Sex Hormone-dependent Renal Cell Carcinogenesis Induced by Ferric Nitrilotriacetate in Wistar Rats

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Ferric nitrilotriacetate (Fe-NTA), an iron chelate, induces necrosis of renal proximal convoluted tubules as a consequence of lipid peroxidation, and a high incidence of renal cell carcinoma (RCC) is also observed in rats and mice. The incidence of RCC and the extent of lipid peroxidation are greater in males than females. In the present study, the effects of castration or ovariectomy, and sex hormone treatment on Fe-NTA-induced renal carcinogenesis in rats were examined. Male and female Wistar rats were each divided into 5 groups. In group 1, rats were sham-operated and treated intraperitoneally (i.p.) with nitrilotriacetate (NTA). In group 2, sham-operated rats were treated with Fe-NTA (5–10 mg iron/kg/day, i.p.). Castrated or ovariectomized rats treated with Fe-NTA served as group 3. Group 4 or 5 was treated in the same way as group 3, but in addition received either testosterone (group 4) or estradiol (group 5). NTA, Fe-NTA or sex hormone treatments were initiated 4 weeks after the operation. NTA or Fe-NTA treatments were conducted for 12 weeks, and sex hormones were administered for 10 months. After 10 months of treatment, all rats were autopsied and both kidneys were examined histopathologically. In NTA-treated groups, there was no pathological change in the kidneys. In Fe-NTA-treated groups (groups 2–5), testosterone treatment or ovariectomy increased the incidence of RCC, and estradiol treatment or castration decreased the incidence of RCC (male: sham operation, castration and testosterone treatment > castration > castration and estradiol treatment, female: ovariectomy and testosterone treatment > ovariectomy > sham operation, ovariectomy and estradiol treatment). These results indicate that sex differences observed in the incidence of RCC induced by Fe-NTA are dependent upon sex hormones.

Key words: Sex difference — Sex hormone — Renal cell carcinoma — Ferric nitrilotriacetate — Rat

NTA[4] is a synthetic aminopolycarboxylic acid that efficiently forms water-soluble chelate complexes with several metal cations at a neutral pH, and experimental models of iron overload[1] and copper toxicosis[2] have been developed by using Fe-NTA and cupric NTA. We have reported that Fe-NTA induces renal proximal convoluted tubular necrosis in rats and mice as a consequence of lipid peroxidation,[3,4] and leads to a high incidence of RCC.[5-7] In these studies, males are far more susceptible to subacute toxicity than females, and the effect of Fe-NTA on renal carcinogenesis is also greater in males than females. Lipid peroxidation of PCT cells induced by Fe-NTA also displays a clear sex difference.[8,9] In addition, it has been reported that lipid peroxidation induced by Fe-NTA was decreased by castration and/or estrogen treatment, and ovariectomy and/or testosterone treatment increased lipid peroxidation induced by Fe-NTA.[10] However, the effect of castration, ovariectomy and/or sex hormone treatments on Fe-NTA-induced renal carcinogenesis has not been examined. In this study, we therefore examined the effects of castration, ovariectomy and/or sex hormone administration on renal carcinogenesis induced by Fe-NTA.

MATERIALS AND METHODS

Four-week-old male and female Wistar rats were purchased from Charles River, Japan and fed a commercial rat chow (CREA, Tokyo) and tap water *ad libitum*. Male and female animals were each randomly divided into 5 groups. The animals of groups 1 and 2 were sham-operated, and the animals of groups 3–5 were castrated or ovariectomized under pentobarbital anesthesia within one week after purchase, and then allowed to recover untreated for 4 weeks.

Fe-NTA was prepared by the method described by Awai *et al.*[1] Nitrilotriacetic acid disodium salt (NTA, Nacalai, Kyoto) and ferric nitrate (Wako, Osaka) were dissolved in Milli-Q water (Millipore-Japan, Osaka), and the Fe solution was mixed with the NTA solution. The pH was adjusted to 7.0 with sodium bicarbonate (Wako).

