CARCINOGENICITY IN RATS OF A MUTAGENIC COMPOUND, 3-AMINO-1,4-DIMETHYL-5H-PYRIDO[4,3-b]INDOLE, FROM TRYPTOPHAN PYROLYSATE

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3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) is a mutagenic principle isolated from a tryptophan pyrolysate. When Trp-P-1 was given to male and female F344 rats at concentrations of 0.015% and 0.02%, respectively, in the diet, it induced hepatocellular carcinomas in high incidence within a year. No tumor was found in control rats.

Key words: Tryptophan pyrolysate — Rat — Hepatocarcinogenicity

A series of new mutagenic heterocyclic amines has been isolated from pyrolysates of amino acids and proteins1,2 and all of these heterocyclic amines so far tested have been found to be carcinogenic to mice and/or rats.3-8 Of these mutagens, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) from a tryptophan pyrolysate were the first to be isolated and identified.9 Both of them are also found in small quantities in broiled sardines and broiled beef.10,11 When injected subcutaneously, Trp-P-1 induced sarcomas at the injection site in golden hamsters and rats.12 Furthermore, in long-term feeding studies both Trp-P-1 and Trp-P-2 induced hepatomas in CDF1 mice,3 and Trp-P-2 induced neoplastic liver nodules in ACI rats.13 In the present work, the carcinogenic effect of oral administration of Trp-P-1 was studied in F344 rats.

MATERIALS AND METHODS

Six-week-old F344 rats of both sexes were obtained from Charles River Japan Inc., Kanagawa. They were housed 4 to a plastic cage and their body weights were measured once a month. Food and tap water were available ad libitum.

Synthetic Trp-P-1 acetate was obtained from Nard Institute, Osaka. The compound was analyzed by high-performance liquid chromatography, mass spectroscopy and elementary analysis, and found to be more than 99.5% pure. A sub-acute toxicity test showed that males were more sensitive than females. Accordingly, Trp-P-1 acetate was added to pellet diet (CE-2; CLEA Japan, Tokyo) at a concentration of 0.015% for male rats and 0.02% for females. The presence of more than 90% of the added Trp-P-1 in the diet was confirmed by high-performance liquid chromatography after extraction of the compound with methanol.

Groups of 40 rats of each sex (initially 8 weeks old) were given diet containing Trp-P-1 acetate, while control groups of 50 rats of each sex were given basal diet only. Autopsies were performed when animals died or were killed after becoming moribund. All surviving animals were killed on day 365. Organs were fixed in 10% neutralized formalin, and embedded in paraffin. Paraffin blocks were sectioned, and stained with hematoxylin and eosin.

Histologic types of liver tumors were classified as recommended in the report on “Histologic Typing of Liver Tumors of the Rat.”14) The $X^2$ test was used for statistical analysis of differences in tumor incidences.

RESULTS

The average body weights of male and female rats given Trp-P-1 were 33% and 20%, respectively, than those of control rats. The average consumptions of diet per day in control groups were 16.5 g for males and 10.0 g females. The average intakes of food and carcinogens, respectively, per day per rat, in Trp-P-1 treated groups were 12.3 g and 1.9 mg for males, and 8.4 g and 1.7 mg for females.
On day 184, the first hepatic tumor was found in a female rat given Trp-P-1. The survival rates on day 184 of male and female rats given Trp-P-1 and male and female control rats were 90%, 98%, 100%, and 100%, respectively. However, subsequently the survival rate of rats fed Trp-P-1 decreased sharply, mainly due to the development of liver tumors. The survival times (days; mean ± SD) of Trp-P-1-treated groups were 279 ± 25 for males and 237 ± 22 for females. Control rats maintained until day 365, when surviving rats were sacrificed.

The incidences of liver tumors in the experimental and control groups are summarized in Table I. In the groups treated with Trp-P-1, liver tumors were found in 30 of 40 males and in 37 of 40 females. There was a significant difference in the incidences of liver tumors in the two sexes.

Histologically, most liver tumors were hepatocellular carcinomas. In male rats treated with Trp-P-1, most hepatocellular carcinomas showed a mixed pattern of trabecular carcinomas and adenocarcinomas, but a few were either trabecular or poorly differentiated carcinomas. Metastasis of hepatocellular carcinomas to the lung was observed in one male rat. Most hepatocellular carcinomas in females treated with Trp-P-1 were trabecular carcinomas or a mixture of trabecular carcinomas and adenocarcinomas, but a few poorly differentiated carcinomas were also found.

The other tumors found in rats treated with Trp-P-1 were 2 adenocarcinomas of the small intestine and 2 adenomas of the colon. No tumor was found in the control group.

**DISCUSSION**

In the present work we demonstrated that oral administration of Trp-P-1 induced hepatocellular carcinomas at high incidence in F344 rats.

The hepatocarcinogenicities of Trp-P-1 and Trp-P-2 have been established in CDF₁ mice. The present results in rats are therefore consistent with those in mice, although a difference in the spectrum of target organs in rats and mice has been observed with Glu-P-1, Glu-P-2 and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ).³,⁴ In mice, however, the incidence of liver tumors induced by Trp-P-1, as by other heterocyclic amines³-⁵ was higher in females than in males. No clear sex difference in the incidences of liver tumors could be demonstrated in the present experiment because different concentrations of Trp-P-1 were added to the diets of males and females, but Glu-P-1, Glu-P-2 and IQ induced liver tumors at higher incidence in male rats than in females.³,⁴

Intraperitoneal injection of Trp-P-1 induced significantly more ATPase-deficient foci in the liver of rats than intraperitoneal injection of Trp-P-2.¹³ This finding suggests that Trp-P-1 is a stronger hepatocarcinogen than Trp-P-2 in rats. In ACI rats given diet containing 0.01% Trp-P-2, most of the tumorous lesions observed were neoplastic nodules in the liver.¹³ Further experiments in rats with higher doses of Trp-P-2 are necessary to confirm the hepatocarcinogenicity of this compound.

A few intestinal tumors were found in the experimental group. However, it is uncertain whether these tumors were induced by Trp-P-1, since adenomas of the intestine and colon develop spontaneously at low incidence in F344 rats.¹⁰

Trp-P-1 and Trp-P-2 were reported to be present at 13.3 and 13.1 ng/g, respectively, in broiled sardines.¹⁰ These amounts seem too small to be carcinogenic to humans on the basis of the carcinogenic potency found...
in the present experiment and in the other work. However, there are also other mutagenic and carcinogenic heterocyclic amines in cooked foods. The effects of combined administrations of these chemicals at below their respective carcinogenic doses should, therefore, be evaluated. Experiments on administration of tumor promoters after treatment with heterocyclic amines are also necessary to evaluate the actual carcinogenic risk to humans of heterocyclic amines present in cooked foods.

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