Jpn. J. Cancer Res. **81**, 1222–1231, December 1990

Carcinogenicity of Captafol in F344/DuCrj Rats

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Captafol was administered at dietary levels of 0 (control), 750 and 1,500 parts per million (ppm) to groups of 50 male and 50 female F344/DuCrj rats for 104 weeks, and then all animals were maintained without captafol for a further 8 weeks, and killed in week 113. Renal cell carcinoma was found in eight of 50 male rats treated with 1,500 ppm and in one of 50 male rats treated with 750 ppm of captafol. The incidences of renal adenomas, including micro-adenomas, and basophilic altered cell tubules were significantly higher in both sexes treated with captafol than in controls, and the increases were apparently dose-dependent except that of adenomas in females. The incidences of neoplastic and preneoplastic lesions of the kidney in captafol-treated animals were higher in males than in females. Captafol also induced hepatocellular carcinomas in four of 50 female rats in the 1,500 ppm group. The incidences of hyperplastic (neoplastic) nodules and foci of cellular alterations in the liver were also significantly increased in both sexes treated with captafol, the increases being dose-dependent. In conclusion, captafol induced renal cell carcinomas in male rats and hepatocellular carcinomas in female rats.

Key words: Captafol — Fungicide — F344 rat — Carcinogenicity — Renal/liver tumor

Captafol has been widely used as a fungicide, because it inhibits mycelial growth from germinating fungal spores. It appeared not to be mutagenic in a dominant lethal mutagenicity study in mice and in host-mediated assay in rats.[1-3] However, it was mutagenic in mutation tests on *S. typhimurium* and *E. coli* in the absence of S-9 mixture.[4]

Captafol was found to increase the incidence of neoplastic lesions in the kidneys of rats of both sexes, and the incidence of neoplastic nodules in the liver of female rats.[5] Furthermore, in a medium-term bioassay system of 8 weeks duration in male rats which we developed[6] for detection of liver carcinogens and modifiers of hepatocarcinogenesis, captafol at 3,000 ppm in the diet was classified as a possible liver carcinogen.

Moreover, we have conducted a 2-year carcinogenicity study in B6C3F$_1$ mice on captafol at dietary levels of 0, 750, 1,500 and 3,000 ppm.[7] In that study it induced hemangioendotheliomas in the heart, hemangiomas or hemangioendotheliomas in the spleen, papillomas and squamous cell carcinomas in the forestomach, adenomas and adenocarcinomas in the small intestine, and hyperplastic nodules and hepatocellular carcinomas in the liver, indicating that it has a broad spectrum of carcinogenicity.

Captan, which is a similar compound to captafol, also has mutagenic activity.[8] In carcinogenicity tests on captan, the combined incidence of duodenal tumors (adenocarcinomas and adenomatous polyps) in male B6C3F$_1$ mice treated with 16,000 ppm of captan was significantly higher than that in controls, but captan did not show any carcinogenicity in Osborne-Mendel rats.[8]

Recently, an epidemiologic survey of a population occupationally exposed to pesticides was reported.[9] The results suggested that exposure to pesticides might have adverse effects on reproductive ability; further, the development of hemangiomas was noted. These results are important in providing information for extrapolating data on experimental animals to assessment of risk in humans.

The aims of the present study were to examine the carcinogenicity of captafol in rats and to evaluate its toxic potential.

MATERIALS AND METHODS

Test chemicals Captafol (CAS No. 2425-06-1, batch No. SX-1671), which is *cis*-N-[(1,1,2,2-tetrachloroethyl)-thio]-4-cyclohexene-1,2-dicarboximide, was produced by Chevron Chemical Co. (Richmond, CA). The purity of the preparation, which was a white crystalline solid, was 97.5%.

Animals and maintenance Male and female F344 rats, 5 weeks old, were purchased from Charles River Japan Inc. (Atsugi) and quarantined for 7 days before the experiment. Animals of the same sex were housed five to a polycarbonate cage (26.0W×41.2L×19.5H cm) with hardwood Beta Chips (Northeastern Product Co., Warrensburg, NY) for bedding. The animals were supplied with feed (Oriental MF powdered diet, Oriental Yeast Co., Ltd., Tokyo) and tap water *ad libitum*. Bed-

ding and cages were changed 3 times a week. The room temperature and relative humidity were controlled at 22±2°C and 55±10%, respectively. The room air was changed more than 15 times per hour. A 12-hr light/dark cycle was provided, with fluorescent lighting. Animals were weighed and randomly assigned to dose groups the day before starting the study.

Diet preparation Diets containing three concentrations of captafol were prepared by mixing weighed quantities of captafol and Oriental MF powdered diet with 2% corn oil in a stainless-steel mixer for 30 min. The diets were prepared every 1 or 2 weeks and stored at 4°C until use. Five analyses of samples of the blended diets showed that the actual levels of captafol in mixtures containing 750 and 1,500 ppm were 65–100% (mean±SD; 646±125 ppm) and 80–100% (1,400±141 ppm), respectively, of the nominal concentrations (analyzed by Japan Food Research Laboratories).

