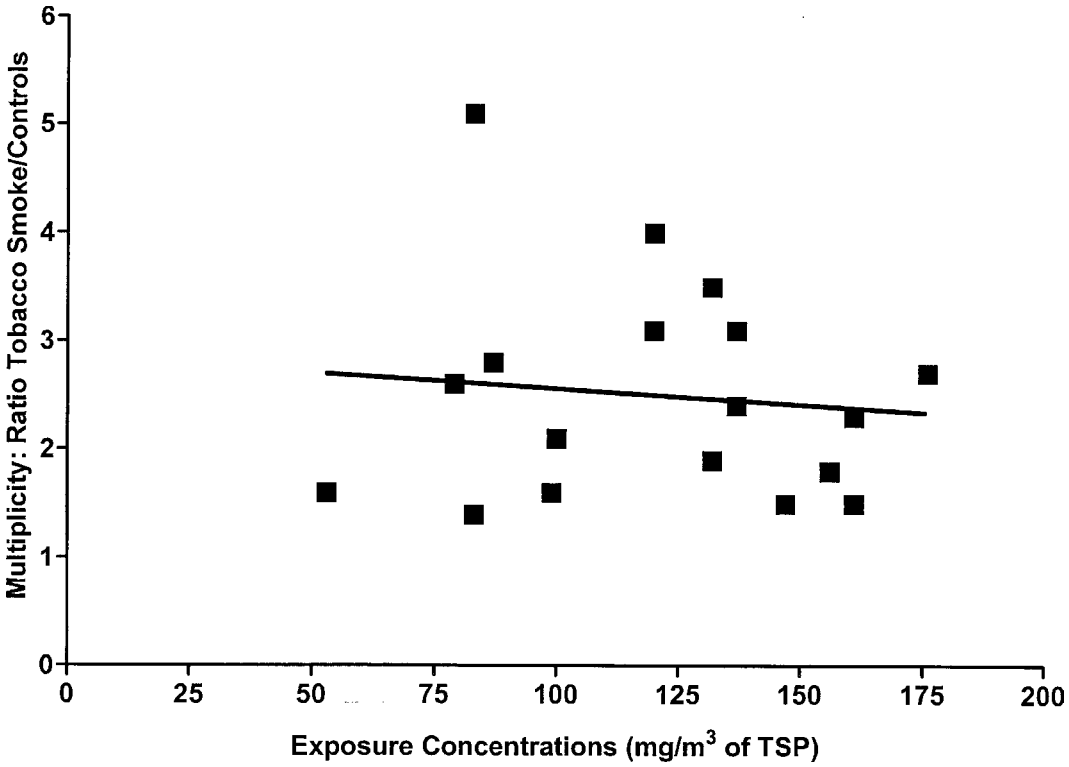

FIGURE 2 Same data as in Figure 1, except that lung tumor multiplicities in smoke-exposed animals are plotted as fractions of corresponding controls. The slope of the regression line does not deviate from 0.

THE STRAIN A MOUSE LUNG TUMOR AS A SHORT-TERM CARCINOGENICITY TEST

The renewed interest in strain A mouse lung tumorigenesis deserves a general discussion on this particular carcinogenicity model. The test was initially recommended by Shimkin and Stoner as a general short-term screen for carcinogens. Their recommendation was based on an extensive body of work with multiple compounds in a standardized assay and has been summarized repeatedly [20, 21]. Most assays were performed by injecting the test compounds by the intraperitoneal route. After 4 to 6 months, the tumorigenic response was assessed by counting lung tumors visible on the surface under a dissecting microscope. Lung tumor multiplicity can then be calculated and is defined as the average number of lung tumors per experimental animal, including non–tumor-bearing animals. Table 2 summarizes the criteria by which the results of the assay should be interpreted. Lung tumor incidence was judged to be of lesser importance because it would not yield additional useful information. Interestingly, the assay was used only in a very few inhalation experiments. An increase in lung tumor multiplicity was observed following inhalation of bis(chloromethyl)ether,

