Malignant Tumors in Rats Given Lasiocarpine

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SUMMARY

Lasiocarpine was injected i.p. into rats in doses of 7.8 mg/kg body weight (50% lethal dose of 0.1) twice weekly for 4 weeks and once a week for an additional 52 weeks. Of 18 rats that survived, 16 developed tumors between weeks 60 and 76 after the beginning of the experiment. Approximately 61% (11 of 18) developed hepatocellular carcinomas, and 33% (6 of 18) developed well-differentiated squamous cell carcinomas of the skin of the back. These tumors were transplanted successfully through five generations. Other tumors included pulmonary adenoma (in 28%), well-differentiated adenocarcinoma of the small intestine (in two rats), and cholangiocarcinoma and adenomyoma of the ileum (in one rat).

It is clear that lasiocarpine is a complete carcinogen capable of inducing a significant number of malignant tumors of the liver and skin of rats. A suitably long experimental interval for administration of the alkaloid (52 weeks) plus a period of observation after the dosing is discontinued (4 to 20 weeks) may be necessary to allow cessation of the antimitotic effect of the alkaloid and to permit expression of its carcinogenicity.

INTRODUCTION

The pyrrolizidine (Senecio) alkaloids constitute a large group of naturally occurring environmental toxins derived from several botanical families distributed in various parts of the world (8, 11). The chemistry, metabolism, and pathological alterations resulting from acute and chronic alkaloid poisoning in animals have been reviewed by McLean (11). In acute toxic doses, several of the pyrrolizidine alkaloids cause centrolobular hemorrhagic necrosis of the liver, segregation of the nucleolus, and disaggregation of polyribosomes associated with rapid inhibition of RNA polymerase activity and of RNA synthesis (14). Chronic administration results in progressive enlargement of liver parenchymal cells, focal perportal fibrosis, bile duct proliferation and, occasionally, cirrhosis of the liver. Several of the alkaloids have an antimitotic and antitumor effect (5, 13).

Some of the pyrrolizidine alkaloids have long been known to cause acute and chronic liver injury in grazing animals and, although the possibility of contamination of foodstuffs with these alkaloids and their use in various indigenous medicines in several developing countries have been reported (17), their possible importance in causing cirrhosis and liver tumors in man has not been ascertained.

The literature currently available reveals marked variation in results and conflicting opinions regarding the hepatocarcinogenicity of certain pyrrolizidine alkaloids. In reviewing specific experiments, we find that there are several factors which might explain the divergent results and interpretations that have arisen with respect to carcinogenicity of these alkaloids. First, there is marked variation in the length of experiments, dose schedules, concentration of alkaloid, route of administration, strain of rat, and periods of observation. Second, in some studies mixtures of alkaloids were used and, because of the unspecified concentrations of each, it was impossible to determine whether one or more of the agents was responsible for the pathological changes. Third, inadvertent contamination of the experimental diet with aflatoxin may have been a factor in studies in which groundnut meal was used. Finally, in those reports of alleged tumor induction, the pathological descriptions were equivocal, or attempts to determine definitely the malignancy of the tumors were not made, thus rendering definite conclusions impossible.

Cook et al. (3) reported liver tumors or nodules in 3 rats fed an unspecified mixture of pyrrolizidine alkaloids from Senecio jacobaea for 8 months. The biological behavior of the tumors was unknown, and the authors were not certain that the changes were true neoplasms. In a later publication, Schoental et al. (18) reported 14 hepatornas in rats given retrorsine or isatidine. One of the tumors developed metastases. In this experiment, however, the animals were given a diet containing 8.5% groundnut meal, and it is possible that the diet was contaminated with aflatoxin. Campbell (2) reported malignant liver tumors in 3 chickens fed a mixture of senecephylline and jacobine, and Harris and Chen (7) reported malignant tumors in rats fed a diet containing Senecio longilobus. In contrast, in 2 experiments, Sedlmeier et al. (19) found no tumors in rats given Senecio vulgaris for 3 to 6 months and concluded that the alkaloids do not have a direct carcinogenic action.