Experimental procedure A 13-week oral toxicity test was carried out on F344 rats of both sexes to determine the highest dose of captafol to use in the carcinogenicity study. Groups of 10 rats of each sex were given diets containing 0, 750, 1,500, 3,000 and 6,000 ppm of captafol. Degenerative and proliferative changes in potential target organs such as the forestomach, liver and kidney were observed, and the results indicated that 1,500 ppm of captafol in the diet was the maximum tolerable dose for the carcinogenicity study.[10]

Groups of 50 rats of each sex were given diets containing 0 (control), 750 or 1,500 ppm of captafol for 104 weeks and then normal diet for a further 8 weeks. The animals were observed daily for abnormalities; rats showing signs of ill-health were isolated, and returned to their group if their condition improved but otherwise killed and autopsied. Individual body weights were recorded weekly for the first 14 weeks and then every other week. Food and water consumptions were measured for 2-day periods before each weighing. In week 112, urine samples were obtained from 12 rats in each group and their pH, protein, glucose, bilirubin, ketones, occult blood and urobilinogen contents were measured with Multistix. After 112 weeks, surviving animals were deprived of food, but not water, overnight and then killed under ether anesthesia by exsanguination from the abdominal aorta. Hematological examinations of the blood included an erythrocyte count, a leukocyte count, measurements of the hemoglobin concentration and hematocrit value and a platelet count (Sysmex microcell-counter CC-180A, Towa Iryo Denshi Co., Ltd., Tokyo). The serum levels of glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, alkaline phosphatase, total bilirubin, total cholesterol, total protein, the albumin: globulin ratio, urea nitrogen, glucose and albumin were measured.

Gross examination was performed at autopsy, and detailed examinations of the luminal surfaces of intestine and urinary bladder were also carried out after fixation. The following organs of each rat were weighed and the organ-to-body weight ratios were determined: the brain, heart, liver, spleen, kidneys, adrenals and testes or ovaries.

Samples of these organs and of the salivary glands, trachea, lungs, thymus, lymph nodes, stomach, small intestine, large intestine, pancreas, urinary bladder, pituitary, thyroid, prostate, seminal vesicle, skin, mammary gland, skeletal muscle, spinal cord, sciatic nerve and any other tissues with abnormal appearance were fixed in 10% buffered formalin. For microscopic examination, tissues were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. Histopathological examinations were also performed on rats that died or were killed when they became moribund during the experiment. The numbers of altered tubules and adenomas, including microadenomas in the kidneys were counted and their total area (cm^2) in six sections of the kidneys was measured with a color image processor (Spicca-II; Nippon Avionics Co., Ltd., Tokyo).

Statistical analysis Data on cumulative mortality were analyzed by means of the generalized Wilcoxon test and Cox-Mantel test.[11, 12] Tumor incidence was analyzed by using the one-sided Fisher's exact probability test. Other data were analyzed by using Student's t test.

RESULTS

No clinical signs related to captafol treatment were apparent in any of the rats during the 112 week experiment. In the experiment, the survival rates of males and females fed 0, 750 and 1,500 ppm of captafol were 58, 62 and 58% and 76, 62 and 68%, respectively. There were no significant differences between the mortalities of controls and captafol-treated animals during the 112 week experiment (Fig. 1).

The mean body weights of rats of both sexes given 1,500 ppm of captafol and females given 750 ppm of captafol were consistently less than those of controls (Fig. 2). The food consumptions of controls and captafol-treated animals were not significantly different. In the two captafol-treated groups, the intake of captafol, calculated from the nominal dietary level, the mean food consumption and the mean body weight of each group, was higher in the first 3 months than during the remainder of the experiment. Excluding the first 3 months, the mean captafol intakes of males and females, respectively, were 25.4 and 30.7 mg/kg body weight/day with 750 ppm of captafol, and 55.2 and 64.1 mg/kg body weight/day with 1,500 ppm of captafol. Water consumption of animals given 1,500 ppm of captafol was slightly in-

