Carcinogenic Effects of Cycasin in Syrian Golden Hamsters and the Transplantability of Induced Tumors

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SUMMARY

Three groups of newborn hamsters received a single s.c. injection of 0.2, 0.4, and 0.6 mg cycasin/g body weight within 24 hr after birth. Two groups of adult hamsters each received one dose of 0.1 or 0.15 mg cycasin/g body weight by stomach tube. Another three groups of adult hamsters received two to four doses of 0.1 mg cycasin/g body weight by stomach tube at intervals of 1 month.

There was no significant difference in the incidence of tumors between newborn and adult hamsters receiving a single administration of cycasin and between adult hamsters receiving single and repeated administrations. However, adult hamsters receiving the larger single dose of cycasin showed a higher incidence of tumors than those receiving the smaller single dose.

The most important changes induced in hamsters with cycasin were seen in the liver and consisted of proliferative changes of the intrahepatic bile ducts. Bile duct carcinomas were produced both in newborn and adult hamsters. Although hepatocellular carcinomas are as readily induced as bile duct carcinomas in newborn hamsters, hepatocellular carcinomas were not found among adult animals.

Eight liver tumors induced by cycasin were transplanted, and five transplantable tumor lines were successfully established. Four lines originated in intrahepatic bile duct carcinomas, and the fifth originated in an anaplastic carcinoma, presumably of liver cell origin.

Kidney and intestinal tumors, which were frequently induced in rats by cycasin, were observed in a much lower incidence in hamsters.

INTRODUCTION

Cycasin (β-D-glucosyloxyazoxymethane) is a toxic glucoside contained in cycads, Cycas revoluta, and Cycas circinalis. The carcinogenic effect of cycasin in rats was first reported in the papers of Laqueur (10) and Laqueur et al. (11). Adult rats treated with a single p.o. dose of cycasin developed tumors mainly in the kidney and the large intestine, whereas a single s.c. injection into newborn rats within 24 hr after birth produced a high incidence of lung and liver cell adenomas and kidney tumors (5). Kidney and intestinal tumors were rare in mice, but hepatocellular carcinomas were common, especially in newborn mice of the C57BL/6 strain that survived for more than 280 days after the administration of cycasin (6). Thus, tumors of the intestine or kidney, frequently observed in cycasin-treated rats, were rarely found in mice. On the other hand, hepatocellular carcinomas, which were rare in rats treated with a single dose of cycasin, were frequently encountered in mice.

Obviously, rats and mice treated with a single s.c. dose of cycasin at birth developed tumors at different sites later in life. The autopsy findings in Syrian golden hamsters that had similarly received a single s.c. dose of cycasin at birth and had been used in another study (7) have now become available for inclusion in a study of the carcinogenic effects of cycasin on newborns of different laboratory animals. They are reported together with some observations on older hamsters that had received cycasin by stomach tube.

MATERIALS AND METHODS

Chemicals. The cycasin as crystals used in these experiments was supplied by Dr. H. Matsumoto, Department of Agricultural Biochemistry, University of Hawaii, and was dissolved in 0.9% NaCl solution to make 2.5 and 5.0% solutions, respectively. The solutions were filtered through a Millipore filter before use.

Animals. Noninbred hamsters of both sexes were originally obtained from a commercial animal supply source in Nagoya, Japan, and were bred in our laboratory. Their offspring were used in the experiments. A total of 150 newborn hamsters from 18 mothers received a single s.c. injection of cycasin at 3 dose levels within 24 hr after birth; i.e., 96 newborns received 0.2 mg cycasin/g body weight (Group 1), 40 received 0.4 mg/g body weight (Group 2), and the remaining 14 newborns received 0.6 mg/g body weight (Group 3). The 2.5% cycasin solution was used in these experiments. Following the injection, the newborns were returned to their respective mothers and were weaned when 1 month old. These hamsters, as well as those of Group 4, were included in an earlier report describing neurotoxicity of cycasin (7).

Ninety-nine 2-month-old hamsters of both sexes were divided into 5 groups. Twenty-three (Group 4) and 25 hamsters (Group 5) received a single intragastric dose of 0.15 or 0.1 mg cycasin/g body weight by stomach tube. Eighteen (Group 6), 17 (Group 7), and 16 hamsters (Group 8) received 2, 3, and 4 administrations, respectively, of 0.1 mg cycasin/g body weight by stomach tube at intervals of 1 month.
A 5.0% cycasin solution was used in the experiment with adult hamsters. Food was withheld overnight prior to administration of cycasin.

Thirteen newborn hamsters served as a control group without treatment. All animals, except for the newborns, were maintained on a diet of CLEA (Central Laboratory of Experimental Animals, Tokyo, Japan) and water. Animals that survived beyond 150 days after the beginning of the experiment were autopsied at death or were killed when moribund. All organs were fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin. Experiments were terminated 480 days after the start of cycasin administration.

**Transplantation of Tumors.** Tissue homogenates were prepared from 8 liver tumors with a tissue press equipped with a metal sieve. As a rule, 0.5 ml of this material was s.c. injected into the back of 2- to 3-month-old hamsters. The sex of the donors near the end of their life-span.

