Potent carcinogenicity of cigarette smoke in mice exposed early in life

Roumen Balansky1,2, Gancho Ganchev3, Marietta Iltcheva3, Vernon E.S.Steele1, Francesco D’Agostini1 and Silvio De Flora1,∗

1Department of Health Sciences, University of Genoa, Via A. Pastore 1, I-16132 Genoa, Italy. 2National Center of Oncology, Sofia 1756, Bulgaria and 3National Cancer Institute, Rockville, MD 20892, USA

∗To whom correspondence should be addressed. Tel: +39 010 3538500; Fax: +39 010 3538504; Email: sdf@unige.it.

In spite of the dominant role of cigarette smoke (CS) in cancer epidemiology, all studies performed during the past 60 years have shown that this complex mixture is either negative or weakly tumorigenic in experimental animals. We implemented studies aimed at evaluating whether exposure of mice early in life may enhance susceptibility to CS carcinogenicity. A total of 98 newborn Swiss albino mice were either untreated (controls) or received a subcutaneous injection of benzo(a)pyrene [B(a)P] (positive control) or were exposed whole-body to mainstream cigarette smoke (MCS) for 120 days, starting within 12 h after birth. Complete necropsy and histopathological analyses were performed at periodical intervals. In contrast with the lack of lung tumors in controls, MCS-exposed mice developed microscopically detectable tumors, starting only 75 days after birth and reaching an overall incidence of 78.3% after 181–230 days. The mean lung tumor multiplicities were 6.1 and 13.6 tumors per mouse in males and females, respectively, showing a significant intergender difference. Most tumors were microadenomas or adenomas, but 18.4% of the mice additionally had malignant lung cancer, MCS also induced bronchial and alveolar epithelial hyperplasia, and blood vessel proliferation. Furthermore, malignant tumors, some of which may have a metastatic origin, were detected in the urinary tract and liver of MCS-exposed mice. A somewhat different spectrum of tumors was observed in B(a)P-treated mice. In conclusion, MCS is a potent and broad spectrum carcinogen in mice when exposure starts early in life, covering stages of life corresponding to neonatal, childhood and adolescence periods in humans. This animal model will be useful to explore the mechanisms involved in CS-induced carcinogenesis and to investigate the protective effects of dietary agents and chemopreventive drugs.

Introduction

After pioneer studies performed many decades ago (1–3), today there is overwhelming evidence that tobacco smoking plays a major role in the epidemiology of lung cancer, cancer at other sites and a variety of chronic degenerative diseases (4–7). Among the multiple components of cigarette smoke (CS), 20 chemical compounds have convincingly been shown to induce lung tumors (8). Laboratory animals have extensively been used for evaluating the carcinogenicity of typical CS components, such as benzo(a)pyrene [B(a)P], as a prototype of polycyclic aromatic hydrocarbons, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, as a prototype of tobacco-specific nitrosamines (6–8).

Less attention has been paid to CS, either mainstream cigarette smoke (MCS) or sidestream CS or environmental cigarette smoke (ECS). This depends on the general difficulty to test complex mixtures rather than individual compounds as well as on various problems inherent to exposure by inhalation of rodents. Thus, although the earliest attempts to reproduce CS tumorigenicity in mice trace back to >70 years ago (9,10), most carcinogenicity studies in a variety of animals species showed that inhaled CS is either negative or just weakly positive (6,7,11–14).

The most convincing medium-term bioassay for CS tumorigenicity has been developed during the last decade by Witschi et al. (12,13,15). This bioassay involves the whole-body exposure of A/J mice or other mouse strains to ECS for 5 months, followed by recovery in filtered air for an additional 4 months. As reviewed by Witschi et al. (12), 18 studies performed in their laboratory and other three laboratories, including ours (16–19), showed statistically significant increases in the yield of surface lung tumors in the majority of the experiments performed. The tumorigenic effects are almost exclusively due to the ECS gas phase and mainly due to 1,3-butadiene (13,20). The increase of ECS-related tumor multiplicity is low, from an average of 1.1 lung tumors per mouse in controls to an average of 2.8 in ECS-exposed mice (12). By using the same methodology, a weak tumorigenic response was also observed in MCS-exposed mice (21). Evidence that MCS is moderately carcinogenic in rodents was generated in recent lifetime studies, involving the whole-body exposure for 30 months either of F344 rats, in which the incidence of both lung and nasal tumors was significantly increased (22), and in B6C3F1 mice, in which the incidences of benign pulmonary neoplasms, adenocarcinomas and metastases were significantly increased (23).

