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Promotion of Rat Hepatocarcinogenesis by Praziquantel

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Praziquantel, the widely used anti-helminthic agent, was investigated for hepatocarcinogenesis-promoting potential using a medium-term liver bioassay system for carcinogens. F344 male rats were given a single intraperitoneal injection of diethylnitrosamine (DEN, 200 mg/kg) and then starting 2 weeks later, received praziquantel in the diet at concentrations of 1.5 or 0.5%, or intragastrically at a dose of 1,500 mg/kg once a week for 6 weeks. Control groups received DEN or praziquantel alone. All rats were subjected to two-thirds partial hepatectomy at week 3 and killed at week 8. Development of glutathione *S*-transferase placental form-positive foci in the liver was significantly increased in terms of both number and area with the 1.5% dose, while only area was affected by the 0.5% dose. The results thus indicate that praziquantel at high dose has promoting potential in rat hepatocytic tumorigenesis.

Key words: Praziquantel — Rat — Liver carcinogenesis — Promotion

Liver fluke infestation of man caused by *Opisthorchis viverrini* is endemic in the northeast region of Thailand[1,2] and a close association between liver flukes and development of cholangiocarcinoma has been reported.[3–5] Cholangiocellular carcinomas have been reported to comprise approximately 95% of liver malignant tumors in this area[6] whereas it is only about 5% in westernized countries which are free from liver fluke infection.[7] Animal experiments have clearly shown that infection with *Opisthorchis viverrini* causes biliary proliferative and inflammatory changes equivalent to those caused in man[8,9] and that it enhances the development of cholangiocellular carcinomas after dimethylnitrosamine or N-bis(2-hydroxypropyl)nitrosamine initiation.[10–12]

Praziquantel is an anthelminthic drug with activity against all species of schistosomes and a wide range of cestodes pathogenic to man. It has already been introduced on a large scale in affected areas of Thailand[13,14] because of the relative lack of undesired pharmacodynamic effects.[15] It is commonly used not once but a number of times because of the prevalence of reinfection.[16] Maximum tolerable doses are very high[15] and a number of tests using different submammalian assays have revealed no genotoxic activity.[17–20] Mutagenicity was reported only after very high dose application,[21] and mutagenic metabolite(s) were found in the urine of mice treated with praziquantel.[22] No carcinogenicity was demonstrated in a 2-year rat study and an 80-week study in Syrian hamster.[23]

The present investigation was undertaken using a medium-term bioassay system[24] to clarify whether praziquantel has the potential to promote hepatocarcinogenesis in rats. The question of possible toxic effects on the liver was also examined in a preliminary experiment.

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MATERIALS AND METHODS

Praziquantel (Embay 8440, Droncit), 2-cyclohexylcarbonyl - 1,2,3,6,7,11b - hexahydro - 4*H* - pyrazino[2,1 - *a*]-isoquinolin-4-one, purchased from Atlantic Laboratories Cor. Ltd., Bangkok, Thailand, was suspended in corn oil or supplemented in the diet. Male F344 rats, 5-week-old, purchased from Charles River Japan Inc., Atsugi, were maintained in plastic cages with woodchip bedding in an air-conditioned animal room at 24±2°C and 55±5% humidity. Food (Oriental M, Oriental Yeast Co., Tokyo) and water were available *ad libitum* throughout the experiment. Experiments were started at the age of 6 weeks.

Experiment 1 The rats were divided into 5 groups of 5 rats each. Praziquantel, suspended in corn oil, was administered once intragastrically at a dose of 3,000, 1,000, 300, 100 or 0 mg/5 ml corn oil/kg body weight. Four days after the administration, rats were killed for analysis of serum levels of GOT, GPT and GGT. Some samples of the liver and kidney were fixed in buffered formalin for subsequent histological examination.