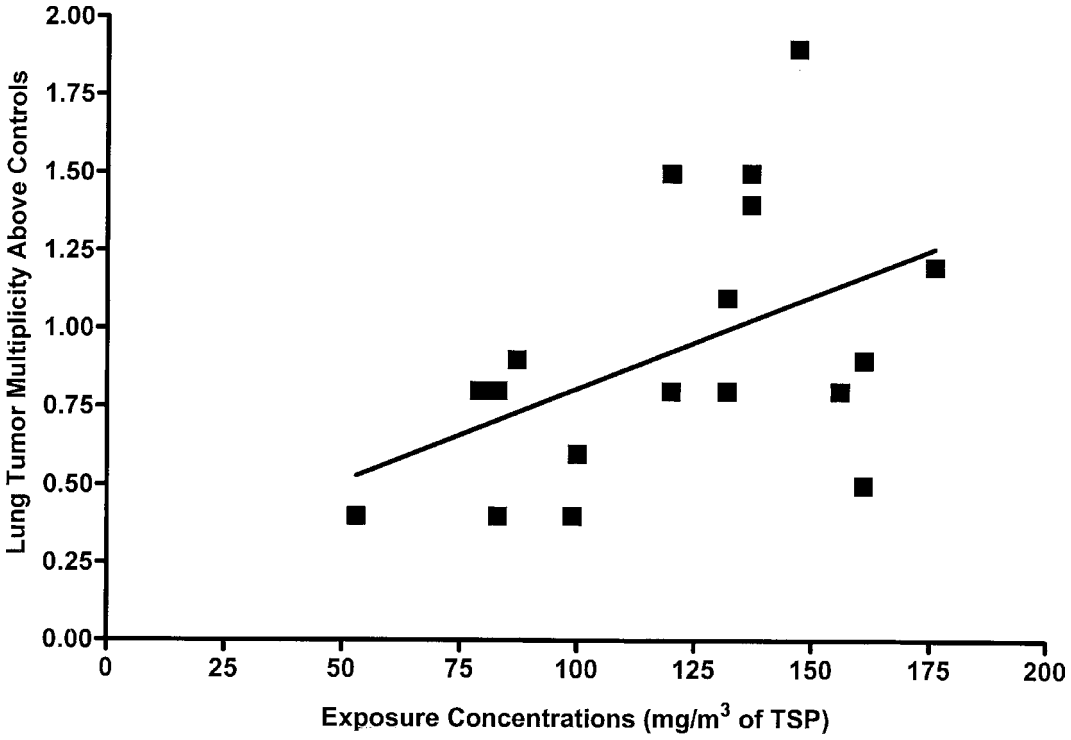

FIGURE 3 Lung tumor multiplicities in strain A/J calculated as number of tumors above corresponding control values in mice exposed to tobacco smoke as a function of average concentration of smoke (mg total suspended particulates/m^3). The slope of the regression line is significantly different from 0 ($P<.05$).

urethane, 1,2-dibromoethane, ethylene oxide, diesel exhaust, ozone, and oxygen, whereas no positive response was obtained with chloromethyl methyl ether, carbon disulfide, and naphthalene (data summarized in [6]). In a later study, no evidence for carcinogenicity of ozone was found in the strain A mouse [22].

In 1986 a study was published in which the strain A lung tumor test was compared to results obtained in full-time bioassays conducted by the National Toxicology Program [23]. The results were disappointing. In 2 different laboratories, a total of 65 chemicals was evaluated in a blind fashion. It was found that there was poor agreement for the results of 65 chemicals, given by the intraperitoneal route to strain A mice, and the results of chronic feeding 2-year bioassays. Eventually, it had to be concluded that strain A mice might show little sensitivity towards aromatic amines, naphtalenes, or metals. Although the strain A mouse seems to have some genetically mediated sensitivity to certain carcinogens, after this evaluation it did loose some of its previous appeal as a short-term general screening test. Whether the proposed newer models of selected and increased genetic susceptibilities have indeed the potential to do better remains to be established [24]. On the other hand, strain A mice are very susceptible to certain

TABLE 2 Shimkin's Criteria for Interpretation of Data in the Strain A Mouse Lung Tumor Assay

1	Lung tumor multiplicity is significantly higher than in controls and is preferably higher than 1.0.[a] (Lung tumor multiplicity is calculated by dividing the number of tumors found in one particular treatment group divided by the number of animals at risk, in cluding non-tumor bearing animals.)
2	Evidence for a dose-response relationship is required. ''Negative'' tests should be repeated with higher doses.
3	Lung tumor multiplicity in controls (spontaneously developing tumors) should be approximately the anticipated number for untreated mice of the same age.[b]
4	It is not acceptable to claim statistical significance whenever control values are lower than anticipated.
5	Preferably include positive controls with each experiment. (One single intraperitoneal injection of 1 mg of urethane per strain A/J mouse usually produces one tumor.)

