Transplacental and Lactational Carcinogenesis by Safrole

S. D. Vesselinovitch, K. V. N. Rao, and N. Mihailovich

ABSTRACT

The carcinogenicity of safrole following transplacental exposure of the mouse fetus and exposure of the neonatal mouse via the mother's milk was investigated in C57BL/6J × C3HeB/FeJ F1 mice (hereafter called B6C3F1) by intragastric administration of the agent to pregnant and lactating C57BL/6J females. Safrole (120 μg/g body weight per treatment) was administered to (a) pregnant mice (4 times on Days 12, 14, 16, and 18 of gestation); (b) lactating mothers (12 times every second day following parturition); or (c) 4-week-old offspring (180 times twice weekly for 90 weeks). Two additional groups of offspring received a, b, and c combination treatments. All survivors were killed at 94 weeks of age. Renal epithelial tumors were observed in 7% of female offspring exposed to safrole in utero; none of the other experimental and control animals developed these tumors. Only male offspring nursed during the preweaning period by mothers treated with safrole developed hepatocellular tumors (34%). In contrast, direct administration of safrole, beginning at the time of weaning and continuing for the duration of the experiment, led to a significantly high incidence of hepatocellular tumors in females (48%), but not in males (8%). Eighty-six% of the liver tumors observed in females were hepatocellular carcinomas with a high rate of pulmonary metastases (42%). The data suggest that safrole or its metabolites came into contact with fetuses by crossing the placenta and with infants through its excretion in milk to exert the perinatal carcinogenicity.

INTRODUCTION

The carcinogenicity of safrole was originally demonstrated in rats (5,9). Innes et al. (6) found that p.o. administration of safrole to mice for 18 months, starting at 7 days of age, led to the development of liver tumors in 65% of male and 100% of female B6C3F1 mice. Epstein et al. (3) observed that infant male Swiss albino mice were highly susceptible to the carcinogenic effects of parenteral administration of this agent. No similarly treated females developed liver tumors. Subsequent studies by Borchert et al. (1,2) implicated 1'-hydroxysafrole and its ester as proximate and ultimate carcinogenic metabolites. These investigators also showed that female infant mice were refractory to induction of liver tumors by safrole, 1'-hydroxysafrole, and 1'-acetoxysafrole (15). Adult female mice were, however, more susceptible than were males to hepatocarcinogenesis following p.o. administration of safrole and its active metabolites (6,15). Because of the age- and sex-associated variation in induction of liver tumors by safrole and because of our interest in perinatal carcinogenesis, we investigated the carcinogenic risk to offspring following administration of safrole to mothers during gestation, lactation, or both. In addition, groups of offspring were treated directly with safrole over their life span, with or without preceding indirect perinatal exposure to this agent.

MATERIALS AND METHODS

Mice. Breeding stock, consisting of 6-week-old female C57BL/6J and male C3HeB/FeJ mice was purchased from The Jackson Laboratories (Bar Harbor, Maine). The mice were bred in our laboratory at 8 weeks of age, and the mothers and their offspring were treated by gastric intubation with 120 μg of safrole per g body weight.

Safrole (purity, >99%) was obtained from Aldrich Chemical Co. (Milwaukee, Wis.). It was emulsified in a steroid-suspending vehicle (supplied by Dr. J. M. Rice of the National Cancer Institute) shortly before each treatment.

Method of Treatment. A 1-ml tuberculin syringe was fitted with a 26-gauge needle which was slightly bent and provided with a sleeve of polyethylene tubing. When this tubing had been introduced into the stomach, 0.01 ml (120 μg) of safrole emulsion per g of body weight was delivered from the syringe. Pregnant mothers received their first treatment on the 12th day of gestation. Three additional doses were delivered at 2-day intervals. The offspring exposed to safrole in utero were nursed by their natural mothers. Lactating mothers received their first treatment with safrole on the day of delivery, and 11 subsequent doses were given at 2-day intervals. All offspring were weaned at 28 days of age, at which time those allocated to postweaning treatment received their first dose of safrole by direct intubation. This treatment was continued twice weekly for 90 weeks. Controls received steroid-suspending vehicle by the same method. All survivors were killed at the age of 94 weeks.

Course of Experiment. Animals were weighed at 2-week intervals and inspected for symptoms indicating poor health or the presence of internal tumors. Daily inspection of all cages permitted prompt autopsy of dead animals. Moribund animals were removed and killed. Detailed autopsies were carried out on all animals, and specimens were taken from lungs and from all grossly visible tumors. The specimens were stained by the hematoxylin and eosin method for histological evaluation. Liver and kidney tumors were classified according to criteria reported earlier (8, 11). Sections of whole lungs were examined systematically for the presence of metastases (7).

Statistical Analysis. The χ² method was used for assessment of the significance of the observed neoplastic lesions. The Yates correction factor was applied routinely. The significance of the age at which tumors were identified was estimated by means of the t test.

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RESULTS

Mice exposed to safrole or its metabolites prenatally across placenta and/or during infancy in milk had a high survival rate (≥90%; Table 1). However, mice subjected to direct gastric intubation beginning at the time of weaning, with or without previous perinatal treatment, had significantly lower survival rates (males, 50%; females, 31%; Table 1).

