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Combining cisplatin with cationized catalase decreases nephrotoxicity while improving antitumor activity

S-F Ma[1], M Nishikawa[2], K Hyoudou[1], R Takahashi[3], M Ikemura[1], Y Kobayashi[1], F Yamashita[1] and M Hashida[1]

[1]*Department of Drug Delivery Research, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan;* [2]*Department of Biopharmaceutics and Drug Metabolism, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan and* [3]*Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan*

Cisplatin is frequently used to treat solid tumors; however, nephrotoxicity due to its reactive oxygen species-mediated effect limits its use. We tested the ability of cationized catalase, a catalase derivative, to inhibit nephrotoxicity in cisplatin-treated mice. Immunohistochemical analysis showed that the catalase derivative concentrated in the kidney more efficiently than native catalase. Repeated intravenous doses of cationized catalase significantly decreased cisplatin-induced changes in serum creatinine, blood urea nitrogen, nitrite/nitrate levels, lactic dehydrogenase activity, and renal total glutathione and malondialdehyde contents. In addition, cationized catalase effectively blunted cisplatin-induced proximal tubule necrosis but had no significant effect on the cisplatin-induced inhibition of subcutaneous tumor growth. Repeated doses of catalase, especially cationized catalase, significantly increased the survival of cisplatin-treated tumor-bearing mice preventing cisplatin-induced acute death. Our studies suggest that catalase and its derivatives inhibit cisplatin-induced nephrotoxicity, thus improving the efficiency of cisplatin to treat solid tumors.

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Correspondence: *M Nishikawa, Department of Biopharmaceutics and Drug Metabolism, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan. E-mail: makiya@pharm.kyoto-u.ac.jp*

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Cisplatin (*cis*-diammine-dichloroplatinum II) is one of the most frequently used anticancer agents for the treatment of solid tumors, including ovarian, testicular, bladder, head and neck, osteogenic, and uterine cervix carcinomas. Unfortunately, chemotherapy using high-dose cisplatin is often accompanied by serious side effects that affect the peripheral neurons and cochlea of the kidney, and these side effects sometimes lead to termination of the treatment or a reduction in the dose.[1,2] Although the mechanism underlying cisplatin-induced nephrotoxicity is not yet fully understood, reactive oxygen species (ROS) have been widely implicated in the toxicity.[3–5] Therefore, various antioxidant enzymes and antioxidants, including superoxide dismutase, catalase, glutathione peroxidase, glutathione (GSH), selenium, flavonoids, and diethyldithiocarbamates, have been investigated as compounds able to protect against cisplatin-induced nephrotoxicity.[6–8]

Although various ROS could be involved in the ROS-mediated, cisplatin-induced nephrotoxicity, hydrogen peroxide is considered to be an effective target molecule to inhibit this toxicity. This is because (i) superoxide anion, which is initially produced, is spontaneously or enzymatically (by superoxide dismutase) converted to hydrogen peroxide and (ii) hydrogen peroxide is stable and has a long half-life. If this is the case, catalase, an enzyme that degrades hydrogen peroxide into oxygen and water, would be a good candidate for the inhibition of cisplatin-induced nephrotoxicity. However, catalase (from bovine liver) is not significantly distributed to the kidney after intravenous injection[9,10] and this severely limits its therapeutic efficacy against such toxicity.

A series of catalase derivatives with different physicochemical properties have been developed in our laboratory, each of which showed unique tissue distribution characteristics.[10] Our previous studies using these catalase derivatives[10–21] clearly indicate that catalase can be highly effective in inhibiting various ROS-mediated injuries once delivered to the region of interest. Therefore, a catalase derivative targeting the kidney would be useful for inhibiting cisplatin-induced nephrotoxicity.

Cationic macromolecules have the ability to interact with the kidneys after entering the blood circulation, as clearly demonstrated with charged dextrans.[22] We have recently synthesized a cationized derivative of bovine liver catalase, that is, ethylenediamine (ED)-conjugated catalase (ED-catalase), and showed that it can protect hepatocytes from carbon tetrachloride-induced acute liver failure in mice.[14] Although ED-catalase does not accumulate to a high degree in the kidneys because of its extensive uptake by the liver, an increase in the amount of catalase delivered to the kidney may be sufficient to inhibit cisplatin-induced nephrotoxicity.