[a]See references 64 and 65 for documentation that counting of surface tumors, as originally recommended by Shimkin, gives excellent correlation with all tumors present in a mouse lung.

[b]See references 20 and 21 for information on historical data of lung tumor multiplicity in strain A mice.

important classes of carcinogens found in tobacco smoke, such as polycyclic aromatic hydrocarbons and nitrosamines

HISTOPATHOLOGY

Most human lung cancers are located in the bronchial tree and can be classified as squamous cell carcinoma, adenocarcinoma, large cell carcinoma, or small cell lung cancer. Bronchoalveolar adenocarcinoma, located in the peripheral lung, occurs less frequently. At one time, squamous cell carcinoma was the tumor most often seen in smokers. During the last decades, a gradual shift toward increased incidence of adenocarcinoma has occurred. This was thought to be due, in part, to the introduction of the low-tar low-nicotine cigarette [25].

The mouse lung tumor assay, in general, has often been criticized for not representing human lung cancer. Strictly speaking, this is correct. Lung tumors in mice do not display the vigorously aggressive and highly malignant disruption of cellular patterns seen, for example, in human lung adenocarcinoma. Also, distant metastases to the brain or other organs are practically never observed. The histomorphology of tobacco smoke–induced lung tumors in mice was originally classified as had been done in previous studies with murine lung tumors [26]. The lesions were distinguished into focal alveolar epithelial hyperplasia, alveolar/bronchiolar adenomas, and alveolar/bronchiolar adenocarcinomas. In the control group, 20% and in the tobacco smoke–exposed group 17% of all tumors could be classified as adenocarcinomas [8]. When the results from several independent studies were compiled later, it was found that in air-exposed animals, 18% of all

tumors were adenocarcinomas, as opposed to 7% in smoke-exposed animals, a statistically significant difference [27].

In strain A mice, chemically induced lung tumors impress as adenomas within the first 12 months after carcinogen administration. The percentage of adenocarcinomas then increases steadily and eventually accounts for more than half the lung tumors found in 14-month-old mice. It appears to increase in a continuing fashion [28]. Thus it is not surprising that in experiments with tobacco smoke, where animals are about 1 year old when killed, most tumors still present as adenomas. It could be anticipated that should these mice be allowed to live for 2 years, most tumors would develop features of malignancy. The apparently paradoxical observation that tobacco smoke exposed animals have fewer malignant tumors than air controls may be attributed to the fact that tobacco smoke exposure, with its accompanying stress, initially slows down tumor growth [8, 11]. Interestingly, administration of chemopreventive agents has also on occasion found to delay the development of adenomas into adenocarcinomas [29, 39].

Distant metastasis that originate from primary lung tumors are in general a rare occurrence in mice and other laboratory rodents exposed to carcinogens. As summarized by Shimkin and Stoner [20], 2 investigators found among more than 5000 mice with induced or spontaneous lung tumors only 3.6% with distant metastases. In urethane treated, 17- to 19-month-old Balb/c mice, 45% of the tumors were carcinomas and about 33% of them had given rise to metastases. In our experiments, we observed in the tobacco smoke–exposed mice occasionally an invasion of adjacent tissue or of lymphatic vessels by tumor cells. Lung tumors in mice, as observed in the studies discussed in this article, represent thus an early stage of progression from hyperplasia to adenoma to adenocarcinoma, whereas in human lung tumors the histiopathological diagnosis usually is only made in the terminal, most progressed stages of the disease process.