The apparently weak tumorigenicity of CS contrasts with the evident alterations of a variety of intermediate biomarkers in the lung and other organs of MCS- or ECS-exposed rodents, among which we investigated bulky adducts to nuclear DNA and mitochondrial DNA, hemoglobin adducts, oxidative DNA damage, transcriptome and proteome alterations, apoptosis and proliferation of bronchial epithelial cells and cytogenetical damage (reviewed in refs 24,25).

Susceptibility to carcinogens may be enhanced during certain stages of life. At birth, there is a sudden transition from the maternal-mediated respiration of the fetus to the autonomous pulmonary respiration of the newborn, which is expected to produce a tremendous oxidative stress. We provided evidence that the simple fact of coming to life causes strong nucleotide alterations in mouse lung, as shown by a 5-fold increase of bulky DNA adducts and a 2-fold increase of oxidative DNA damage (26). In parallel, the analysis of 746 genes by cDNA arrays showed the up-regulation of 33 genes (4.4%) involved in adaptive functions, such as glutathione metabolism, cellular stress and damage to DNA and proteins (26). In another laboratory, Affymetric analyses showed that ~1300 genes had a differential expression in mouse fetal lung versus post-natal lung (27). Although these transcriptional mechanisms tend to compensate the ‘spontaneous’ alterations due to oxidative stress and DNA damage, it is evident that birth is per se a critical event. In humans, there is adequate reason to suspect that perinatal exposures to carcinogens contribute to both childhood cancers and cancers appearing later in life (28). Mutagenicity of genotoxic carcinogens was much higher in neonatal than in adult Big Blue mice (29). Administration of phenobarbital to rat pups increased the incidence of tumors and other diseases and reduced life expectancy, which suggests that certain adult diseases may have their origins early in life (30). Moreover, exposure of rodents during the first weeks of life, which corresponds to infancy, childhood and adolescence in humans, may enhance susceptibility to ECS. In fact, early age smoking has been hypothesized as an independent risk factor for lung cancer (31) and, mechanistically, adolescence may constitute a critical period during which tobacco carcinogens can induce fields of genetic alterations (32). In mice, a neonatal tumorigenicity bioassay was proposed for the first time in 1959 (33) and was recently recommended as an alternative tumorigenicity bioassay (34). Although the liver is the main target in this model, encouraging results have also been obtained with pulmonary carcinogens, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (35) and especially polycyclic aromatic hydrocarbons and their derivatives (36–43).

Abbreviations: B(a)P, benzo(a)pyrene; CS, cigarette smoke; ECS, environmental cigarette smoke; MCS, mainstream cigarette smoke.
Based on these premises, we implemented studies aimed at evaluating whether exposure of mice to CS early in life may enhance the carcinogenic response. To this purpose, we used Swiss albino mice, which are next to A/J for sensitivity to lung carcinogens but are more suitable for reproduction and have a lower spontaneous background of lung tumors (24,44). The results obtained in the present study provide evidence that, when exposure starts immediately after birth and continues early in life, MCS behaves as a potent carcinogen by inducing, with a short latency time, a high incidence and multiplicity of lung tumors as well as malignant lung tumors and tumors of the liver and urinary tract.

Materials and methods

Mice

A total of 16 adult Swiss albino mice, 5 males and 11 females (Animal Laboratory, National Center of Oncology, Sofia, Bulgaria), aged 12–15 weeks and weighing 28–30 g (males) and 23–25 g (females) each, were used for breeding. The animals were housed in Makrolon cages on sawdust bedding, and maintained on standard rodent chow and tap water ad libitum. The animal room temperature was 23 ± 2 °C, with a relative humidity of 55% and a 12 h day–night cycle. After 1 week of acclimatization, the mice were mated (three females and one male per cage for 3 days) to produce F1 mice. Housing, breeding and treatment of mice were in accordance with national and institutional guidelines.