Bull et al. (1) rejected the concept that pyrrolizidine alkaloids cause malignant tumors of hepatic parenchymal cells. They interpret the suspected hepatomas reported by Schoental et al. as regenerating liver and, in their own experiments, found no hepatomas in 65 rats.

McLean (11) recently reviewed all data regarding the possible carcinogenicity of the pyrrolizidine alkaloids and concluded that at present there is no evidence, from in vivo experiments, that lasiocarpine (Chart 1) is carcinogenic to the liver. Similarly, Rogers and Newberne (16) did not detect tumors in rats given repeated doses of lasiocarpine for a year.

In view of the prevailing controversy regarding the possible effects...
carcinogenicity of pyrrolizidine alkaloids and, particularly, the lack of conclusive evidence of carcinogenicity of lasiocarpine specifically, the present studies were done.

MATERIALS AND METHODS

Fifty inbred male Fischer 344 rats (A. R. Schmidt Co., Madison, Wis.) weighing 110 to 125 were maintained in separate cages. They were divided into 2 groups, each consisting of 25 animals. Rats in both groups were given free access to water and were fed a synthetic diet having the following composition: casein, 16%; full vitamin mixture, 2.5%; Salt Mixture XIV, 4%; sugar, 62.5%; corn oil, 10%; and Alphacel, 5%.

Freshly prepared lasiocarpine was given i.p. to 1 group of rats in a dose of 7.8 mg/kg body weight (0.1 50% lethal dose) twice weekly for the first 4 weeks and once a week for an additional 52 weeks, after which the administration of lasiocarpine was discontinued. The 2nd group of animals served as controls and were given 0.9% NaCl solution, 0.1 ml/100 g body weight, by i.p. injection. Laparotomies were performed under methoxyflurane (Metofane; Pitman-Moore Div., Fort Washington, Pa.) anesthesia at selected intervals, and liver biopsies were obtained for light and/or electron microscopic examination. Autopsies were done when the animals were moribund or had palpable tumors. A majority of the animals treated with lasiocarpine were sacrificed between Weeks 60 and 76.

Microscopic Studies. For light microscopy, tissues were fixed in formalin and embedded in paraffin, and all the sections were routinely stained with hematoxylin and eosin. When indicated, periodic acid-Schiff, mucicarmine, oil red O, Masson’s trichrome, Van Gieson’s stain, and the Gordon-Sweet reticulum stains were used. The sections were examined with a Zeiss Ultraphot II microscope and were photographed with the use of a green filter.

For electron microscopy, tissue blocks of approximately 1 cu mm were fixed for 1 to 2 hr in osmium tetroxide buffered with s-collidine to pH 7.4, at 0–4°. The blocks were dehydrated in a graded series of alcohols and embedded in Epon 812 containing 5% Araldite. Thin sections were stained with lead hydroxide and examined by electron microscopy.

For cytochemical demonstration of microbody peroxidase activity, samples of liver, of intrahepatic liver tumors and their metastases in lungs, and of s.c. transplants of liver tumors were fixed in 3% glutaraldehyde; buffered with 0.1 M sodium cacodylate buffer (pH 7.4), containing 0.22 M sucrose; and incubated for peroxidase activity at pH 9.0 in the reaction medium of Graham and Karnovsky (5), as modified by Novikoff and Goldfischer (12).

Transplantation. Weanling male Fischer 344 rats weighing between 60 and 80 g were used as tumor transplant recipients. All procedures were done under sterile conditions. Portions of intrahepatic primary liver tumors and of squamous cell carcinomas of skin were removed from the donor rats and minced in sterile 0.9% NaCl solution. Two to 3 small blocks of tumor approximately 2 mm in diameter were placed s.c. in the inguinal region of the recipient rats under Metofane anesthesia.