MOUSE LUNG TUMORS AND Ki ras MUTATIONS

In both human and mouse lung adenocarcinomas, Ki ras mutations are often detected early and are frequent [30]. Regarding human lung cancers, the observation of a strong association with tobacco smoking is particularly interesting [31]. Therefore it was legitimate to look for similar events in mouse lung tumors induced by tobacco smoke. In 1995, we analyzed 11 tumors from strain A mice exposed to a comparatively low concentration of tobacco smoke ($4\,mg/m^3$). We found an apparent target for tobacco smoke in exon 2 (codon 61), where more than 90% of mutations were located, whereas in control animals (14 tumors) mutations in exons 1 and 2 appeared to be evenly distributed [6]. However, a later and somewhat more detailed analysis of lung tumors from mice exposed to a much higher

tobacco smoke concentration failed to reveal any differences in the mutational spectrum between controls and tobacco smoke–exposed animals [27]. A more recent study came to the essentially same conclusions [32]. Thus the strain A model so far has essentially been unable to confirm the observations made in humans.

CHEMOPREVENTION

During the past decades, multiple studies have been designed to examine the effectiveness and toxicology of putative chemopreventive agents with the strain A mouse lung tumor assay [33–37]. In practically all of these studies, the effectiveness of a given agent was measured in strain A mice treated with what are considered to be important tobacco smoke carcinogens, most often NNK, benzo(a)pyrene, or a mixture thereof. Typically, these carcinogen regimens induce a 100% incidence of tumors and tumor multiplicities from between 10 to 30 or more tumors per lung, with standard deviations usually in the 10% to 20% range. This allows to obtain significant results with comparatively small numbers of animals per group ($n = 10$ to 30; see [38–40] for representative experimental protocols).

Although these investigations have successfully identified several highly promising chemopreventive agents, it has been found to be much more difficult to show the same effectiveness against the full complex mixture of tobacco smoke. Table 3 summarizes the data obtained in our laboratory during the last 6 years. First it must be noted that, with 2 exceptions (acetylsalicylic acid and green tea), we got good and above all significant responses in experiments in which the effect of a given chemopreventive agent was examined in mice treated with a single carcinogen. However, against tobacco smoke, only a mixture of dexamethasone and *myo*-inositol was effective, whether given during the entire experiment [41] or even only once smoke exposure had ceased [42]. With several other agents (Bowman-Birk protease inhibitor, *d*-limonene, *myo*-inositol, phenethyl isothiocyanate [PEITC], a mixture of PEITC and benzyl isothiocyanate [BITC], and 1,4-phenylenebis(methylene)selenoisocyanate [pXSC], reductions in tumor multiplicities from 10% to 20% were found. None of these results was statistically significant at a level of $P < .05$.

In a clinical trail, a reduction of lung cancers by 20% would be considered to be an encouraging development. Unfortunately, it does not seem possible to observe such an effect in the strain A mouse model of tobacco carcinogenesis. We have previously pointed to a certain lack of sensitivity and that it might be difficult to document less than 100% efficiency [43, 44]. For example, the average differences in lung tumor multiplicities, in chemoprevention assays where a 20% efficiency is observed, lies mostly between 0.2 and 0.5. Given the usually observed standard deviations for

TABLE 3 Summary of Chemopreventive Studies with Tobacco Smoke

Agent[a]	Concentration (mg/kg diet)	Multiplicity[b]	Incidence[c]	Positive controls: multiplicity[d]	Reference
ASA	300	105%	107%	107%	41
BBIC	1000	90%	135%	62%[e]	57
β-Carotene	5000	105	95	N.D.[f]	50
d-Limonene	6500	93%	94%	60%[e]	42
Green tea	1250 (extract in drinking water)	100%	114%	131%	46
Myo-Dexa	10,000 and 0.5	48%[e]	73%[e]	14%[e]	41
Myo-Dexa	10,000 and 0.5	42%[e]	70%[e]	N.D.[f]	42
Myo alone	10,000	81%	85%	31%	44
Myo alone	30,000	86%	96%	25%	44
NAC	2000	123%	111%	65%[e]	46
PEITC	500	85%	102%	14%[e]	46
PEITC and BITC	250/250	88%	108%	N.D.[f]	42
p-XSC	20	86%	89%	20%[e]	42

[a] *Abbreviations:* ASA, acetylsalicylic acid; BBIC, Bowman-Birk protease inhibitor concentrate; Myo, myo-inositol; Dexa, dexamethasone; NAC, N-acetylcysteine; PEITC, phenethyl isothiocyanate; BITC, benzyl isothiocyanate; p-XSC, 1,4-phenylenebis(methylene)selenoisocyanate.

[b] Tumor multiplicities found in animals exposed to tobacco smoke and fed control diet = 100%.