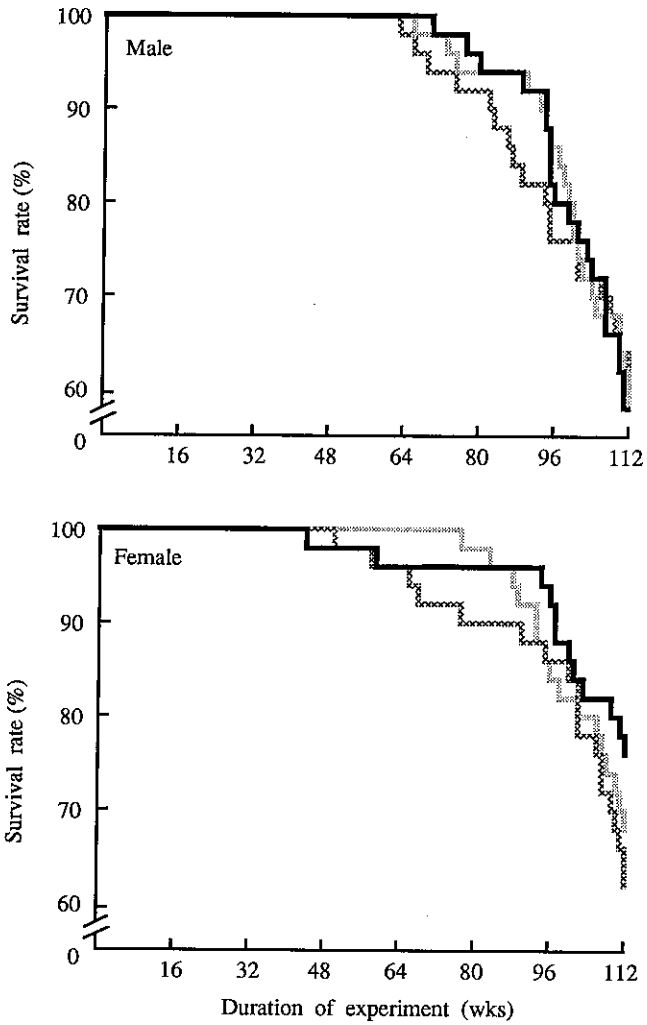

Fig. 1. Survival rates of male and female F344 rats on diets containing captafol at concentrations of 0 (■), 750 (▩) and 1,500 (▨) ppm.

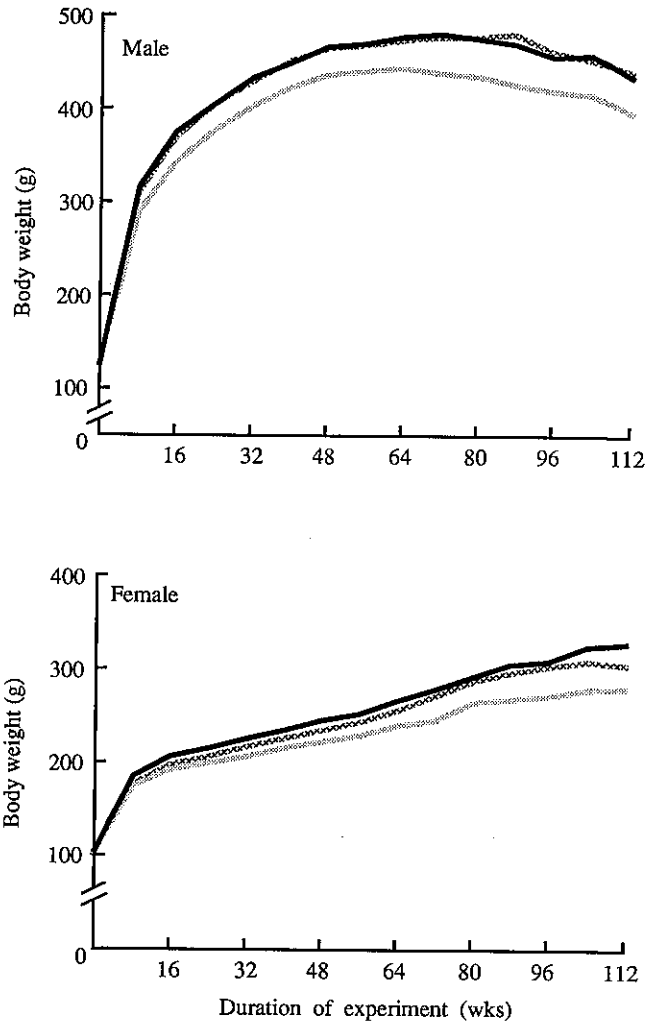

Fig. 2. Growth curves of male and female F344 rats on diets containing captafol at concentrations of 0 (■), 750 (▩) and 1,500 (▨) ppm.

Table I. Clinical Data on Captafol-treated F344 Rats (Mean±SD)

Sex	Level (ppm)	No. of rats	GOT (IU/liter)	GPT (IU/liter)	ALP (IU/liter)	T. BIL (mg/dl)	BUN (mg/dl)	GLU (mg/dl)	T. CHOL (mg/dl)
Male	0	12	84.4±23.3	24.0±6.9	178.3±53.1	0.31±0.07	19.54±2.41	128.9±12.1	118.0±44.8
	750	12	84.8±23.6	24.2±6.2	150.1±23.0	0.29±0.05	19.74±2.64	121.4±19.7	102.6±23.0
	1500	12	139.7±130.9	37.3±28.0	198.0±85.7	0.29±0.07	20.59±2.26	126.1±13.1	97.6±22.9
Female	0	12	87.8±61.7	28.3±17.3	168.8±38.5	0.28±0.04	20.88±6.07	131.2±12.6	119.0±11.7
	750	12	79.4±67.5	22.6±6.7	155.5±40.8	0.33±0.13	19.73±3.03	125.4±17.8	101.0±15.7[b]
	1500	12	89.1±71.7	24.8±8.5	266.5±160.0	0.35±0.13	22.73±4.44	116.2±15.5[a]	93.5±14.4[b]

a, b) Significantly different from control value at $P<0.05$ and 0.01, respectively.

creased from week 94 in males and week 98 in females until the end of the experiment.