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[4] The abbreviations used are: NTA, nitrilotriacetic acid; Fe-NTA, ferric nitrilotriacetate; RCC, renal cell carcinoma; PCT, proximal convoluted tubular.
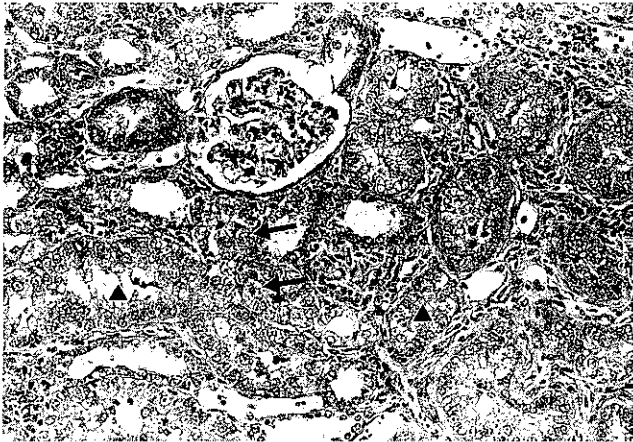
Fig. 1. Subacute effect of Fe-NTA on PCT cells of male Wistar rats (group 2, 5 to 10 mg/iron/kg, three times/week for 4 weeks). Mitotic cells are observed (arrows) and some of the regenerative cells are large with prominent nucleoli (arrowheads). The glomerulus is intact. H & E, ×200.
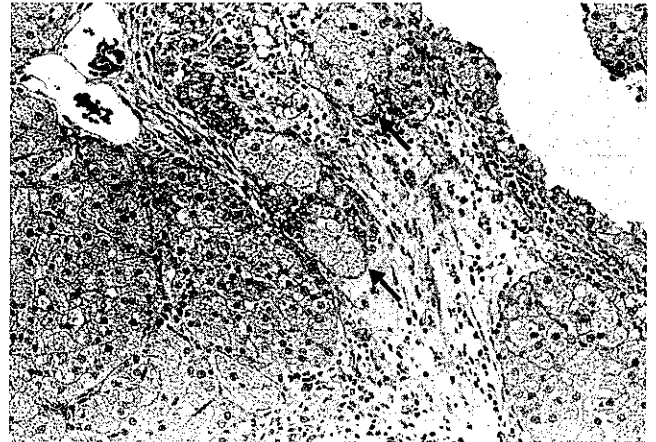

Fig. 2. Basophilic granular-type cells of renal cell carcinoma in male rats at 30 weeks after the last Fe-NTA treatment (group 2, 5 to 10 mg/iron/kg, three times/week for 12 weeks). Invasion into the surrounding stroma is seen (arrows). H & E, ×200.

Table I. Incidences of Animal Death and Renal Cell Carcinoma in Wistar Rats

No. of group	Sex	Treatment	No. of rats used	No. of dead or moribund animals during NTA or Fe-NTA treatment	No. of rats bearing renal cell carcinoma/ No. of rats tested*
1	Male	Sham+NTA	20	0	0/20
2		Sham+Fe-NTA	22	7	4/15
3		Castration+Fe-NTA	21	5	1/16
4		Castration+Fe-NTA+Testosterone	22	4	5/18
5		Castration+Fe-NTA+Estradiol	21	1	1/20
1	Female	Sham+NTA	12	0	0/12
2		Sham+Fe-NTA	20	5	1/15
3		Ovariectomy+Fe-NTA	20	6	4/14
4		Ovariectomy+Fe-NTA+Testosterone	20	4	4/16
5		Ovariectomy+Fe-NTA+Estradiol	19	5	0/14

* Significant difference ($P<0.05$) in the incidence of renal cell carcinoma by the cumulative χ^2 test in the following order: males, groups 2 or 4 > group 3 > group 5; females, group 4 > group 3 > group 5.