[c] Tumor incidence found in animals exposed to tobacco smoke and fed control diet = 100%.

[d] Tumor multiplicities found in animals injected with NNK = 100%.

[e] Significantly different ($P < .05$) from corresponding controls.

[f] N.D., no data.

multiplicity, it might under these circumstances easily take more than 150 animals per group to show a significant difference at $P < .05$ in the range of 0.7 to 0.9 of power. Although this power is considered to be reasonable for this kind of studies [45], the number of animals that would have to be used would not only be impractical, but unacceptable from an animal welfare standpoint. On the other hand, in animals treated with NNK or other carcinogens, many more tumors are produced and differences of 30% or more between controls and treated animals can usually be found with 20 to 25 animals per group.

The limited response in tumor development to tobacco smoke inhalation suggests that in preclinical chemoprevention evaluations this particular assay might easily yield false negatives. Such an interpretation of the data could wrongly preclude the evaluation of potentially useful chemopreventive agents in man. In this context, *N*-acetylcysteine (NAC) remains a particularly puzzling problem. Although in our experiments we did not find an effect [46], there exists a large body of excellent evidence that this compound substantially decreases multiple biomarkers of exposure and of effect in laboratory rodents exposed to tobacco smoke [47, 48]. When fed to female SWR mice during tobacco smoke exposure, lung tumor multiplicities

were reduced to 27% of the number found in controls and incidences to 22%. Such findings are highly suggestive of a significant effect. However, due to large interindividual variations and a comparatively small number of animals used, statistical significance was not observed [48]. In a large clinical trial, NAC had no effect [49]. Beta-carotene was another chemopreventive agent found to be negative in the strain A mouse lung tumor assay [50]. Clinical trials with the same agent were also negative [51]. Some results with the mouse lung tumor assay have thus so far been ''validated'' by the human experience.

WORK WITH DIFFERENT MOUSE STRAINS AND OTHER SPECIES

Ever since tobacco smoke carinogenesis became an issue, attempts were made to reproduce the disease in experimental animals, mostly rats, hamsters, mice, and on occasion dogs and monkeys [52]. In 1930 Mertens [53] reported the result of a study in which individual mice had inhaled cigarette smoke. Smoke was forced by a rubber bulb into a glass desiccator where the animals were kept from 1 to 4 hours daily, for up to 15 months. Although Mertens found at the end of the experiment inflammatory changes in the lungs, he thought it remarkable that large areas showed no pathological alterations. Neoplastic lung lesions were found in only 2 animals. Multiple small adenocarcinomas, found in 1 mouse, were considered to have been preexisting. The second animal showed several small nodules and one large adenocarcinoma, 4 mm in diameter, originating in a bronchus and invading the adjacent parenchyma. Mertens doubted that this tumor had been caused by tobacco smoke inhalation.

This early study was a harbinger of things to come. It proved exceedingly difficult to produce tumors with tobacco smoke in the respiratory tract of laboratory animals. Although many histopathological changes, such as inflammation, evidence for deposition of paniculate material and its becoming engulfed by macrophages, and, on occasion, metaplastic changes in the airway epithelia were seen in the respiratory tract of mice, rats, hamsters, and dogs, tumor response was practically nil. In 1978, the conclusion was reached: ''No researcher has succeeded as yet in producing a significant incidence of pulmonary tumors'' [54]. In 2004, the International Agency for Research on Cancer reviewed the existing evidence [14]. One rat study (out of 4) gave some evidence for the carcinogenicity of tobacco smoke, although tumor incidence in the exposed group was below 10%. Hamsters developed laryngeal tumors, but no tumors in the lower respiratory tract. Studies in mice showed that out of a total of 1703 mice exposed to tobacco smoke in various laboratories, only 108 animals (6.3%) developed lung tumors. In control animals, the overall incidence was 3.9% (39 out of 998). Despite these low incidence numbers, the difference was

TABLE 4 Lung Tumor Response to Tobacco Smoke in Different Strains

Strain	TSP	Air[a]	Smoke[a]	TS/Air[b]	Reference
SWR	122	0.04 ± 0.20 1/26 (4%)	0.35 ± 0.78 6/31 (19%)	8.8	27
SWR		0.14 ± 0.47 2/22 (9%)	0.57 ± 0.73 9/21 (43%)	4.1	48
Balb/c	122	0.20 ± 0.38 6/30 (20%)	0.44 ± 0.66 9/27 (33%)	2.2	27
CByB6F1	153	0.17 ± 0.43 4/30 (13%)	0.23 ± 0.49 6/30 (20%)	1.4	Unpublished observation

[a]Lung tumor multiplicity $\pm$ SD, and lung tumor incidence, number of animals bearing tumors/total number of animals at risk (%).