Benign and malignant hepatocellular tumors developed in mice of both sexes with various frequencies, depending upon the route of exposure to safrole. These tumors were detected late in the experiment, 2 in mice at 56 and 57 weeks of age, 14 by the 72nd week, and the remaining 121 between the 73rd and 94th weeks. Table 1 also presents the incidence of liver tumors based on the number of mice alive at 52 weeks. However, since 98.6% of all tumor-bearing mice were identified after their 60th week of life, the incidence based on 60-week-old survivors is listed for comparison in the last column of the table. The data show that transplacental exposure to safrole or its metabolites was ineffective in inducing liver tumors in either sex. However, animals which consumed this agent in milk as a result of gastric intubation of the mother developed liver tumors (33.7% in males and 2.5% in females). The combination of transplacental exposure and exposure in milk led to a similar incidence of liver tumors in both sexes (46.3% in males and 59.7% in females; p > 0.05). Under those conditions, the induction of liver tumors was triggered during infancy in males and during adulthood in females.

The distribution of benign and malignant hepatocellular tumors, classified according to recently described criteria (11), is presented in Table 2. In all of the safrole-exposed groups, 33 of 75 (44%) tumor-bearing males and 46 of 62 (74%) tumor-bearing females had hepatocellular carcinomas. Thus, significantly more hepatocellular carcinomas were observed in females (p < 0.01). The average age at death (Table 2, Column 11) shows that this higher incidence is not associated with greater longevity of females (p > 0.05). The overall rate of pulmonary metastases in safrole-exposed groups was 24% for males and 39% for females (p > 0.05).

In addition to liver tumors, 3 other types of neoplasms were observed: renal epithelial tumors, pulmonary adenomas and carcinomas, and malignant lymphomas. The incidence of kidney tumors was low; these tumors were seen only in females (Table 2). They occurred only in animals exposed to safrole indirectly in utero alone or in combination with the other treatment modes, which by themselves did not induce renal tumors. Thus, apparently only kidneys of female fetuses were susceptible to transplacental carcinogenesis by safrole. Lung tumors were observed with about the same incidence in safrole-treated groups as in solvent-treated controls. Malignant lymphomas were observed in one male and 2 female mice.

DISCUSSION

The present studies demonstrated for the first time the carcinogenic risk to offspring when safrole is administered to female mice during gestation, lactation, or both. The results also confirmed earlier observations regarding the modifying effect of sex and of age at treatment on liver carcinogenesis. The finding that administration of safrole to pregnant mice during the second half of gestation led to the development of epithelial kidney tumors in female offspring is biologically significant. Such tumors were not seen in the vehicle-treated controls or in females exposed to safrole or its metabolites directly or in milk. The tumors were observed, however, in groups exposed to safrole transplacentally in combination with other routes of administration. In the 3 “transplacental” groups, kidney tumors developed in 14 of 199 (7%) of females, whereas none of the 126 females exposed to safrole intra-gastrically or in milk and none of the 171 males exposed

<table>
<thead>
<tr>
<th>Exposure to safrole</th>
<th>No. of mice alive at age (wk)</th>
<th>No. of mice with liver tumors by (wk)</th>
<th>% incidence based on 60-wk survivors</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>52</td>
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<tr>
<td>Controls</td>
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<td></td>
<td>F</td>
<td>98</td>
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<td>Transplacentally</td>
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<td>63</td>
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<td>71</td>
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<td>In milk</td>
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<td>Gastric intubation</td>
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<td>Transplacentally and in milk</td>
<td>M</td>
<td>68</td>
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<td>Transplacentally, in milk, and by gastric intubation</td>
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<td>F</td>
<td>75</td>
<td>62</td>
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* Incidence based on 52-week survivors.
"transplacentally" developed such tumors. Thus, a strong age and sex difference \( p < 0.01 \) in renal carcinogenesis by safrole was observed. In contrast, in the case of the direct alkylating agent, the situation was different (10), and preweaning and adult mice were equally sensitive to renal carcinogenesis (8, 14).

Exposure of offspring to safrole or to its metabolites in milk induced hepatocellular tumors only in males. The lack of metabolic competence in females was most probably related to the absence of hepatocarcinogenesis in infant females. Gastric intubation of offspring from weaning until sacrifice, however, resulted in development of liver tumors only in females. This observation is at variance with most studies on the role of sex in hepatocarcinogenesis, in which females were found to be consistently less responsive than males. Nevertheless, the present study confirms earlier observations regarding safrole (6, 15) and benzidine (12) hepatocarcinogenicity, in which adult females were more responsive than males.

At present, it is not known, however, to what extent hepatocarcinogenesis is influenced by the variation in safrole metabolism, the degree of macromolecular binding of its derivatives in the liver, concurrent cellular replication, and the influence of imposed sex-hormonal shift on the neoplastic expression. Nevertheless, these studies suggest that perinatal exposure to safrole or its metabolites may represent a carcinogenic risk to offspring. Since safrole occurs naturally in several plants (2) and has been used as a flavoring agent (4), consideration should be given to such a possibility in exposed human populations.

REFERENCES

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