Design of the study

The neonatal mice composing each litter were kept with their dam, in a single cage, throughout the weanling period, lasting ~5 weeks. At birth, a total of 98 newborn mice, 49 males and 49 females, were divided into three groups, including (a) 36 untreated mice (15 males and 21 females) used as negative controls, (b) 38 mice exposed to MCS (22 males and 16 females), and (c) 24 mice treated with B(a)P (12 males and 12 females) used as positive controls. After weanling, males and females were housed separately.

Exposure to MCS

Exposure to MCS of the mice belonging to group b started within 12 h after birth and continued daily for 120 days. A whole-body exposure was obtained by using filter-tipped commercial cigarettes (Arda—Bulgartabac) that have a declared content of 15 mg tar and 1.0 mg nicotine each. Smoking one cigarette delivers 9 mg CO in MCS. The mice of each litter and their dam were placed in a 22.5 l sealed glass chamber that was subsequently filled with MCS, as described previously (45). Briefly, MCS was generated by drawing 15 consecutive puffs, each of 60 ml, employing a 60 ml syringe connected with the exposure chamber. Each puff lasted 6 s. Each MCS treatment (15 mice per session) lasted 65 min, involving six exposures of 10 min each with a 1 min intervals, during which a total air change was made. The concentration of total particulate matter in the exposure chamber was, on average, 818 mg/m³ air. This exposure method proved to be effective in inducing a variety of alterations in rodents, such as early histopathological changes (46), cytogenetic damage (45,47), biochemical alterations (48), adducts to both nuclear DNA and mitochondrial DNA (49) and hyperproliferation and apoptosis in respiratory tract cells (50).

Treatment with B(a)P

Within 12 h after birth, the newborns belonging to group c received a single subcutaneous injection of B(a)P (Sigma–Aldrich, St Louis, MO) in the interscapular region. B(a)P (1.0 mg per mouse) was dissolved in olive oil (0.025 ml per mouse).

Necropsy and histopathological analyses

Besides spontaneous deaths (see Results), 12 control mice, 6 B(a)P-treated mice and 14 MCS-exposed mice were killed from day 75 to day 200 (see Figure 3). All remaining B(a)P-treated mice were killed 210 days after birth, whereas controls and MCS-exposed mice were killed after 230 days. The mice were deeply anesthetized with diethyl ether and killed by cervical dislocation. A complete necropsy was performed. Lungs, liver, kidney, stomach and all organs with suspected macroscopic lesions were fixed, cut into standardized sections and subjected to standard histopathological analysis. In particular, the left lung was cut into three pieces and the accessory, cranial, middle and caudal lobes of the right lung were cut into two pieces each, thus accounting for a total of six sections to be analyzed microscopically. Two sections were analyzed per each kidney, and three sections per liver.

Statistical analyses

The yield of tumors and other lesions was expressed in terms of incidence and, in case of multiple tumors, of multiplicity. Body weights and multiplicity data were expressed as means ± SEs of the mice composing each experimental group, and comparisons between groups were made by Student’s t-test for unpaired data. Comparisons between groups regarding survival and incidence were made by χ² analysis. Correlations between time and tumor multiplicity were evaluated by using Spearman’s and simple regression tests.

Results

Survival and body weights

Before termination of the study, there were spontaneous deaths (a) in four control mice (11.1%), including one male (dead after 200 days) and three females (dead after 165, 196 and 212 days); (b) in three B(a)P-treated mice (12.5%), including one male (dead after 180 days) and two females (dead after 190 and 198 days), and (c) in eight MCS-exposed mice (21.1%), including three males (dead after 174, 184 and 202 days) and five females (dead after 131, 143, 183, 187 and 210 days). The difference between controls and MCS-exposed mice was not statistically significant.

As shown in Figure 1, the body weight gain was significantly lower in mice exposed to MCS, as compared with controls, from day 35 to day 120 of the experiment, when exposure was discontinued. The MCS-related loss of body weight gain after 35, 60, 90 and 120 days was of 24.3, 19.6, 12.1 and 11.6% in males and of 17.9, 14.2, 9.4

![Fig. 1. Body weights (means ± SEs) of post-weanling Swiss albino mice at various time intervals after birth and either untreated (empty circles) or treated with a single subcutaneous injection of B(a)P (empty triangles) or exposed whole-body to MCS for 120 days (full circles), starting within the first 12 h after birth. b, P < 0.05, and c, P < 0.01, as compared with untreated mice.](https://academic.oup.com/carcin/article-abstract/28/10/2236/2476287)
and 12.7% in females, respectively. B(α)P significantly decreased the body weight in both males and females, but only 30 days after treatment.