RESULTS

The mortality, tumor incidence, and number and types of tumors resulting from lasiocarpine treatment are summarized in Table 1. During the initial stages of the experiment, lasiocarpine was injected in a 50% lethal dose of 0.1 (7.8 mg/kg body weight) i.p. twice weekly. Three rats died of acute liver necrosis during the first 4 weeks with this regimen. Thereafter, lasiocarpine was injected once a week (7.8 mg/kg body weight) after the 4th week for an additional 52 weeks, at which time treatment was stopped. By this time, each of the 18 surviving rats had received an average cumulative dose of 125 mg of lasiocarpine. Of these 18 rats, 16 developed tumors between 60 and 76 weeks after the beginning of the experiment. Ten of the 16 animals had more than 1 tumor. Of the animals that survived more than 56 weeks of receiving lasiocarpine treatment, approximately 61% (11 of 18) developed liver tumors, 33% (6 of 18) developed squamous cell carcinomas of the skin, and 28% (5 of 18) had pulmonary adenomas. Among the other tumors were adenocarcinoma of the small intestine (2 rats), adenomyoma of the ileum (1 rat), and interstitial cell tumor of the testis (1 rat).

Grossly, the hepatomas appeared as irregular gray to brown nodules of varying size (Fig. 1) and, on section, were well demarcated from surrounding liver parenchyma. Histologically, the predominant pattern was that of a well-differentiated trabecular hepatoma (Fig. 2), although some tumors were less differentiated and were composed of polyhedral cells without a trabecular pattern (Fig. 3). The metastases in the lungs were of similar poorly differentiated pleomorphic cell type (Fig. 4). Electron microscopic examination of blocks of primary hepatic tumors and their pulmonary metastases incubated for peroxidase activity revealed the presence of numerous circular cytoplasmic bodies containing a dense reaction product. These bodies corresponded to microbodies (peroxisomes) that were

Chart 1. The structural formulas of lasiocarpine, a pyrrolizidine alkaloid, (left), its N-oxide (center), and pyrrole derivatives (right). It is believed that the pyrrolizidine alkaloids themselves are not hepatotoxic but that a portion of the alkaloid is metabolized in the liver through the N-oxide to a pyrrole-like derivative, which is considered to be hepatotoxic (10).

3,3-diaminobenzidine (Sigma Chemical Co., St. Louis, Mo.)

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cholangiocarcinoma were successfully transplanted s.c. into weanling Fischer 344 rats. In relation to the number at the start. Control (0.9% NaCl solution) four of these animals died during the first 4 weeks of the experiment and the others died within 9 months.

In relation to the effective number.

Lasiocarpine was given i.p. in a dose of 7.8 mg/kg body weight once every week for 56 weeks and then was discontinued. By that time, each animal had received an average total dose of 125 mg of lasiocarpine. 

Four resulted from hepatocellular carcinoma and 1 resulted from cholangiocarcinoma. In our study, the use of a specified synthetic diet and the absence of tumors in the control group suggest strongly that the tumors were induced by lasiocarpine and that diet contaminants, if present, were a negligible factor. Absence of a carcinogenic effect of lasiocarpine and other pyrrolizidine alkaloids, as reported by others (1, 16, 19), may be related to differing experimental dose schedules. For example, in the experiments of Sedlmeier et al. (19), rats were given alkaloids for only 3 to 6 months, which may be too short a period to elicit an overt carcinogenic response. The lack of tumor induction in rats after 1 year of repeated doses of lasiocarpine, as reported by Rogers and Newberne (16), may be due either to too short an interval or the fact that the dosing was not interrupted. In view of the antimitotic effect of lasiocarpine, treatment may have to be interrupted in order to allow the antimitotic effect to subside and permit growth of a tumor. McLean (11) pointed out that, in those experiments that failed to produce tumors, dosing was regular and continued until death while, to produce tumors, it may be necessary to interrupt or discontinue the alkaloid administration several months before death. In the present experiments, the injection of lasiocarpine was discontinued 4 to 20 weeks before the appearance of most tumors.

It is clear from these findings that lasiocarpine is a complete carcinogen capable of inducing malignant tumors not only of the liver but also of the skin and probably of the small intestine in rats. The malignancy of the tumors of liver and skin is confirmed by successful transplantation through 5 generations, with successively shorter periods being required for the transplant to become palpable. The presence of microbodies (peroxisomes) in the cells of 10 of the liver tumors attests to the derivation of such tumors from hepatic parenchymal cells rather than from bile duct epithelium, since microbodies are characteristically present in the former but absent from the latter. The occurrence of cholangiocarcinoma in 1 rat, however, suggests that lasiocarpine is also carcinogenic to bile duct epithelium.