Successive transplantation was carried out with tumors of donors who survived beyond 16 days and showed neurological signs, including ataxia and gait disturbances.

The tumor incidence observed in animals surviving beyond 150 days is summarized in Table 1. The most important lesions were confined to the liver, especially to the intrahepatic bile ducts. The description of the lesions in the liver followed that of Herrold (4). Proliferative changes of the intrahepatic bile ducts were classified into 3 main categories: bile duct proliferation, cystic lesions, and carcinoma. Adenomatous and cystadenomatous lesions of the bile ducts were included with bile duct proliferative changes. Degenerative, cystic dilatation of the proliferated bile ducts was classified as cystic lesion. In Groups 1 and 2, cystic lesions of the bile duct were most commonly observed in long-term survivors. Other lesions included focal proliferations of bile ducts and of reticuloendothelial cells. Hepatocellular carcinomas (Figs. 1 and 2) and bile duct carcinomas were relatively frequently encountered (Table 1). Only 5 of 14 animals in Group 3 survived beyond 150 days; the other 9 hamsters died of acute toxicity of cycasin and pneumonia.

**Intrahepatic bile duct carcinoma** was usually observed as a grayish-white, relatively soft nodule that frequently invaded the abdominal tissues. Microscopically, it was an adenocarcinoma (Fig. 3) in which mucin production was not prominent. Most hepatocellular carcinomas were of the trabecular type (Fig. 1) and had metastasized to the lung (Fig. 2). Intrahepatic bile duct carcinoma and hepatocellular carcinoma usually developed about 1 year after administration of cycasin. Hemangioblastic sarcomas were also occasionally observed. Lesions in other organs noted in a few animals included lung adenomas (7/73; 9.5%) and cortical adenomas of the adrenal gland (3/73; 4.1%). A carcinoma of the gallbladder was found in only 1 animal. Kidney and intestinal tumors were not induced in the newborns of Groups 1 and 2. One intestinal tumor was noted in an animal of Group 3.

With consideration of only the intrahepatic liver tumors, the relative incidence was 32% in the groups of neonatal hamsters (Groups 1 to 3), whereas the incidence for the mature hamsters receiving a single administration was 24% (Groups 4 to 5). This difference was not significant statistically. Also, in comparison of the overall incidence of tumors, no significant difference was observed. These calculations were obtained after the occasional occurrence of multiple liver tumors of different types was taken into consideration. Such animals were counted only once.

**Table 1**

*Incidence of tumor induced by cycasin in newborn and adult hamsters*

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. surviving beyond 150 days</th>
<th>Liver cell</th>
<th>Liver</th>
<th>Gall bladder carcinoma</th>
<th>Kidney</th>
<th>Intestine</th>
<th>Malignant lymphoma</th>
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<tr>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
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<tr>
<td>Total</td>
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<td>4 (5.4)</td>
<td>6 (8.2)</td>
<td>9 (12.3)</td>
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<td>5 (21.7)</td>
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a Value in parentheses, percentage.
Adult Hamsters. The results are summarized in Table 1. Hepatocellular carcinoma did not occur among adult hamsters in contrast to the observations in newborn hamsters. Tumors of the intrahepatic bile ducts and of reticuloendothelial cells developed with equal frequency in adult and newborn animals. Most intrahepatic bile duct carcinomas were observed at almost the same time after treatment as in the experiment with newborns. Malignant lymphomas were occasionally seen in adult hamsters but were not observed in newborns treated with cycasin. The incidence of intestinal adenomas and adenocarcinomas was low, and the majority of these tumors was observed in the colon. Out of 78 animals (Groups 4 to 8) that survived over 150 days after the start of the experiment, one animal developed a kidney adenoma and 1 developed a renal cell carcinoma; 5 lung adenomas were noted in Group 4 and 1 lung adenoma each was seen in Groups 6 and 7. A comparison of the overall incidence of neoplasms in Groups 4 and 5 indicates that those receiving the larger single dose (Group 4) had about twice as many tumors as those receiving the smaller dose (Group 5). This difference becomes less striking when only tumors of the liver are considered. There was, however, no difference in the tumor incidence between hamsters receiving single and repeated administrations.

Hemorrhagic cysts, characterized by small, blood-filled spaces, were frequently encountered in both newborn and adult animals. Larger doses and more frequent administration of cycasin seemed to induce a higher frequency of these hemorrhagic cysts. In adult hamsters, especially in Groups 7 and 8, which received consecutive administrations of cycasin, 9 hamsters that died from 10 to 60 days after the last administration showed megagolycytosis in the liver with striking enlargement of liver cells and a large nucleus with prominent nucleoli.

Two out of 13 hamsters in the control group had tumors; 1 had a liver adenoma, and 1 had a colonic carcinoma.

