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Subacute Nephrotoxicity and Induction of Renal Cell Carcinoma in Mice Treated with Ferric Nitrilotriacetate[1]

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ABSTRACT

We investigated the induction of renal tumors by the ferric complex of nitrilotriacetic acid (Fe-NTA) in male and female A/J mice. Fifty-three male and 21 female mice received i.p. injections of Fe-NTA, 1.8 to 2.7 mg of iron/kg of body weight/day, 6 days a wk for 12 wk, at the longest. Ten male and ten female mice received nitrilotriacetic acid (NTA) i.p. at the dose equivalent to the NTA portion of Fe-NTA for the same period of time. Twenty male and 20 female mice left untreated served as the controls. Twenty-eight of the 53 Fe-NTA-treated male mice died within 14 days of the treatment. Renal proximal tubular cell necrosis was the major autopsy finding in these mice. On the other hand, all the Fe-NTA-treated female mice and NTA-treated male and female mice survived the 12 wk of treatment. Renal tubular cell carcinoma had developed in 15 of the 25 male mice and in one of the 21 female mice by the 420th day after the start of the experiment. The NTA-treated and control mice did not develop any tumors. In conclusion there is no species specificity in rats or mice in the induction of the renal carcinoma by Fe-NTA, but male mice are far more susceptible to both the acute or subacute toxicity and carcinogenic effect of Fe-NTA than are female mice.

INTRODUCTION

NTA[3] is a chelating agent that has become a major component of some detergent formulas as a substitute for polyphosphates. Mammalian toxicity and certain environmental effects of NTA have been extensively reviewed (1–5). Since NTA exists as a metal complex in a near neutral or basic environment, investigations of the biological effect of metal-complexed NTA are necessary. We have reported severe acute and subacute nephrotoxicity by repeated i.p. injections of either Fe-NTA or Al-NTA in Wistar rats (6–8). Interestingly, a high incidence of renal adenocarcinoma was seen only in rats treated with Fe-NTA (9–11). The renal tumors induced by Fe-NTA in rats appear to be the counterpart of human renal adenocarcinoma. The occurrence of renal adenocarcinoma in either humans or experimental models often shows species specificity or sex predominance. In the present study, we examined the induction of renal tumors by Fe-NTA in mice of both sexes.

MATERIALS AND METHODS

Four-wk-old A/J mice (inbred strain at the Laboratory Animal Center, Kyoto University) were used. The animals were provided commercial mouse chow (Funahasi, Chiba, Japan) and soft water *ad libitum*. The animals were randomized into groups that were given Fe-NTA (53 males and 21 females), NTA (10 males, 10 females), or no treatment (20 males, 20 females). The Fe-NTA solution was prepared daily by the method of Awai *et al.* (12). Briefly, the $Fe(NO_3)_3 \cdot 9H_2O$ (Ishizu, Osaka, Japan) solution was mixed by a magnetic stirrer in a 4-fold molar excess of nitrilotriacetic acid disodium salt (Nakarai, Kyoto, Japan), and the pH was adjusted to 7.4 with sodium bicarbonate (Wako, Osaka, Japan). NTA was of guaranteed reagent quality, and no further analysis was done to determine its purity. The Fe-NTA dose was 1.8 to 2.7 mg of iron/kg of body weight/day, given i.p. 6 days a wk for 12 wk, and the NTA dose was equivalent to the NTA portion of Fe-NTA. After 12 wk of treatment, all the animals were kept without treatment. They were observed carefully, and the animals that appeared to be dying were killed. All the remaining animals were killed 420 days from the start of the experiment. Hematoxylin-eosin stain was used for light microscopic observations.

RESULTS

Twenty-eight of the 53 male mice treated with Fe-NTA died between 1 and 14 days of treatment (Table 1). Degeneration, necrosis, sloughing off, and many mitoses of the renal PCT cells were found at autopsy (Fig. 1). Also many of the PCT cells were regenerative, and some of the regenerative cells were very large and atypical. They were marked by prominent nucleoli and mitoses. Some of the regenerative cells lacked tubular formation (Fig. 2). These changes were comparable to those seen in rats with Fe-NTA treatment (9–11). All the Fe-NTA-treated females, NTA-treated males, and controls survived the treatment for 12 wk (death rate for Fe-NTA-treated males *versus* females, $P < 0.005$, χ^2 test). Fifteen of the 25 surviving male mice (60%) developed renal tubular cell tumors between the 50th and 420th days of the experiment. One of the 21 female mice given Fe-NTA developed a renal tubular cell tumor by the 420th day after the start of the experiment (males *versus* females, $P < 0.005$, χ^2 test; Table 1). Fig. 3 shows a small tumor seen in the renal cortex of a male mouse in the earlier stage. It consists of cystic lesions with multilayered atypical cells. The invasive growth pattern is seen even in this small lesion. The typical gross appearance of renal tumors was solid, cystic, or hemorrhagic. The microscopic appearance was variable (Fig. 4), *i.e.*, clear, granular, or spindle cells forming solid aggregates, papillae, cysts, or glandular patterns. There was no distant metastasis. None of the NTA-treated or control mice developed renal tumors by the 420th day of the experiment.