The molar ratio of Fe to NTA was 1:4. Fe-NTA was given three times a week for 12 weeks by intraperitoneal injection.

The animals of group 4 were given an intramuscular (i.m.) injection of 10 mg/kg testosterone propionate (Testinon; Mochida, Tokyo) twice a week for 10 months. Five mg/kg estradiol valerate (Pelanin Depot; Mochida) was given i.m. to the animals of group 5, once a week for 10 months. The experimental groups and doses are summarized in Table I.

The animals were observed daily and moribund animals were killed under ether anesthesia. Dead animals were necropsied as soon as possible after discovery. After

10 months of treatment, all remaining animals were killed under ether anesthesia. Both kidneys were dissected out, fixed with 10% phosphate-buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin for histological examination.

RESULTS

In the NTA-treated group (group 1), there was no mortality and no renal histopathological changes were seen at microscopy. Mortality in other Fe-NTA treated groups was equivalent, except for the male Fe-NTA and estradiol group (group 5). Microscopically, degenera-

tion, necrosis and many mitoses in the PCT cells were noted in male animals killed when moribund during Fe-NTA treatment (Fig. 1). Regenerative and atypical PCT cells were also found in these rats, characterized by prominent nucleoli and mitoses. After 10 months of treatment, renal tumors were found in several animals; they were characterized grossly by a solid, cystic or hemorrhagic appearance. The histopathological features were similar to human RCC and clear or granular-type cells were observed (Fig. 2). Tubular cystic changes were also observed adjacent to the RCC. The incidence of renal carcinogenesis was increased in Fe-NTA-treated groups and in testosterone-treated or ovariectomized groups, and estradiol treatment or castration decreased the incidence of RCC (male: sham operation, castration and testosterone treatment > castration > castration and estradiol treatment, female: ovariectomy and testosterone treatment > ovariectomy > sham operation, ovariectomy and estradiol treatment). There was no appearance of RCC in NTA-treated groups. The incidence of RCC is summarized in Table I.

DISCUSSION

Treatment with Fe-NTA causes severe nephrotoxicity as a consequence of lipid peroxidation,[3, 4] and administration for 12 weeks induced RCC in both rats and mice.[5-7] In these studies, males are far more susceptible to the subacute toxicity and carcinogenic effects of Fe-NTA than females. It has been reported that the extent of lipid peroxidation is greater in males,[8, 9] and that this sex difference in lipid peroxidation is dependent

upon the sex hormones.[10] In the present study, it is shown that the incidence of RCC induced by Fe-NTA is affected by sex hormones, as is the case for lipid peroxidation.

The precise mechanism of the effect of sex hormone on renal carcinogenesis induced by Fe-NTA is unknown. It has been reported that estrogen acts as an antioxidant and inhibits lipid peroxidation.[11-13] However, we thought that estrogen may alter Fe-NTA-induced lipid peroxidation by changing a metabolic pathway rather than by direct scavenging activity, because i.v. treatment with water-soluble conjugated estrogen, 5 min prior to the Fe-NTA treatment, has no significant effect on lipid peroxidation.[10] On the other hand, Fe-NTA-induced lipid peroxidation in renal PCT cells requires a glutathione cycle.[14, 15] Ruiz-Larrea et al. reported that estrogen decreases reduced glutathione (GSH) level.[16] Therefore estrogen may affect Fe-NTA-induced lipid peroxidation and may modulate renal carcinogenesis through altering the glutathione cycle. Wachnik et al. found that testosterone accelerates hepatic lipid peroxidation in rats,[17] and this finding may be related to our data suggesting that testosterone increased renal lipid peroxidation and renal carcinogenesis induced by Fe-NTA treatment. Since this sex difference is also observed in human renal cell carcinoma,[18] this animal model could be very useful for the analysis of renal carcinogenesis in humans, though the precise mechanism of sex hormone function in carcinogenesis in this animal model still needs to be further investigated.

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