**Tumors and other lesions in lung**

Table I summarizes incidence data and Table II summarizes multiplicity data for tumors and other histopathological alterations in the variously treated mice, as detected in a period ranging between 75 and either 210 days [B(α)P] or 230 days (MCS) after birth. Both B(α)P and MCS caused a significant increase of hyperplasias of alveolar epithelial cells, frequently congesting the alveolar spaces and tending to obliterate them (see an example in Figure 2A) as well as hyperplasias of the bronchial epithelium (see an example in Figure 2B) in both males and females. Moreover, MCS induced foci of capillary or cavernous proliferation of blood vessels in lung, kidney and liver.

In contrast with the total lack of tumors in control mice (Tables I and II), both MCS and B(α)P induced the formation of macroscopically detectable lung microadenomas, which were more frequent in MCS-exposed mice than in B(α)P-treated mice, and of macroscopically visible adenomas (see an example in Figure 2C), which conversely were more frequent in B(α)P-treated mice than in MCS-exposed mice. Note that the adenomas detected in B(α)P-treated mice were mainly papillary or tubular, whereas those detected in MCS-exposed mice were mainly alveolar. Characteristically, one only of the lung tumors in MCS-exposed mice was macroscopically visible on the lung surface, but all of them were detected at the analysis of lung sections. Moreover, in MCS-exposed mice but not in B(α)P-treated mice, there was a significant increase of malignant lung tumors in B(α)P-treated mice, there was a significant increase of malignant lung tumors in B(α)P-treated mice. In fact, half of the MCS-exposed mice died either spontaneously or killed at periodical intervals before termination of the study (Figure 3). By stratifying the data according to time intervals, lung tumors were detected in two of five mice (40.0%) after 75–120 days, in two of four mice (50.0%) after 121–150 days, in four of six mice (66.7%) after 151–180 days and in 18 of 23 mice (78.3%) after 181–230 days. There was a statistically significant correlation (r = 0.414, P = 0.05) between the time of exposure (x) and the multiplicity of lung tumors (y) in ECS-exposed male mice, the regression line being y = −5.623 + 0.062x. Conversely, the correlation was not significant in females (r = 0.064), presumably because a high multiplicity of tumors was even recorded after short periods of exposure (Figure 3).

**Tumors and other lesions in organs other than lung**

Prenecrotic or neoplastic lesions were detected in organs other than the lung of MCS-exposed mice. In fact, half of the MCS-exposed females bearing lung tumors had at the same time tumors in other organs, five of them having tumors in both lung and either liver or kidney and two of them having tumors in all three organs. In most

Table I. Incidence of histopathological alterations observed, in a period ranging between 75 and 210–230 days after birth, in male (M) and female (F) A/J mice, either untreated (controls) or receiving a subcutaneous injection of B(α)P or exposed to MCS for 4 months

<table>
<thead>
<tr>
<th>Organ or anatomical site</th>
<th>Controls</th>
<th>B(α)P</th>
<th>MCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial epithelial hyperplasia</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Alveolar epithelial hyperplasia</td>
<td>0</td>
<td>2 (9.5)</td>
<td>2 (5.6)</td>
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<tr>
<td>Microadenoma</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adenoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malignant tumors</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>All tumors</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kidney</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adenomas</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malignant tumors</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>All tumors</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bladder</td>
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<td>0</td>
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<tr>
<td>Transitional cell papilloma</td>
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<td>0</td>
</tr>
<tr>
<td>Liver</td>
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<tr>
<td>Cavernous hyperplasia</td>
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<td>0</td>
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<tr>
<td>Hemangioma</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malignant tumors</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Forestomach</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Keratosis</td>
<td>1 (6.7)</td>
<td>0</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>Hiperplasia</td>
<td>1 (6.7)</td>
<td>0</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>Papilloma</td>
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<td>1 (4.8)</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>Thorax</td>
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<td>0</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
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<td>0</td>
</tr>
<tr>
<td>Hematopoietic system Tumors</td>
<td>2 (13.3)</td>
<td>5 (23.8)</td>
<td>7 (19.4)</td>
</tr>
<tr>
<td>Ovary</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cyst</td>
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Treatments started within the first 12 h after birth. Values in parentheses indicate the percent of mice bearing the indicated lesion. Significantly increased (\*P < 0.05, \**P < 0.01 and \***P < 0.001), compared with the corresponding control.
cases, extrapulmonary tumors had histopathological patterns similar to those detected in lung. In particular, as reported in Tables I and II, MCS but not B(α)P caused an increased yield of kidney adenomas (see an example in Figure 2l) in female mice, both in terms of incidence and of prevalence. Moreover, one MCS-exposed female was affected by a malignant renal tumor (Figure 2l) and one male was affected by a transitional cell papilloma of bladder.