Urine analyses and hematological examinations did not reveal any marked changes related to captafol treatment. Serum glucose values were decreased in females given 1,500 ppm of captafol, and total cholesterol values were slightly decreased dose-dependently in both captafol-treated groups of females. There were no other differences in serum chemistry including total protein, the albumin:globulin ratio and albumin between control and captafol-treated groups of either sex (Table I).

On gross observation, unilateral kidney nodules/masses in one male given 750 ppm of captafol and in seven males given 1,500 ppm of captafol were usually tan-colored and solid in consistency, and projected from the cortex (Fig. 3). White spots/areas and tan-colored nodules in the liver were observed in both captafol-treated groups of males and in females fed 1,500 ppm of captafol. Significant increase in the relative weight of the kidneys was noted in both sexes fed 1,500 ppm of captafol, and in the relative weight of the liver in both captafol-treated groups of females. Slight increase in the relative weight of the heart was observed in females given 1,500 ppm of captafol, and this increase seemed to be related to the retardation of increase in body weight. A statistically significant increase in the relative weight of the testes was observed in the captafol-treated groups, but its significance was not clear because the incidences of tumors of the testis in the captafol-treated groups were not different from those in controls and no particular histopathological changes were seen in the testes of these animals. The relative weights of the ovaries in the captafol-treated groups were higher than that of controls because large tumor masses developed, although these were apparently unrelated to captafol treatment (Table II).

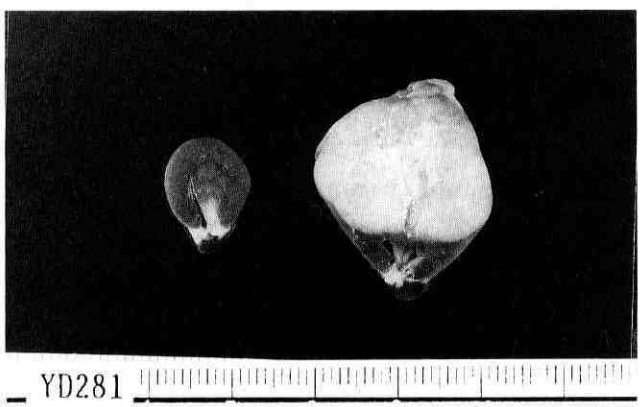

Fig. 3. Macroscopical appearance of a unilateral kidney nodule/mass. The solid, tan-colored mass projected from the cortex of a male F344 rat given 1,500 ppm of captafol.

The nonneoplastic, preneoplastic and neoplastic lesions of the kidneys, liver and forestomach that developed in the present study are summarized in Table III.

Renal cell carcinomas developed in eight males fed 1,500 ppm of captafol (Fig. 4) and in one male fed 750 ppm of captafol, but not in females. One male fed 1,500 ppm of captafol had a metastasis in the lung and another had metastases in liver, lymph nodes and abdominal fat tissue.

The incidence and number of adenomas, including microadenomas, per square centimeter of kidney area in captafol-treated males increased dose-dependently. Their number was also significantly increased in females given captafol, but the increase was not dose-dependent. Adenomas were of the cystic-papillary or solid type (Fig. 5), but rarely the clear cell type and contained a small amount of fiber tissue.

Scattered, basophilic, altered cell tubules (Fig. 6), thought to be preneoplastic lesions, were seen. The numbers of altered cell tubules per square centimeter of kidney area in the captafol-treated males and females also increased dose-dependently. The total incidences and densities of neoplastic and preneoplastic lesions in captafol-treated animals were higher in males than in females. The incidence of karyocytomegaly[13] was markedly increased in both sexes given 1,500 ppm of captafol

Table II. Final Body Weights (g) and Organ Weights (% Body Weight) of Captafol-treated F344 Rats (Mean ± SD)

Sex	Level (ppm)	No. of rats	Final body weight (g)	Heart	Liver	Kidneys	Testes/Ovaries
Male	0	28	411 ± 45	0.29 ± 0.03	2.85 ± 0.50	0.72 ± 0.14	1.29 ± 0.39
	750	31	416 ± 42	0.28 ± 0.04	2.78 ± 0.33	0.78 ± 0.37	1.58 ± 0.43[a]
	1500	30	374 ± 37[b]	0.30 ± 0.04	2.88 ± 0.40	0.94 ± 0.45[a]	1.94 ± 0.62[b]
Female	0	37	315 ± 42	0.29 ± 0.05	2.38 ± 0.33	0.61 ± 0.06	0.04 ± 0.03
	750	30	292 ± 38[a]	0.30 ± 0.06	2.77 ± 0.87[a]	0.65 ± 0.13	0.13 ± 0.48
	1500	33	268 ± 32[b]	0.31 ± 0.05[a]	2.72 ± 0.41[b]	0.69 ± 0.09[b]	0.14 ± 0.41

a, b) Significantly different from control value at $P < 0.05$ and 0.01, respectively.

