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Prevention by 2-Mercaptoethane Sulfonate and *N*-Acetylcysteine of Renal Oxidative Damage in Rats Treated with Ferric Nitrilotriacetate

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Ferric nitrilotriacetate (Fe-NTA) is a renal toxicant and carcinogen in rats and mice. We found that its administration results in formation of 4-hydroxy-2-nonenal (HNE) in the renal proximal tubule cells of rats, and 8-hydroxydeoxyguanosine (8-OHdG) adducts in their DNA, suggesting a role for oxidative stress. Since 2-mercaptoethane sulfonate (MESNA) and *N*-acetylcysteine (NAC), administered orally, have been shown to increase the kidney levels of free thiol groups, their influence on the renal toxicity and carcinogenicity induced by Fe-NTA was examined in the present study. Male Wistar rats were intraperitoneally injected with Fe-NTA (12 mg Fe/kg), and MESNA (100 mg/kg) or NAC (200 mg/kg) was given orally 1 h before and 1 h after this treatment. The animals were killed for tissue analyses 3 h after the Fe-NTA exposure. In accord with our previous reports, HNE-modified protein was detected in the proximal tubules of Fe-NTA-treated rats by means of immunohistochemistry. Likewise, levels of 8-OHdG in the renal nuclear DNA, lipid peroxides as thiobarbituric acid-reactive substances in the kidneys, and blood urea nitrogen and creatinine in the serum were significantly increased by the Fe-NTA treatment. All of these changes were completely inhibited by oral administration of MESNA or NAC. These results suggest that both of these compounds can prevent the oxidative stress induced by Fe-NTA.

Key words: Ferric nitrilotriacetate — 8-Hydroxydeoxyguanosine — 4-Hydroxy-2-nonenal — Mercaptoethane sulfonate — *N*-Acetylcysteine

Ferric nitrilotriacetate (Fe-NTA) is a potent nephrotoxin, which also induces high incidences of renal cell carcinomas in rats and mice.[1,2] We have found that 4-hydroxy-2-nonenal (HNE), a major aldehydic product of lipid peroxidation, is present in the proximal tubules of rats treated with Fe-NTA.[3] It is believed that HNE causes lipid peroxidation-associated toxicity, possibly due to its ready reactivity with cellular proteins.[4] We have also established that Fe-NTA administration results in several kinds of renal DNA damage, including the formation of 8-hydroxydeoxyguanosine (8-OHdG) through the generation of active oxygen radicals.[5–7] Since 8-OHdG adducts can cause misreading of the DNA sequence during replication, due to G-for-T base substitutions,[8] they might result in significant activation of oncogenes.[9] Accordingly, it seems likely that both of the established products play key roles in Fe-NTA-induced toxicity and carcinogenicity.

2-Mercaptoethane sulfonate (MESNA) has been widely used to ameliorate the urotoxic effects of oxazaphosphorine-type cancer chemotherapeutic agents[10] because of its ability to increase the levels of free thiol groups, especially in the kidneys, after oral administration.[11] *N*-Acetylcysteine (NAC), a precursor of intracellular cysteine (Cys) and glutathione (GSH), has also been clinically tested as a chemopreventive agent because of its antioxidant properties.[12] Therefore, in the present study, we examined whether these thiol compounds can prevent the formation of 8-OHdG and HNE-modified proteins, and reduce lipid peroxidation and renal toxicological parameters in the kidney of rats treated with Fe-NTA.

MATERIALS AND METHODS

Animals Five-week-old male Wistar rats (specific pathogen-free) were purchased from Japan SLC, Inc. (Shizuoka) and kept under quarantine for 1 week before starting the experiment. The animals were housed in plastic cages, 4 rats/cage, and maintained under controlled conditions of temperature (23±2°C), and relative humidity (55±5%), with a 12 h light/dark cycle.

Chemicals MESNA was obtained from Tokyo Kasei Industry (Tokyo), and NAC, $Fe(NO_3)_3$ and Na_2NTA were purchased from Wako Chemical Industry (Kyoto). The Fe-NTA solution was prepared by the method of Awai *et al.*,[13] $Fe(NO_3)_3$ being mixed in a 4-fold molar excess of Na_2NTA, and the pH adjusted to 7.4 with

$NaHCO_3$. An 8-OHdG standard was kindly donated by Professor H. Kasai (University of Occupational and Environmental Health, Kitakyushu) and 2′-deoxyguanosine was purchased from Sigma Inc. (St. Louis, MO).