Various histopathological alterations, and especially parenchymal dystrophies, were detected in the liver of both B(α)P-treated mice and MCS-exposed mice of both genders, whereas no alteration was detected in controls. Two cases of severe hepatocellular dysplasia (see an example in Figure 2k) and two cases of malignant liver tumors (see an example in Figure 2l) were detected in MCS-exposed females.

The incidence of forestomach keratosis was significantly increased in B(α)P-treated mice, especially in females. There was no significant difference among the three experimental groups regarding the incidence of hematopoietic tumors and of ovary cysts. A single case of rhabdomyosarcoma was detected in the thorax of a B(α)P-treated female.

Discussion

The results obtained provide evidence, for the first time, that CS can behave as a potent carcinogen in rodents when exposure starts soon after birth. In fact, the carcinogenic response was characterized (i) by a short latency time, (ii) by a high yield of preneoplastic lesions and benign tumors in the lung, contrasting with a complete lack of tumors in unexposed mice, (iii) by induction of pulmonary malignancies and (iv) by occurrence of primary lesions and metastases outside the respiratory tract.

In addition to a very high incidence and multiplicity of lung microadenomas and adenomas, a fair proportion of the mice suffered from bronchial and alveolar hyperplasias. Moreover, foci of caver-nous angiogenesis-related proteins in rat tissues (53). A significant increase in the concentration of angiogenesis-related proteins in rat tissues (53). A characteristic of the MCS-induced adenomas was that most of them were alveolar and almost all of them could only be detected following microscopical examination of lung sections. In contrast, B(α)P-induced adenomas were mainly papillary and tubular and were often detected on the lung surface. This conclusion supports the suggestion that serial sectioning of the lung is necessary for a better detection of MCS-induced tumors (18). Clearly, this requirement renders the bioassay more laborious and time consuming. It is also evident that sensitivity of the assay is affected by the number of sections examined.

It is noteworthy that, in spite of the young age (5–7 months), malignant tumors were detected in MCS-exposed mice but not in B(α)P-treated mice. Two tumors had the histopathological appearance of carcinoids. However, the exact nature of these tumors should be confirmed by suitable immunohistochemical analyses. Interestingly, carcinoids are the most common neuroendocrine tumors and have been associated with exposure to CS constituents, such as nicotine and 4-methylaminobenzene (54). One of the most common smoking-associated lung cancer types, small cell carcinoma, expresses phenotypic and functional features of pulmonary neuroendocrine cells, and these cells have been found to be increased in several other smoking-associated pediatric lung disorders (54). Interestingly, pulmonary neuroendocrine cells, which are abundant in the neonate, function as hypoxia-sensitive receptors and are thought to be important mediators of pulmonary neonatal adaptation, particularly the onset of breathing (54,55).

The MCS-related lung tumor multiplicity was significantly higher in female mice than in male mice. A similar conclusion was drawn in the lifetime study in rats. In fact, exposure to MCS resulted in a significant incidence of lung neoplasias in females, in contrast with the lack of tumors in the control group, whereas non-significant increases were observed in males (22). The data generated in animal models support the view that the risk for smoking-related lung cancer is higher for women than for men (56,57), although this issue is still under debate (58). A greater susceptibility of females has been ascribed to a higher expression of CYP1A1 and levels of DNA adducts in female lung (59) and to the implication of estrogen in lung carcinogenesis in female smokers (60). Estrogen can directly stimulate the transcription of estrogen-responsive genes in the nucleus of lung cells, and it can also transactivate growth factor signaling pathways, in particular the epidermal growth factor pathway (61).