DISCUSSION

Renal adenocarcinomas have been produced often as a part of multiorgan carcinogenesis in experimental animals by chemical, physical, and viral agents (13). Fe-NTA induction of renal adenocarcinoma in rats was reported only recently (9–11). The uniqueness of Fe-NTA as a carcinogen is that the Ames test for Fe-NTA is negative,[4] and biotransformation of NTA is not known (1). Most renal tumors induced by Fe-NTA in rats and mice show necrosis and hemorrhage in the central area. Cells

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[3] The abbreviations used are: NTA, nitrilotriacetic acid; Fe-NTA, ferric nitrilotriacetate; Al-NTA, aluminum nitrilotriacetate; PCT, proximal convoluted tubular (ee).

[4] Y. Kikuchi, personal communication.

Table 1 *Incidence of acute death and renal tubular cell tumors in male and female A/J mice treated with Fe-NTA*

Treatment	Sex	No. of mice used	No. of deaths within 14 days	No. of mice bearing renal tumors/no. tested
Fe-NTA	Male	53	28[a]	15/25[a]
	Female	21	0	1/21
NTA	Male	10	0	0/10
	Female	10	0	0/10
None	Male	20	0	0/20
	Female	20	0	0/20

[a] Significantly different from Fe-NTA-treated female mice, NTA-treated, and untreated mice by the χ^2 test ($P < 0.005$).

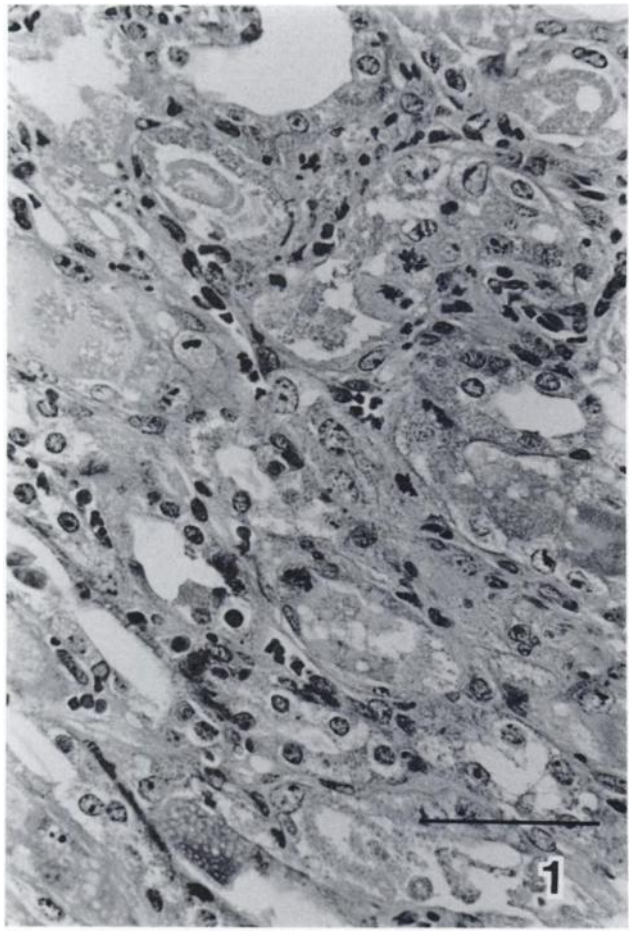

Fig. 1. PCT of male A/J mouse treated with Fe-NTA (2.7 mg of iron/kg of body weight/day for 2 days). Degeneration, necrosis, and many mitoses of the PCT cells are seen. H & E, × 400. Bar, 50 μm.