Table III. Nonneoplastic, Preneoplastic and Neoplastic Lesions in the Kidney, Liver and Forestomach of Captafol-treated F344 Rats

Sex	Males			Females		
level (ppm)	0	750	1,500	0	750	1,500
Kidneys						
Effective No. of rats	50	49	50	50	50	50
Chronic nephropathy	28 (56)	30 (61)	36 (72)	0	1 (2)	1 (2)
Infarction	0	0	1 (2)	1 (2)	3 (6)	21 (42)[d]
Karyocytomegaly	0	2 (4)	48 (96)[d]	0	4 (8)	50 (100)[d]
Altered tubules	0	41 (84)[d]	46 (92)[d]	0	10 (20)[d]	20 (40)[d]
(Number/cm^2)[e]	0	2.3±2.4[c]	2.6±1.7[c]	0	0.3±0.3[c]	0.3±0.4[c]
Renal cell adenoma[a]	0	26 (53)[d]	38 (76)[d]	0	8 (16)[c]	6 (12)[b]
(Number/cm^2)[e]	0	0.5±0.8[c]	1.1±1.2[c]	0	0.1±0.2[c]	0.1±0.2[b]
Renal cell carcinoma	0	1 (2)	8 (16)[c]	0	0	0
Liver						
Effective No. of rats	50	50	50	50	50	50
Nuclear pleomorphism	0	0	2 (4)	0	16 (32)[d]	43 (86)[d]
Oval cell proliferation	0	0	0	0	0	8 (16)[c]
Bile duct hyperplasia	36 (72)	43 (86)	33 (66)	4 (8)	5 (10)	0
Foci of cellular alteration	14 (28)	13 (26)	30 (60)[d]	15 (30)	27 (54)[c]	38 (76)[d]
Hyperplastic (neoplastic) nodule	2 (4)	8 (16)[b]	21 (42)[d]	3 (6)	14 (28)[c]	34 (68)[d]
Hepatocellular carcinoma	2 (4)	0	1 (2)	0	0	4 (8)
Forestomach						
Effective No. of rats	50	50	49	50	48	50
Basal cell hyperplasia	0	0	6 (12)[b]	0	0	1 (2)
Squamous cell hyperplasia	2 (4)	3 (6)	13 (27)[c]	0	7 (15)[c]	10 (20)[d]
Squamous cell papilloma	0	0	3 (6)	0	1 (2)	0
Leiomyosarcoma	1 (2)	0	0	0	0	0

a) Including microadenoma.
b, c, d) Significantly different from the corresponding value for the control group at $P<0.05$, 0.01, and 0.001, respectively.
e) Mean±SD.
Numbers in parentheses are percentages.

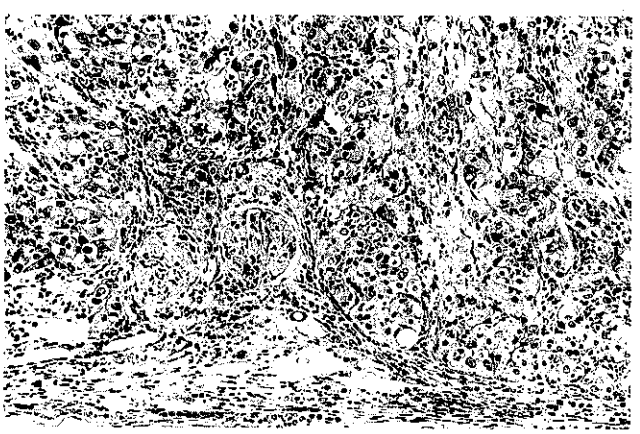

Fig. 4. A renal cell carcinoma in a male F344 rat given 1,500 ppm of captafol. The tumor was solid, consisted of large and small basophilic cells and invaded the surrounding tissue. H-E, ×175.