Experiment 2 The experimental protocol has been described in detail in previous papers.[24] The animals were divided into 7 groups. Groups 1 to 4 (16 or 15 rats each) were given a single intraperitoneal injection of diethylnitrosamine (DEN) at a dose of 200 mg/kg body weight. Starting 2 weeks later, the rats in Groups 1 and 2 were given praziquantel supplemented into powdered

diet at a concentration of 1.5 or 0.5% for 6 weeks, while Group 3 received weekly intragastric administration of praziquantel suspended in corn oil at a dose of 1,500 mg/kg body weight, for the same period. The doses of praziquantel chosen for experiment 2 were based on the data gained from experiment 1. Group 4 was the carcinogen control group without administration of praziquantel. Groups 5 to 7 were the corresponding controls to Groups 1 to 3 given only praziquantel without prior DEN. All rats were subjected to two-thirds partial hepatectomy at the end of week 3 and killed at week 8. The livers were immediately excised and sections 2–3 mm thick cut with a razor blade. Three slices, one from the caudate lobe and two from the right anterior lobe, were fixed in ice-cold acetone solution for immunohistochemical examination of glutathione *S*-transferase placental form (GST-P) expression. Additional slices were fixed in 10% phosphate-buffered formalin solution for routine staining with hematoxylin and eosin.

GST-P-positive liver foci were visualized by the avidin-biotin-peroxidase complex (ABC) method as described previously.[24] The numbers and the areas of GST-P-positive foci of more than 0.2 mm in diameter and the total areas of the liver sections examined were measured by using a color video image processor (VIP-21C). The results were assessed by comparing the values for foci in control and experimental groups.

Statistical analysis of the observed values was carried out by using Student's *t* test in combination with the *F*-test for variability.

RESULTS

Experiment 1 Liver weight was significantly increased in rats given 3,000 and 1,000 mg/kg praziquantel compared to the control values, although no remarkable changes in body and kidney weights were found at any dose (Table I). Blood analysis revealed that the highest dose of praziquantel induced significant elevation ($P<0.05$) of serum GPT and GGT levels (Table II). GOT was also increased but without statistical significance. Centrilobular liver cell enlargement was observed histopathologically. No hepatocyte degeneration and/or death was evident.

Experiment 2 The estimated average intakes of praziquantel in Groups 1 to 3 were 9.5, 3.6 and 1.8 g, respectively and those in Groups 5 to 7 were 10.0, 3.7 and 1.9 g, respectively. Data for five rats each in Groups 3 and 7 were accidentally lost. The body weights of rats in Groups 1 to 3 were significantly lower than the control Group 4 value at the time of death. Absolute and relative liver weights of rats in Groups 1 and 2 and relative liver weights of rats in Group 3 were significantly higher than those of the control group (Table III). Liver weights of rats in Groups 5 to 7 showed similar trends. Histological examination revealed prominent liver cell enlargement in the centrilobular area in the rats given praziquantel in the diet.

Numbers and areas of GST-P-positive foci per unit area of liver section are summarized in Table IV. Administration of DEN alone resulted in the formation of an average of 6.71 foci with an area of 0.49 mm^2/cm^2. When DEN was followed by 1.5% praziquantel, the number and area of foci increased to 9.36 and 0.96, respectively, in both cases a significant elevation. The dose of 0.5% significantly increased only the area of foci. Weekly administration of 1,500 mg of praziquantel did not increase either the number or area of foci. Praziquantel treatment without prior DEN did not induce foci (Groups 5 to 7).

Table II. Changes in GOT, GPT and GGT in Rats Given a Single Administration of Praziquantel

Praziquantel dose (mg/kg)	GOT (IU/liter)	GPT (IU/liter)	GGT (IU/liter)
3,000	199.3±151.0	139.3±89.7[a)]	4.3±2.9[a)]
1,000	78.6±10.2	40.6±4.5	0.4±0.5
300	86.6±8.3	45.0±2.6	0.4±0.5
100	81.8±6.2	41.4±3.0	0.2±0.4
Control	80.6±13.2	43.2±5.2	0.2±0.4

a) Significantly different from control value at $P<0.05$. Mean±SD.

Table I. Body and Organ Weights of Rats Given a Single Administration of Praziquantel

Praziquantel dose (mg/kg)	Weights (g)				
	Body		Liver		Kidneys
	Initial	Final	g	% of body wt.	
3,000	99.4±2.9	106.7±4.4	7.27±0.82	6.82±0.75[a)]	0.99±0.07
1,000	100.5±3.9	109.8±3.3	5.54±0.33	5.05±0.17	0.94±0.02
300	98.4±1.9	108.9±2.6	4.97±0.40	4.56±0.29	0.94±0.03
100	99.3±2.4	112.5±4.4	5.06±0.32	4.50±0.24	0.96±0.04
Control	101.4±4.0	110.3±2.8	4.98±0.26	4.51±0.15	0.95±0.04

a) Significantly different from control value at $P<0.001$. Mean±SD.