Animal treatments The Fe-NTA solution (12 mg as Fe/kg BW) was injected intraperitoneally into 4 rats in each group. A solution of MESNA (100 mg/kg in distilled water (DW)) or NAC (200 mg/kg on 0.2 *M* phosphate buffer, pH 6.5) was administered orally to these animals 1 h before and 1 h after the injection of Fe-NTA solution. The Fe-NTA alone group and the control group were given saline instead of the compounds. All animals were anesthetized under ether 3 h after the treatment with Fe-NTA (or saline), and blood was collected from the jugular vein to measure blood urea nitrogen (BUN) and creatinine (CRN). The kidneys were immediately removed, rinsed with cold 1.15% KCl, cut into pieces, quickly frozen, and stored at −80°C for the subsequent analyses. Slices of the kidneys were fixed in methanol-Carnoy for immunohistochemical examination.

Analyses To detect HNE-modified proteins immunohistochemically, routinely prepared sections were stained using an LSAB kit (DAKO Co., Santa Barbara, CA) and previously described procedures.[3] Nuclear kidney fractions were obtained by homogenizing the tissue and centrifuging at 800*g*; DNA was isolated using a 341 Nucleic Acid Purification System (Applied Biosystems, Foster City, CA). 8-OHdG levels were assessed as detailed earlier.[14] Thiobarbituric acid-reactive substances (TBARS), measured as lipid peroxide levels in the kidneys, were determined by the method of Uchiyama and Mihara.[15] BUN and CRN were measured using commercially available kits with a Hitachi Automatic Analyzer 7150.

Statistics Data were analyzed for homogeneity of variance, and statistical significance was assessed with Student's or Cochran's *t* test.

RESULTS AND DISCUSSION

As shown in Table I, TBARS in the kidneys increased 3-fold after treatment with Fe-NTA alone compared to that in control animals. Co-treatment with MESNA or NAC completely blocked this rise. Increased levels of serum BUN and CRN, as indicators of renal toxicity, were evident after Fe-NTA alone, but not after either of the co-treatments. In addition, immunohistochemical examination revealed HNE-modified proteins in the cytoplasm of the proximal tubules, presumably resulting from the reaction of HNE with cytoplasmic constituents,[16] only in the animals given Fe-NTA alone (Fig. 1) and not in those that received a thiol compound pre- and post-treatment with Fe-NTA (Fig. 2). HNE, which is generated via lipid peroxidation and which interacts with

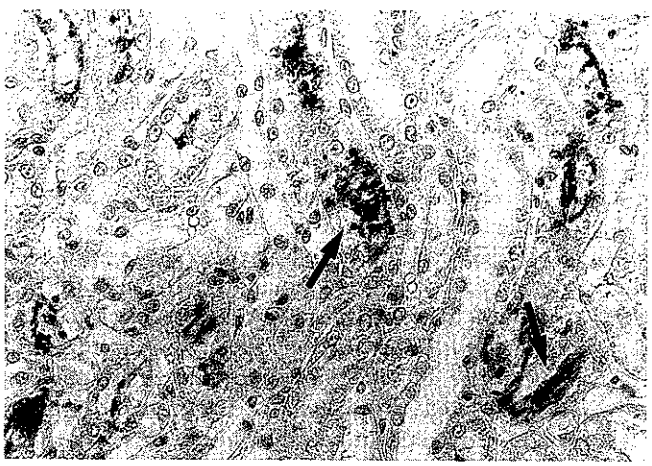

Fig. 1. Immunohistochemical detection of HNE-modified proteins in the proximal tubules of a kidney in an Fe-NTA-treated rat (arrows). ×100.

Table I. Effects of MESNA and NAC on TBARS, BUN, CRN and 8-OHdG Levels in Rats Treated with Fe-NTA

Group	TBARS (mmol MDA/g)	BUN (mg/dl)	CRN (mg/dl)	8-OHdG (/10^5dG)
Control	142±45	11.4±0.6	0.21±0.01	0.74±0.07
Fe-NTA[a)]	446±96[e)]	19.2±6.7[d)]	0.38±0.11[e)]	1.23±0.06[e)]
Fe-NTA+MESNA[b)]	135±15[g)]	10.2±0.5[f)]	0.22±0.03[g)]	0.67±0.13[g)]
Fe-NTA+NAC[c)]	120±10[g)]	8.2±1.4[g)]	0.20±0.01[g)]	0.83±0.17[g)]

a) Fe-NTA were given intraperitoneally at a dose of 12 mg Fe/kg.
b) MESNA (100 mg/kg) were given orally 1 h before and 1 h after the Fe-NTA treatment.
c) NAC (200 mg/kg) were given with the same protocol as the MESNA treatment.
d) $P<0.05$ vs. control.
e) $P<0.01$ vs. control.
f) $P<0.05$ vs. Fe-NTA.
g) $P<0.01$ vs. Fe-NTA.