Transplantation of Cycasin-induced Tumors. Six of 8 tumors were successfully transplanted; by error, 1 of them was not transplanted to the 2nd generation. Thus, 5 tumors (69–549, 69–548, 69–391, 69–615, and 69–483) were established as transplantable tumor lines. These tumors were intrahepatic bile duct carcinomas except 1 line, 69–391, which was tentatively diagnosed as an anaplastic carcinoma. The successful transplantation rate of these tumor lines was 100%. The average life-span of host animals varied from 55 to 110 days depending on the tumor lines. Four of the liver-established tumor lines maintained the same features as the primary tumor during successive transfer generations (Figs. 3 and 4). The 5th (69–391), the highly undifferentiated tumor possibly of hepatic cell origin, widely metastasized in each transfer generation to the lung, kidney, liver, ovary, and lymph nodes. Successful transplantation was not achieved with 1 hepatocellular carcinoma and 1 hemangioendothelial sarcoma.

DISCUSSION

As previously reported, rats treated with a single s.c. or intragastric dose of cycasin developed a high incidence of kidney tumors (5), while mice receiving the same treatment rarely had kidney tumors (6). This study shows that kidney tumors were also rarely found in hamsters. Hepatocellular carcinomas, which were frequent in mice, especially when they were treated as newborns (6), were not induced with a single administration of cycasin in rats except for a few treated as newborns (5). Hepatocellular carcinomas were induced with cycasin only in newborn hamsters, whereas proliferative changes and carcinomas of the intrahepatic bile ducts, rarely encountered in either rats or mice (5, 6), were observed with a high incidence in hamsters when treated either as newborn or as adult animals.

These changes in the liver were not seen in any of our control hamsters nor in another group of about 50 hamsters serving as controls in other experiments.

The carcinomas of the intrahepatic bile ducts induced in hamsters were transplantable. Thus, a single dose of cycasin to hamsters is a convenient method of obtaining bile duct carcinomas for transplantation studies.

Intestinal tumors, which were observed frequently in rats but not in mice, occasionally occurred in hamsters. However, it has been reported (2, 3) that intestinal tumors develop spontaneously in some colonies of hamsters, and 1 of 13 untreated control animals in this study had a colonic carcinoma. It may be difficult, therefore, to conclude that the intestinal tumors observed in this study were caused by cycasin.

For comparison of the target organs of a certain carcinogen among different species of animals, it is necessary to use the same dose and the same route of administration. This requirement for comparative response studies with cycasin can be only partially fulfilled because of the considerably greater sensitivity of the hamster to cycasin and its metabolite when compared with mice and rats. The LD50 of cycasin in adult animals was 562 mg/kg body weight in rats, 500 mg/kg in mice, and less than 250 mg/kg in hamsters (1. Hirono et al., unpublished data). The data indicate that the localization of tumors induced by a single administration of cycasin differed among the species thus far examined. The main target organs in rats were kidney and intestine, and in mice and hamsters the main target organ was the liver. Furthermore, the hepatocellular carcinomas were most frequently found in mice, whereas intrahepatic bile duct alterations represented the main lesion in the liver of hamsters.

Spatz et al. (13) reported that hamsters given a single administration of methylazoxymethanol, the aglycone of cycasin, developed a high incidence of colonic tumors as well as liver tumors. This finding suggests that the incidence of intestinal tumors may depend on the dose of cycasin and the rate of hydrolysis by which the aglycone, the proximate carcinogen, is obtained in vivo.

Three out of 73 hamsters in the experiment on neonates had cortical adenomas of the adrenal gland. However, cortical adenomas develop spontaneously in hamsters of some colonies (8, 9), and thus it is conceivable that these tumors developed spontaneously. Malignant lymphomas were observed in 6 out of 78 hamsters that survived beyond 150 days in the experiment with adult hamsters. Toth (14, 15) reported that spontaneous malignant lymphomas were observed in hamsters.

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and that the incidence was accelerated by 7,12-dimethylbenz(a)anthracene. Since in the control group and the experiment on neonates no malignant lymphoma was observed, the results obtained in this study may be attributable to cycasin.

Most newborn hamsters treated with cycasin showed defective development of the molecular and granular cell layers of the cerebellar cortex, and some of them had developed ataxia and gait disturbances as reported previously (7). However, gliomas were not observed.

Different views have been expressed as to the significance of megalocytosis. Bull and Dick (1) interpreted megalocytosis caused by senecio poisoning as a degenerative change of liver cells. On the other hand, Scheuer (12) suggested that megalocytosis was a preneoplastic form of proliferation rather than a degenerative phenomenon. In this study, hepatocellular carcinomas were not induced in adult hamsters, although megalocytosis was observed frequently. Thus, it seems to be difficult to interpret megalocytosis as a preneoplastic change.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Hepatocellular carcinoma of the trabecular form. H & E, x 255.
Fig. 2. Lung metastases from primary hepatocellular carcinoma shown in Fig. 1. H & E, x 255.
Fig. 3. Primary intrahepatic bile duct carcinoma. H & E, x 255.
Fig. 4. The 2nd transfer generation of intrahepatic bile duct carcinoma shown in Fig. 3. H & E, x 255.
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