Aflatoxin B$_1$, a Hepatocarcinogen in the Infant Mouse

S. D. Vesselinovitch, N. Mihailovich, G. N. Wogan, L. S. Lombard, and K. V. N. Rao

INTRODUCTION

The hepatocarcinogenicity of aflatoxins in rats (24), ducks (2), and rainbow trout (6, 12, 13) has been convincingly demonstrated. In contrast, mice appear to be resistant to the toxic and carcinogenic effects of this otherwise potent, naturally occurring carcinogen (1).2

Our studies in the area of perinatal carcinogenesis (14, 16—21), as well as the work of others (3, 5, 8—11), revealed a significantly higher susceptibility to liver carcinogenesis in newborn and infant animals than in adult mice (23). For this reason, we decided to evaluate aflatoxin B$_1$ hepatocarcinogenicity in both newborn and infant mice.

This paper is a report of these studies, which showed that a limited exposure of 4- or 7-day-old mice to aflatoxin B$_1$ resulted in a high incidence of liver tumors in male mice.

RESULTS

Liver tumors measuring up to 5 mm in diameter already were present in mice killed at 52 weeks. By the 82nd week, livers were practically replaced by the tumors, resulting in a 3-fold increase in weight. The incidence of liver tumors in males is presented in Table 1. In Group 1, to which only trioctanoin had been administered, there were no hepatoma-bearing mice at 52 weeks of age. The 1-day-old animals (Group 2) were given aflatoxin B$_1$ only once, because such treatment resulted in a 55% mortality rate. The survivors of this acute toxic effect of aflatoxin had no tumors when they were sacrificed at 52 weeks. However, by the 82nd week, 22.7% of the males then sacrificed had small but definite hepatomas.

Group 3 animals, which were 4 days old at 1st administration of aflatoxin, did not succumb to the acute toxicity of this agent; therefore, 2 additional injections of aflatoxin were administered (total dose, 6 µg/g body weight). By 52 weeks, 70% of the male animals had hepatomas; each

SUMMARY

Aflatoxin B$_1$ has been administered to newborn and infant C57BL x C3H F$_1$ mice for a further evaluation of its hepatocarcinogenicity in this species. To date, adult animals have been shown to be relatively refractory.

One-, 4-, or 7-day-old groups of animals were exposed to either single or limited numbers of i.p. injections of aflatoxin B$_1$. Animals selected at random were sacrificed at 52 and 82 weeks of age.

Newborn animals appeared to be more prone to the lethal effect of aflatoxin B$_1$ than did the 4- or 7-day-old infants. Male infants developed hepatomas by 52 weeks of age. At 82 weeks, both tumor incidence and tumor mass increased in all male groups. Seven-day-old treated females developed hepatomas, although at a low incidence rate. Thus, it has been demonstrated that aflatoxin B$_1$ is a potent hepatocarcinogen under the experimental conditions.

It has been concluded that the infant age and the male hormonal environment were factors favorable for the inception and expression of liver neoplasia in mice.
Table 1
Incidence of hepatomas in C57BL × C3H F1, male mice at 52 and 82 weeks of age following administration of aflatoxin B1 at infancy

<table>
<thead>
<tr>
<th>Group</th>
<th>Age at start (days)</th>
<th>Quantity (µg/g)</th>
<th>No. of times given</th>
<th>Total (µg/g body wt)</th>
<th>Age at sacrifice (days)</th>
<th>No. of animals inspected</th>
<th>No. of mice with tumors</th>
<th>%</th>
<th>Multiplicity</th>
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<tr>
<td>1</td>
<td>1</td>
<td>None</td>
<td></td>
<td></td>
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<td>3</td>
<td>3</td>
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<td>2</td>
<td>1</td>
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<td>1</td>
<td>2.00</td>
<td>52</td>
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<tr>
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<td>7</td>
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<td>3</td>
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<td>52</td>
<td>10</td>
<td>4</td>
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<tr>
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<td>1.5</td>
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<td>20</td>
<td>15</td>
<td>75</td>
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</tr>
</tbody>
</table>

- a Given i.p. at 3-day intervals.
- b R, tumor mass replacing liver tissue.

animal had an average of 3.4 neoplastic nodules per liver. At 82 weeks, all animals had large hepatomas. Experimental Group 4, which received a total amount of 3 µg of aflatoxin per g body weight, showed a somewhat lower neoplastic response. A further decrease in dose (5 X 0.25 µg/g body weight given to animals in Group 5) resulted in 50 and 89% incidence of hepatomas in mice killed at 52 and 82 weeks of age, respectively.

A single exposure to aflatoxin B1, 6 µg/g body weight, was well tolerated by the 7-day-old mice (Group 6), as indicated by the absence of acute and late mortality. At 52 weeks, 75% of the male mice had an average of 3.6 hepatomas per liver. Eighty-five % of the mice sacrificed at 82 weeks of age bore hepatomas which were replacing the liver mass.

Female mice, which were killed at 82 weeks of age, showed liver tumors only in the group that received aflatoxin B1 at 7 days of age. Two of 28 animals (7.0%) in this group had large hepatomas.

The tumors observed at 52 weeks of age were characterized by solid masses or thick cords of tumor cells resembling hepatocytes, but they lacked the lobular orientation of the normal liver. The extent of cellular pleomorphism and the ratio of nucleus to cytoplasm varied from animal to animal, as well as from one hepatoma to another. At 82 weeks, the entire liver appeared to be replaced by nodular tumorous tissue. On microscopic examination, it was observed that individual hepatomas merged with one another. Because of their close proximity, the hepatomas generally appeared as solid sheets of tumor cells. Only the least compressed, peripherally located hepatoma cells showed trabecular structure. Cellular pleomorphism was a common finding. Mitotic figures were numerous and frequently abnormal in hepatomas of mice killed either at 52 or 82 weeks of age.

**DISCUSSION**

Studies at various laboratories suggested that the adult mouse may tolerate relatively high doses of aflatoxin B1 (up to 60 mg/kg) without manifesting toxic or carcinogenic response and that the metabolism of this agent differs from that found in the other species (23). In contrast, the newborn mice appeared highly sensitive to the acute toxic effect of this agent, since 55% of those treated with 2 µg of aflatoxin died within 4 days. The present data also demonstrated that 4- and 7-day-old mice were highly responsive to the hepatocarcinogenic effect of aflatoxin B1. Apparently, the necessary metabolic competence developed shortly after birth (4, 7), leading to effective aflatoxin B1 metabolism and to generation of carcinogenic species and their interaction with critical, actively replicating (22) macromolecular site(s).

The sensitivity of 4-day-old mice to hepatocarcinogenesis is also manifested by the fact that a total amount of only 0.004 µM aflatoxin B1 (1.25 µg) per g of body weight (given from the 4th to 16th day of age in 5 treatments) was sufficient to induce hepatomas in 89% of the mice.

The extreme hepatocarcinogenicity of aflatoxin for infant mice becomes clear when one compares the effects of the aforementioned amount of this agent with the following doses of 2 other hepatocarcinogens. Mice of the same strain and age required 16.84 µM urethan (18) and 0.51 µM ethylnitrosourea (15) per g of body weight to manifest a similar hepatocarcinogenic effect.

The current findings further substantiated our previous observations that the infant age is a period when the mouse is highly prone to hepatocarcinogenesis by urethan (16, 18) and by X-irradiation (21). Also, the low incidence of liver tumors that developed in females complements the importance of
hormonal environment for the neoplastic expression in that organ (17). It is obvious, therefore, that the outcome of carcinogenesis must be viewed as the result of a multifactorial interaction.

REFERENCES

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