Because of the possibility of aflatoxin contamination of the diet in the study reported by Schoental et al. (18), it was not certain that the hepatomas reported were due solely to retrorsine or isatidine. In our study, the use of a specified synthetic diet and the absence of tumors in the control group suggest strongly that the tumors were induced by lasiocarpine and that diet contaminants, if present, were a negligible factor.

Table 1
Cumulative data on mortality and tumor incidence of male Fischer 344 rats on lasiocarpine

<table>
<thead>
<tr>
<th>Animals with</th>
<th>Livers with</th>
<th>Tumor-bearing animals</th>
<th>Animals with more than 1 tumor</th>
<th>Squamous cell carcinoma of skin</th>
<th>Metastases in lungs</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>Died</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Control (0.9% NaCl solution)</td>
<td>25</td>
<td>25</td>
<td>2</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lasiocarpine</td>
<td>25</td>
<td>18</td>
<td>7</td>
<td>28</td>
<td>16</td>
<td>88.9</td>
</tr>
</tbody>
</table>

a Number alive at the time of appearance of 1st tumor, a hepatocellular carcinoma with lung metastases at 60 weeks. These animals were killed or died between 60 and 76 weeks after the commencement of the experiment.

b In relation to the number at the start.

c In relation to the effective number.

d Of these 11 liver tumors, 10 were hepatocellular carcinomas and 1 was a cholangiocarcinoma. All liver tumors with the exception of the cholangiocarcinoma were successfully transplanted s.c. into weanling Fischer 344 rats.

e Four resulted from hepatocellular carcinoma and 1 resulted from cholangiocarcinoma.

f In relation to the number of liver tumors.

g Four of these animals died during the first 4 weeks of the experiment and the others died within 9 months.

DISCUSSION

It is clear from these findings that lasiocarpine is a complete carcinogen capable of inducing malignant tumors not only of the liver but also of the skin and probably of the small intestine in rats. The malignancy of the tumors of liver and skin is confirmed by successful transplantation through 5 generations, with successively shorter periods being required for the transplant to become palpable. The presence of microbodies (peroxisomes) in the cells of 10 of the liver tumors attests to the derivation of such tumors from hepatic parenchymal cells rather than from bile duct epithelium, since microbodies are characteristically present in the former but absent from the latter. The occurrence of cholangiocarcinoma in 1 rat, however, suggests that lasiocarpine is also carcinogenic to bile duct epithelium.

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From the present results it is clear that lasiocarpine is capable of causing hepatocellular and squamous cell carcinomas in rats. Although in several parts of the world, groups of people as well as farm animals have had at least one episode of pyrrolizidine poisoning, there is as yet no correlation between alkaloid ingestion and subsequent hepatoma. Absence of such correlation may be due to factors similar to those that influence attempts to induce tumors experimentally with the alkaloids, i.e., cumulative dose; dose schedule; mixtures of dietary contaminants; period of observation; death from other earlier effects of the alkaloids, such as venoocclusive disease or cirrhosis; and other complex factors. Accordingly, for the present, the possibility exists that lasiocarpine or related pyrrolizidine alkaloids may be one of several factors involved in the high incidence of liver cancer in populations that consume these alkaloids deliberately or inadvertently.

REFERENCES


Fig. 1. Liver of a male rat given lasiocarpine by i.p. injection for 56 weeks. Several light gray tumor nodules are apparent.
Fig. 2. Most of the liver tumors were well-differentiated trabecular hepatomas that invaded surrounding liver parenchyma. H & E, X 450.
Fig. 3. A few of the liver tumors were composed of pleomorphic polyhedral cells with no well-defined histological pattern. H & E, X 150.
Fig. 4. Pulmonary metastases were typically of the pleomorphic cell type, often with abundant fibrous stroma. H & E, x 180.

Fig. 5. Well-differentiated squamous cell carcinoma of the skin of the back. H & E, x 150.

Fig. 6. Moderately well-differentiated, mucin-secreting adenocarcinoma of the small intestine. H & E, x 120.
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