Tumors were also detected in extra-respiratory organs of MCS-exposed mice. Although transitional cell papilloma of bladder is linked to an effect of MCS in the urinary tract, it is likely that some of the neoplastic lesions observed in liver and kidney may have had a metastatic origin, since these lesions displayed histopathological features similar to those detected in the primary lung tumor. So far, a systemic induction of tumors had only been observed in hamsters 15–25 months after injection of a CS condensate either during pregnancy or in young animals (62). The multiorgan damage produced by CS is documented by the elevated levels of carcinogen–DNA adducts in female lung (59) and to the implication of estrogen in lung carcinogenesis in female smokers (60). Estrogen can directly stimulate the transcription of estrogen-responsive genes in the nucleus of lung cells, and it can also transactivate growth factor signaling pathways, in particular the epidermal growth factor pathway (61).
Fig. 2. Examples of histopathological alterations observed in lung, liver and kidney of MCS-exposed mice. (A) Diffuse hyperplasia of alveolar epithelium. (B) Papillary hyperplasia of bronchial epithelium. (C) Lung adenoma. (D) Carcinoma in situ in lung. (E) Small cell lung carcinoma. (F) Lung tumor containing adenocarcinomatous and small cell areas. (G) Adenosquamous lung carcinoma. (H) Low-differentiated lung carcinoma. (I) Kidney adenoma. (J) Malignant renal tumor. (K) Severe hepatocellular dysplasia. (L) Malignant liver tumor. Stained with hematoxylin–eosin. Original magnification: ×125.
exhibited different characteristics of pulmonary carcinogenicity, which were also distinct from the patterns elicited by the gas phase of ECS (13). Moreover, MCS but not B(a)P was associated with tumors in the urinary tract, whereas B(a)P but not MCS was associated with forestomach tumors, which are typically induced by this polycyclic aromatic hydrocarbons in mice (65). On the other hand, both MCS and B(a)P were associated with an increased incidence of liver distrophies. Thus, it is likely that B(a)P only partially contributes to the effects produced by MCS in this animal model.

The striking susceptibility of mice to carcinogenicity of MCS, when exposure starts few hours after birth, can be ascribed to a variety of mechanisms, such as: (i) the induction of oxidative DNA damage and formation of bulky DNA adducts (26), accompanied by over-expression of a number of genes (26,27) in mouse lung during the transition from the fetal life to the postnatal life. Moreover, oxidative DNA damage in lung is significantly higher in mice exposed to ECS since birth throughout the weanling period than in their dams exposed in the same cages (our study in progress); (ii) the increased proliferative rate in neonatal organs, among which lung, liver and kidney, which facilitates both the fixation of DNA damage and the clonal expansion of initiated cells. In particular, in mice, the proliferation of type-II cells markedly increased after birth, with a decline after day 14 (66), and alveolarization occurred during the first 12 days of post-natal life (67); (iii) alterations of xenobiotic metabolism, such as lower levels of glutathione S-transferases during the first weeks of post-natal life of mice, followed by a several fold enhancement in adult animals (68); (iv) a lower efficiency, in newborn mice, of certain DNA repair mechanisms (69,70); and (v) hypothetically, an increased probability for the involvement of stem cells, which have been shown to have an increased susceptibility to genotoxic carcinogens (71). In addition to the enhanced susceptibility at birth, continuation of exposure to MCS of mice during the first 4 months of life may have contributed to an increased development of tumors, consistently with the hypotheses raised in humans (31,32).

In conclusion, we have now available a suitable medium-term bio-assay that can be used as an animal model for CS-related carcinogenesis and chemoprevention studies. As extensively discussed by Hecht (14), animal models of CS-induced cancer are important for several reasons. We would like to emphasize their importance for exploring the mechanisms involved in CS-related cancers and other degenerative diseases, for studying the interactions between CS and other agents, and for assessing the preventive efficacy of pharmacological and dietary agents. Although it is obvious that the most logical way to prevent CS-related diseases is to quit smoking, chemoprevention provides a comprehensive strategy that can be applied to addicted active smokers, passive smokers and ex-smokers, who today comprise ~50% of all new lung cancer cases (32). Interestingly, by using mice exposed to CS during the first 4 months of life, it is possible to administer chemopreventives either after weanling, thus mimicking the situation in current smokers, or after discontinuation of exposure, thus mimicking the situation in ex-smokers.

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