Table III. Survival, Body and Liver Weights in DEN-initiated Rats Treated with Praziquantel

Treatment	No. of rats		Mean body weight (g)			Liver weight[a]	
	Initial	Final	Week 0	Week 4	Week 8	g	% of body wt.
DEN+Praziquantel 1.5%	16	16	123.2	157.1[d]	232.8[d]	12.7±1.08[d]	5.46±0.21[d]
DEN+Praziquantel 0.5%	16	16	119.2	179.3	259.1[c]	10.2±0.82[d]	3.96±0.43[d]
DEN+Praziquantel 1,500 mg/kg	16	11	120.2	170.1[c]	250.6[d]	9.3±0.60	3.71±0.13[d]
DEN	15	13	123.5	186.1	273.8	9.0±0.41	3.29±0.13
Praziquantel 1.5%	10	10	123.0	184.2	252.2	14.1±0.58	5.60±0.25
Praziquantel 0.5%	10	10	123.3	200.0	281.8[b]	11.5±0.57	4.08±0.13
Praziquantel 1,500 mg/kg	10	5	120.0	195.6	271.8	10.8±0.71	3.97±0.15

a) Mean±SD.
Significantly different from the DEN alone value at *b*) $P<0.05$, *c*) $P<0.01$ and *d*) $P<0.001$.

Table IV. Quantitative Values for Numbers and Areas of GST-P-positive Foci in DEN-initiated Rats Treated with Praziquantel

Treatment dose of praziquantel	Effective No. of rats	GST-P-positive foci	
		No./cm^2	Area (mm^2/cm^2)
1.5% in diet	16	9.36±3.33[a]	0.96±0.50[b]
0.5% in diet	16	7.73±1.86	0.92±0.61[a]
1,500 mg/kg i.g.	11	6.13±2.42	0.46±0.31
none	13	6.71±2.44	0.49±0.28

Significantly different from the DEN value alone at *a*) $P<0.05$ and *b*) $P<0.01$.

DISCUSSION

The toxicity of praziquantel has been shown to be very weak.[15] For example, LD_{50} values for praziquantel given orally to mice and rats were determined to be 2,454 and 2,840 mg/kg, respectively. Daily oral dosing of 1,000 mg/kg to rats for 4 weeks did not induce any treatment-related lesions. In beagle dogs daily oral doses of 180 mg/kg did not cause any organ damage after repeated administration for up to 13 weeks. The present data in experiment 1 showed that a single oral dose of 3,000 but not 1,000 mg/kg induced liver enlargement with elevation of serum GOT, GGT and GPT, suggesting that praziquantel is weakly toxic at a very high dose level. In experiment 2, the dose of 1,500 mg/kg was therefore selected for gastric administration. In addition, dietary supplementation with praziquantel was performed, the daily dose being estimated at about 300 mg per rat with 1.5% concentration. Total intakes of praziquantel per rat in groups given 1.5 and 0.5% in the diet and 1,500 mg/kg were 9.5, 3.6 and 1.8 g, respectively. The praziquantel-related increase in liver weight was dose-dependent.

Quantitative analysis of GST-P-positive foci in the present experiment clearly showed that praziquantel increased both the number and area of the foci when given at 1.5% and the area when given at 0.5%, indicating that it can exert an enhancing effect on rat liver carcinogenesis. Since no praziquantel mutagenicity has been detected in many systems, the mechanism underlying this promotion must be epigenetic.

With regard to the human opisthorchiasis situation, a cure rate of 100% was obtained in a group of patients orally given 3×25 mg/kg on 1 day or 6×25 mg/kg on 2 days.[13] Dosing with 2×25 mg/kg or 1×40 mg/kg resulted in a lower cure rate. Thus, compared to the effective therapeutic dose, 75 or 150 mg/kg, given over one or 2 days, the doses that exhibited a promoting effect in the present experiment are extremely high. Therefore, risk to humans in terms of hepatocellular tumorigenesis is presumably negligible, even with repeated dosing due to reinfection. However, the fact that infestation with liver fluke itself induces liver damage and proliferation of both hepatocytes and bile duct cells indicates that the modifying effect of praziquantel on hepatocellular carcinogenesis in injured liver should be clarified. Furthermore, the effects of praziquantel on bile duct carcinogenesis should be investigated using hamster models to clarify whether or not the compound has a similar promoting activity in human cholangiocarcinogenesis, which is related to *Opisthorchis viverrini* infection.

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