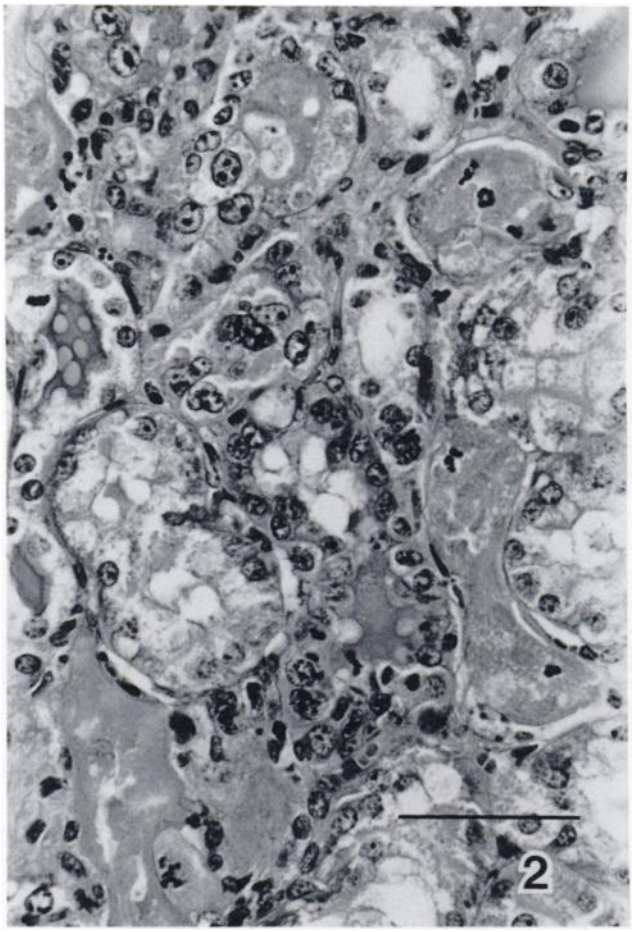

Fig. 2. Subacute effect of Fe-NTA in PCT cells of male A/J mouse (2 to 2.7 mg of iron/kg of body weight/day for 5 days). Some of the regenerative cells are large with prominent nuclei and nucleoli. Regenerative cells without tubular formation are seen in the center field. H & E, × 400. Bar, 50 μm.

in small renal tumors are similar to those in large tumors, which show invasion to surrounding normal tissue (Fig. 3). In Wistar rats, distant metastases were observed in about 50% of the animals, and tumor cells were transplantable and have been cultured in bottles for 1.5 yr.[5] Although it is not easy to classify renal tumors of the experimental animals into adenomas and adenocarcinomas, the above results, the cell morphology, and growth pattern indicate that renal tubular cell tumors induced by Fe-NTA in mice as well as in rats are malignant.

Renal cell carcinoma occurring in experimental animals as well as humans affects more males than females (13). In the present study, we found that acute nephrotoxicity is more pronounced in males. This fact seems to be closely linked with the high incidence of renal neoplasms in male mice, although the male sex hormone may play a role of promoter. As the pathogenesis of Fe-NTA toxicity seems to be lipid peroxidation by activated oxygen species (Footnote 6; Ref. 15), a study is in progress to elucidate sex specificity in terms of lipid peroxidation.

The present work showed that Fe-NTA nephrotoxicity and carcinogenicity occur in species other than rats, and that male mice are more affected than female mice. As Al-NTA, which is nephrotoxic (8) but not carcinogenic (11), is not responsible for the lipid peroxidation,[6] we suspect that lipid peroxidation, which is evoked by Fe-NTA and is responsible for acute toxicity, plays some part in the Fe-NTA carcinogenesis.

[5] S. Okada, unpublished data.

[6] S. Okada, S. Hamazaki, Y. Ebina, J. L. Li, and O. Midorikawa. Ferric nitrilotriacetate promoted lipid peroxidation, submitted for publication.

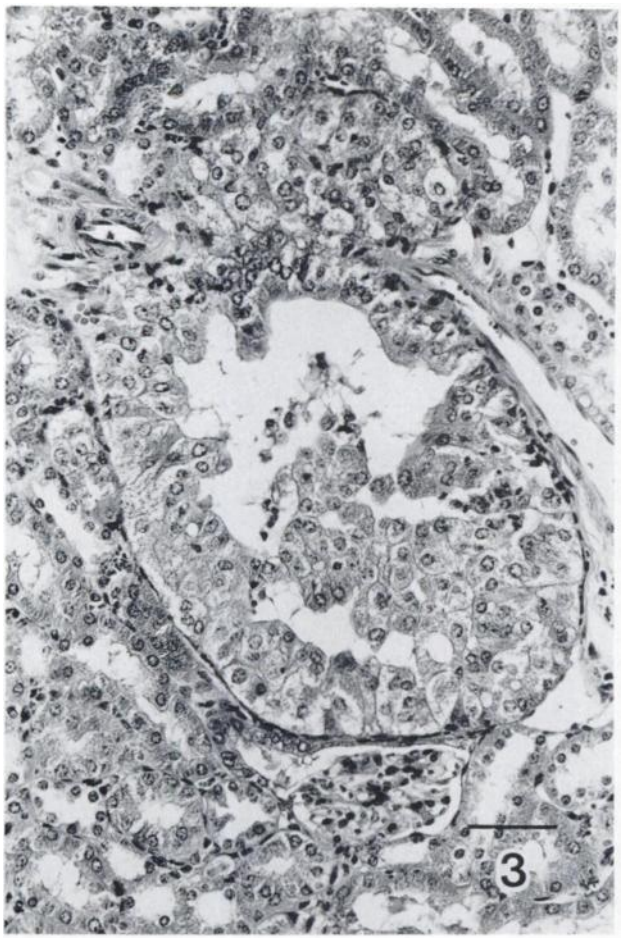

Fig. 3. Cystic tumor with invasion seen in a male A/J mouse at the 149th day (65 days after the last Fe-NTA injection). H & E, × 200. Bar, 50 μm.

Fig. 4. Light microscopic appearance of tumor cells observed in a male mouse at 330 days after the last Fe-NTA injection. Clear and granular cells with a papillary growth pattern are seen. H & E, × 400. Bar, 50 μm.

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