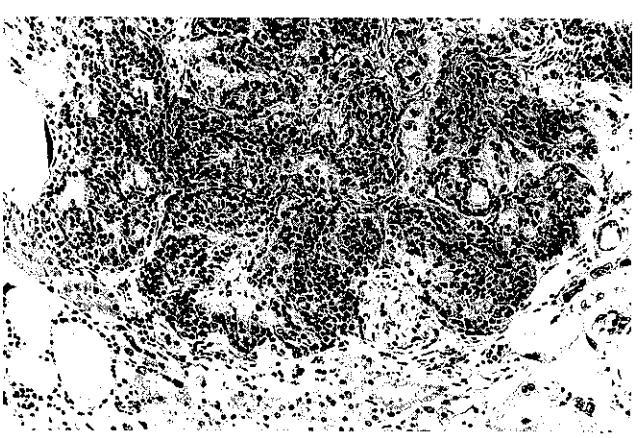

Fig. 5. A solid type of renal cell adenoma in a male F344 rat given 1,500 ppm captafol. The tumor was solid, and consisted of small basophilic cells with a small amount of fiber tissue. H-E, ×175.

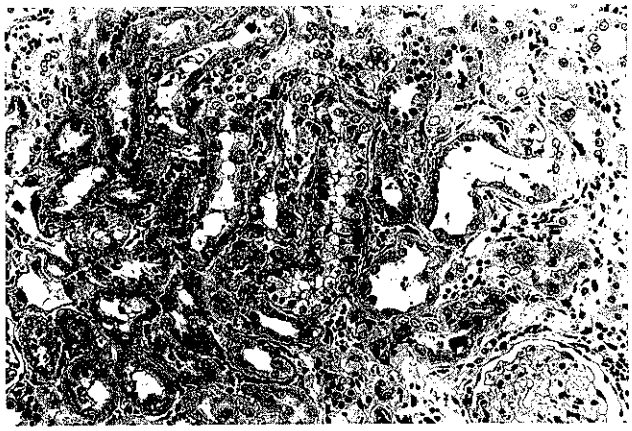

Fig. 6. Basophilic altered renal cell tubules in a male F344 rat given 1,500 ppm of captafol. H-E, ×230.

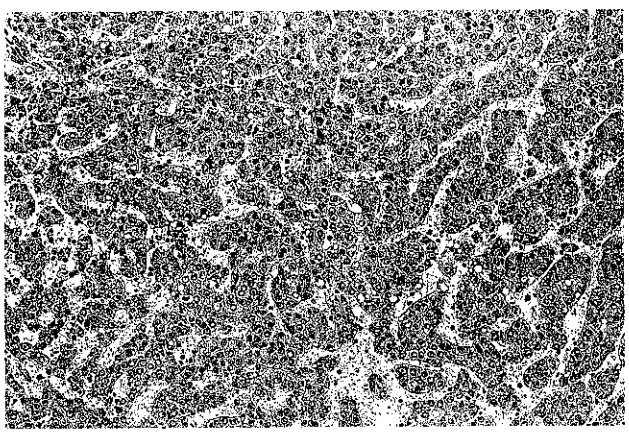

Fig. 7. A well differentiated hepatocellular carcinoma in a female F344 rat given 1,500 ppm of captafol. H-E, ×175.

(96% in males; 100% in females). Chronic nephropathy was not significantly increased by captafol treatment in males, whereas infarction, supposed to be due to toxic effects, was observed at high incidence in females fed 1,500 ppm of captafol.

Well differentiated hepatocellular carcinomas developed in females given 1,500 ppm of captafol, but their incidence was not increased significantly (Fig. 7). The incidences of hyperplastic (neoplastic) nodules and foci of cellular alteration[14-16] in both sexes treated with captafol increased dose-dependently, being higher in females than in males.

In the forestomach, basal cell hyperplasias were significantly increased in males given 1,500 ppm of captafol. The incidence of squamous cell hyperplasia was significantly increased in females given 750 ppm of captafol and in both sexes of the 1,500 ppm group. Papillomas were found in three males given 1,500 ppm of captafol, and one 750 ppm female.

The neoplastic lesions other than those described above are summarized in Table IV. The incidence of C-cell adenomas of the thyroid in females given 1,500 ppm of captafol was significantly higher than that in controls. The statistically significant difference between the 1,500 ppm females and controls was also found in the incidence of C-cell tumors (adenomas plus carcinomas). In the duodenum, one adenocarcinoma was found in a female given 1,500 ppm of captafol.

On histopathological examination, a wide range of nonneoplastic lesions was found (data not shown). The lesions were similar to those considered to be usual in aged F344 rats. An increased incidence of myocardial fibrosis in the captafol-treated males was observed, but the value was within the normal range of compiled data in our laboratory, and so seemed not to be related to captafol treatment.

DISCUSSION

In this study we examined the carcinogenicity of captafol in male and female F344 rats using dietary concentrations of 750 and 1,500 ppm of captafol. Captafol at a dose of 1,500 ppm induced a significant increase in the incidence of renal cell carcinomas in male F344 rats. In males the incidences of carcinomas, adenomas and basophilic altered cell tubules showed dose-related trends. Females also showed significant increases of renal adenomas and basophilic altered cell tubules, but the incidence of adenomas was not dose-dependent. Furthermore, four hepatocellular carcinomas were observed in females treated with the highest dose of captafol. In F344 rats spontaneous hepatic tumors are very uncommon in females.[17-21] We also observed high incidences of hyperplastic (neoplastic) nodules and foci of cellular alteration in the liver of both sexes treated with captafol. These results clearly show that captafol is carcinogenic in F344 rats, primarily affecting the kidney and liver.