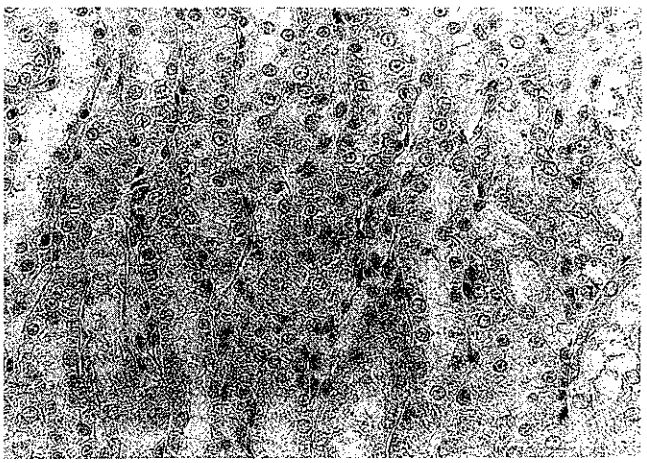

Fig. 2. HNE-modified proteins are undetectable in the proximal tubules of a kidney in an Fe-NTA-treated rat given MESNA. ×100.

cellular proteins via its reactive aldehyde group, is a key factor in the toxicity of Fe-NTA.[17,18] Our data suggest that administration of MESNA or NAC can efficiently prevent the occurrence of lipid peroxidation and consequently, HNE-induced nephrotoxicity following Fe-NTA exposure.

The 8-OHdG level in DNA isolated from renal nuclei was also increased 1.7-fold compared to that of the control rats at 3 h after the Fe-NTA treatment (Table I), in accordance with our previous data.[5-7] Oral administration of either of the chemopreventive compounds completely blocked this increase. The presence of 8-OHdG adducts in replicating DNA has been reported to lead to G-T and A-C transversions[8,19,20] and is also known to induce G-T transversion in codon 12 of c-Ha-*ras*, thereby activating the gene.[9] Considering the number of reports demonstrating a close relation between 8-OHdG adduct formation and carcinogenicity,[14,21-23] it seems likely that 8-OHdG formation might participate in Fe-NTA-induced renal tumorigenesis.[24] In this respect, our data, revealing that MESNA or NAC administration can protect against such an increase in 8-OHdG formation, suggest that these thiol compounds might be effective against Fe-NTA carcinogenicity. Though the mechanism of 8-OHdG formation due to cellular oxidation is unclear, the present data are of interest in highlighting a possible link between lipid peroxidation and oxidative DNA damage. HNE also reacts with deoxyguanosine, resulting in the formation of exocyclic adducts.[25] In the presence of *t*-butyl hydroperoxide, HNE is readily epoxidized to yield 2,3-epoxy-4-hydroxynonanal, which is more mutagenic and tumorigenic than the parent aldehyde.[26]

MESNA has been widely used as a systemic agent which protects against the adverse effects of oxazaphosphorines, such as ifosfamide.[10] NAC is considered to be one of the most promising cancer chemopreventive agents on account of its antioxidant properties.[12,27] In an *in vitro* study, both MESNA and NAC efficiently decreased the formation of exocyclic DNA adducts derived from crotonaldehyde, which is an analogue of α, β-unsaturated aldehyde.[28] Pharmacokinetic studies have demonstrated that MESNA is rapidly oxidized to the disulfide form in plasma, and this in turn is reduced back to MESNA, an active thiol form, by cytosolic enzymes in the renal tubular epithelium.[11] It has been reported that NAC acts extracellularly as an analogue of reduced GSH, and intracellularly as a precursor of this peptide.[29] The reduction of iron from a ferric to a ferrous state is a prerequisite for generating reactive oxygen radicals in Fe-NTA-mediated oxidative stress,[30] and this might depend on the presence of Cys formed during GSH degradation due to γ-glutamyltranspeptidase located on the brush-border surface of the proximal tubule[31-33]; this pathway provides a clue to the organ specificity of Fe-NTA-action. Therefore, we can hypothesize that intracellular, and not extracellular, elevation of thiol groups following MESNA or NAC administration is responsible for preventing Fe-NTA-induced oxidative stress. Considering the fact that 8-OHdG and HNE-modified proteins have been detected in human renal cell carcinomas,[34] our data are of interest as the first to suggest that preventive agents would be effective against renal carcinogenesis involving oxidative stress.

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