[b]Ratio of lung tumor multiplicity in smoke-exposed animals to controls.

statistically significant thanks to the large overall number of animals involved. Nevertheless, these results show a relative increase in lung tumor incidence by almost 100%. In another large study the incidence was not significantly increased, but time to tumor seemed to be shortened [15]. A review of the results of 14 chronic inhalation studies in rats and mice with mainstream cigarette smoke emphasized in 1998 that ''significant increases in the numbers of malignant tumors were not produced in the respiratory tract of rats or mice exposed chronically by inhalation of cigarette smoke'' [55]. Currently, the Syrian golden hamster has been claimed to represent the most reliable model for the induction of respiratory tract cancer by cigarette smoke [56], although lesions have been found only in the larynx and not in the airways or deeper lung.

The introduction of the split-exposure protocol—exposure of the animals first for 5 months to tobacco smoke, followed by a 4 month recovery period in air [8]—gave some expectation that a positive response to tobacco smoke might also be obtained in different mouse strains. Particularly, it was hoped to find a substantial increase of both tumor multiplicity and incidence in strains with a lower background rate of spontaneously occurring tumors than commonly seen in the A mouse. Results of data so far available are summarized in Table 4. It is readily apparent that other mouse strains are afflicted by the same difficulties the A mouse has, i.e., very small differences and large standard deviations between exposed and control animals. Likewise, experiments with genetically manipulated mice so far have not given better results [12, 57].

CONCLUSIONS

In the study of tobacco smoke carcinogenesis, the main feature of the strain A mouse lung tumor assay has been its reproducibility. Increases in

tumor multiplicity were independently found in 4 different laboratories and the aggregated data show a significant response in 83% of all experiments. Increased lung tumor incidences were found in more than half of the studies. This seems to be the first animal model in which cigarette smoke consistently produces tumors in the deep lung (as opposed to laryngeal tumors that have been reproducibly found in hamsters). It also requires only comparatively small number of animals to document a positive effect; group sizes in most cases range from less than 10 to less than 30 animals, again only about half as many as in a conventional bioassay, where usually 50 animals per group are required. Furthermore, no apparent sex difference has been found to exist.

The assay was derived according to principles and procedures suggested many years ago [20, 21]. The short-term duration and the ease of quantitation of the tumorigenic response, which requires only minimal histopathological evaluation, seem to be the main attractive features and thus make the assay an attractive tool for meeting several current goals in the study of cigarette smoke toxicology: development of a modified product [60], finding alternative ways for the satisfaction of nicotine addiction [61], or discovery of effective chemopreventive agents [62]. Unfortunately, the assay so far has been found to be less than ideal for this purpose. Although 100% efficacy of certain chemopreventive agents has been found, the majority of experiments did not allow to detect a statistically significant reduction in lung tumor multiplicity in animals treated with putative chemopreventive agents and exposed to tobacco smoke. The statistical power of the assay, when relying on lung tumor multiplicities induced by a weak carcinogen, is not strong enough.

This poses a dilemma, because there is a risk of false-negative data, which might preclude further investigations, particularly clinical trials, of a given chemopreventive agent. On the other hand, clinical trials are not easily designed and performed and, above all, so far have been disappointing when it came to examine chemoprevention of tobacco smoke–induced lung cancer. This was said rather eloquently: ''Hypothesis-driven chemoprevention of lung cancers, when put to the test of randomized large-scale clinical trials, so far has been disappointing, unlike important successes with selective estrogen receptors modulators for breast cancer and nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors for colon cancer'' [63]. At least, the strain A mouse lung tumor model of tobacco smoke carcinogenesis provides us with a system, where lung tumors can actually be produced, as opposed to all other studies with inhaled tobacco smoke effects in experimental animals. Whether it will yield novel or better mechanistic clues for prediction of efficacy remains to be established.

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