The results of a two-year study on the effect of long-term treatment of rats with 1,500 and 5,000 ppm of captafol, available only in abstract form, indicated that the main changes in the kidney were alterations of the proximal and distal tubular cells, and the presence of many giant cells with large irregular nuclei.[3] The main changes of the liver were degeneration of hepatic cells, vacuolization, incipient fat alteration, and infiltration of mononuclear cells.[3] An FAO/WHO report, also available only in abstract form, indicated that captafol caused an increased incidence of neoplastic lesions in the kidneys of male rats in a high-dose group, that these also

Table IV. Incidences of Tumors Developing in Organs Other than the Kidneys, Liver and Forestomach of Captafol-treated F344 Rats

Sex	Males			Females		
Dose (ppm)	0	750	1,500	0	750	1,500
Effective No. of rats	50	50	50	50	50	50
Heart						
Fibroma	0	1 (2)	3 (6)	1 (2)	0	0
Spleen						
Lymphoma	0	0	0	0	1 (2)	0
Hemangiosarcoma	0	0	0	0	0	1 (2)
Thymus						
Thymoma	0	0	0	0	1 (2)	0
Pituitary						
Adenoma, pars distalis	5 (10)	9 (18)	6 (12)	19 (38)	19 (38)	14 (28)
Adenoma, pars intermedia	1 (2)	0	0	0	0	0
Carcinoma, pars distalis	1 (2)	0	1 (2)	0	1 (2)	0
Thyroid						
C-cell adenoma	10 (20)	11 (22)	10 (20)	0	3 (6)	8 (16)[b]
C-cell carcinoma	0	0	2 (4)	2 (4)	0	0
Adrenals						
Cortical adenoma	1 (2)	0	0	0	0	0
Pheochromocytoma	15 (30)	15 (30)	12 (24)	3 (6)	0	3 (6)
Ganglioneuroma	0	0	1 (2)	0	0	0
Malignant pheochromocytoma	1 (2)	0	3 (6)	1 (2)	1 (2)	1 (2)
Lungs						
Adenoma	1 (2)	1 (2)	4 (8)	1 (2)	1 (2)	0
Adenocarcinoma	0	0	1 (2)	0	0	1 (2)
Tongue						
Squamous cell papilloma	0	0	1 (2)	0	0	1 (2)
Small intestine						
Leiomyoma	1 (2)	1 (2)	0	1 (2)	0	0
Adenocarcinoma	0	0	0	0	0	1 (2)
Large intestine						
Adenoma	1 (2)	0	0	0	0	0
Pancreas						
Islet-cell adenoma	2 (4)	0	1 (2)	1 (2)	0	1 (2)
Acinar-cell adenoma	0	1 (2)	2 (4)	0	0	0
Islet-cell carcinoma	2 (4)	0	3 (6)	0	0	0
Urinary bladder						
Transitional cell papilloma	2 (4)	0	0	0	2 (4)	0
Transitional cell carcinoma	1 (2)	0	0	0	0	0
Testis						
Interstitial cell tumor	49 (98)	48 (96)	47 (94)	—	—	—
Prostate						
Adenoma	0	0	1 (2)	—	—	—

Table IV – continued

Sex	Males			Females		
Dose (ppm)	0	750	1,500	0	750	1,500
Effective No. of rats	50	50	50	50	50	50
Preputial/clitoral gland						
Adenoma	9 (18)	1 (2)[b]	6 (12)	4 (8)	4 (8)	9 (18)
Carcinoma	0	1 (2)	0	0	0	1 (2)
Mammary gland						
Adenoma	2 (4)	1 (2)	1 (2)	6 (12)	3 (6)	2 (4)
Fibroadenoma	5 (10)	2 (4)	2 (4)	10 (20)	12 (24)	5 (10)
Adenocarcinoma	0	0	0	1 (2)	2 (4)	0
Ovary						
Luteoma	—	—	—	0	1 (2)	0
Granulosa cell tumor	—	—	—	0	1 (2)	1 (2)
Granulosa/theca cell tumor	—	—	—	0	0	1 (2)
Uterus						
Endometrial stromal polyp	—	—	—	15 (30)	7 (14)[a]	11 (22)
Leiomyoma	—	—	—	0	0	1 (2)
Hemangioma	—	—	—	0	1 (2)	0
Teratoma	—	—	—	0	0	1 (2)
Adenocarcinoma	—	—	—	1 (2)	1 (2)	1 (2)
Endometrial stromal sarcoma	—	—	—	3 (6)	0	1 (2)
Skin/subcutis						
Squamous cell papilloma	1 (2)	1 (2)	0	0	1 (2)	0
Basal cell epithelioma	0	0	1 (2)	0	0	0
Keratoacanthoma	0	0	1 (2)	0	0	0
Trichoepithelioma	0	0	1 (2)	0	0	0
Fibroma	10 (20)	11 (22)	6 (12)	5 (10)	3 (6)	0[a]
Squamous cell carcinoma	2 (4)	1 (2)	0	0	0	0
Fibrosarcoma	0	0	2 (4)	0	0	1 (2)
Malignant fibrous histiocytoma	1 (2)	1 (2)	3 (6)	0	0	1 (2)
Hemangiosarcoma	0	0	0	0	1 (2)	0
Leiomyosarcoma	0	1 (2)	0	0	0	0
Sarcoma, NOS	0	0	0	0	1 (2)	0
Ear/Zymbal's gland						
Squamous cell papilloma	0	0	0	0	0	1 (2)
Squamous cell carcinoma	1 (2)	1 (2)	0	0	0	3 (6)
Peripheral nerve						
Neurinoma	1 (2)	1 (2)	0	0	0	0
Abdominal cavity						
Mesothelioma	3 (6)	3 (6)	5 (10)	0	0	0
Malignant fibrous histiocytoma	0	0	0	0	0	1 (2)
Sarcoma, NOS	0	0	0	0	1 (2)	0
All sites						
Malignant lymphoma/leukemia	11 (22)	5 (10)	8 (16)	5 (10)	11 (22)	4 (8)
Others						
Rhabdomyoma	0	0	1 (2)	0	0	0
Osteosarcoma	1 (2)	1 (2)	1 (2)	0	0	0

a, b) Significantly different from the control group values at $P < 0.05$ and 0.01, respectively.
Numbers in parentheses are percentages.

developed in females at lower dose levels, and that neoplastic nodules in the liver were significantly increased in females in the high-dose group.[5]

Recently, Nyska *et al.*[22] reported that merpafol (captafol) induced renal cancer in male F344 rats when given at levels of 500, 2,000 and 5,000 ppm for two years, but they did not report changes in other organs.

Renal cell tumors can be induced by chemical carcinogens,[23] and induction of renal tumors in animals is modified by various chemicals, hormones, and other factors.[23] Captafol mainly induced a basophilic, solid type of carcinoma in the kidney, like those induced by other compounds.[24-29] Renal cell carcinomas were associated with numerous basophilic altered cell tubules, which are thought to be preneoplastic lesions.[30, 31] In the present study, the number of these tubules increased dose-dependently. These findings suggest that the observed preneoplastic lesions would develop into adenomas and carcinomas. A high incidence of karyocytomegaly was observed in the present study, but karyocytomegaly does not appear to be linked to neoplastic transformation.[13, 32]

In non-tumorigenic areas of the liver, nuclear pleomorphism was observed in captafol-treated rats. Some cells had giant, hyperchromatic hepatic nuclei, often with multiple nucleoli and binuclear hepatocytes were also seen. These cells were more numerous in periportal areas than in the centrilobular region and were clearly observed in captafol-treated females. These findings indicate that polyploidy may be associated with neoplastic transformation of liver cells.[33]

In the forestomach, basal cell hyperplasias and squamous cell hyperplasias were significantly increased in males in the high-dose group, and the incidence of the latter in females also significantly increased dose-dependently. Further, three papillomas were found in males given 1,500 ppm of captafol, but their development was not clearly related to captafol administration. Thus, these findings suggest that the observed preneoplastic lesions of the forestomach would not significantly develop into tumors at either dose level of captafol. Forestomach tumors have not been reported to be induced by captafol.[5, 8, 22]

The significantly higher incidence of C-cell adenomas in the thyroid of females treated with 1,500 ppm of captafol is not thought to be related to captafol because it was within the normal range of compiled data in our laboratory.[19] Captafol did not induce thyroid tumors because C-cell cercinomas were found in controls, but not in females given captafol.

In this study only one adenocarcinoma was found in the duodenum of a female given 1,500 ppm of captafol. Thus captafol does not appear to induce tumors of the small intestine in rats, in contrast to its effect in B6C3F$_1$ mice.[7] Captafol also had no apparent tumorigenicity in mammary tissues of rats. This information was requested by the US Environmental Protection Agency (EPA) before a complete assessment could be made of the risk of captafol.[34]

No captafol-related tumors were found in the heart, spleen or forestomach, or in the small intestine, in which they were induced by captafol in B6C3F$_1$ mice.

In conclusion, in the present study captafol induced renal cell carcinomas in male rats and hepatocellular carcinomas in female rats. It also induced benign tumors and preneoplastic lesions in the kidney and liver of both sexes.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid from the Ministry of Health and Welfare of Japan, and also by a grant from the Society for Promotion of Pathology of Nagoya.

(Received July 27, 1990/Accepted September 27, 1990)

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