In this study, we first demonstrated that ED-catalase is more efficiently delivered to the kidneys than catalase. Then, the effects of ED-catalase on cisplatin-induced nephrotoxicity were examined in mice by evaluating blood urea nitrogen (BUN), serum creatinine, nitrite/nitrate levels, lactate dehydrogenase (LDH) activity, and the total GSH content and malondialdehyde (MDA) levels in the kidney. Its effects on the antitumor activity of cisplatin were also investigated in tumor-bearing mice.

RESULTS

Renal accumulation of catalase and ED-catalase in mice

Figure 1 shows the immunohistochemical staining of catalase derivatives in mouse kidneys. Compared with the kidney section from an untreated mouse (Figure 1a), one from a mouse receiving an intravenous injection of catalase at a dose of 1 mg kg^{-1} (40 000 U kg^{-1}) showed faint catalase staining in the proximal tubule regions of the kidney (Figure 1c). To confirm the renal distribution of catalase, a very high dose of 100 mg kg^{-1} (4 000 000 U kg^{-1}) was used, which gave detectable signals in the proximal tubules (Figure 1b). On the other hand, the injection of ED-catalase at the low dose of 1 mg kg^{-1} (18 000 U kg^{-1}, 450 U mouse^{-1}) resulted in an intense staining in the same regions (Figure 1d), suggesting that ED-catalase is more efficiently delivered to the kidney than unmodified catalase. The total renal catalase activity was hardly changed by administration of catalase derivatives (data not shown), because of a very high endogenous catalase activity in mouse kidneys ($14\,200 \pm 2200$ U g^{-1} kidney, 5230 ± 840 U kidney^{-1}).

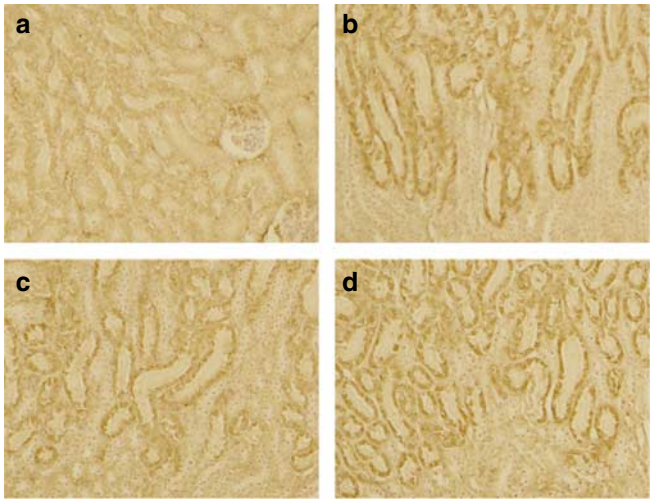

Figure 1 | Immunohistochemical staining of catalase and ED-catalase in mouse kidneys (× 100 original magnification). Catalase or ED-conjugated catalase was injected into the tail vein of mice at a dose of 1 mg kg^{-1}. At 30 min after injection, the kidneys were excised and paraffin-embedded sections were immunostained for bovine catalase using biotin-conjugated anti-bovine catalase antibody, followed by incubation with peroxidase-conjugated antibody. (**a**) Untreated, (**b**) high-dose catalase (100 mg kg^{-1}), (**c**) catalase, and (**d**) ED-catalase.

Single-dose cisplatin-induced nephrotoxicity and its inhibition by catalase

An intraperitoneal (i.p.) injection of cisplatin at a dose of 20 mg kg^{-1} induced serious injuries in mice, and all mice had died by 5 days after the injection. Cisplatin also caused significant weight loss in mice (Figure 2a). In addition, the BUN and serum creatinine levels were significantly increased by the injection of cisplatin 2 days after injection (Figure 2b and c). Administration of catalase (5000−50 000 U kg^{-1} shot^{-1}, seven injections) significantly reduced the cisplatin-induced changes in body weight (Figure 2d) and the BUN and serum creatinine levels (Figure 2e and f) in a dose-dependent manner.

Effect of ED-catalase on single-dose cisplatin-induced nephrotoxicity

Figure 3 shows the effects of catalase and ED-catalase on the nephrotoxicity induced by a single dose (20 mg kg^{-1}, i.p.) of cisplatin. Both catalase and ED-catalase (10 000 U kg^{-1} shot^{-1}, seven injections) significantly reduced the increase in BUN (Figure 3a), serum creatinine (Figure 3b), nitrite/nitrate (Figure 3c) levels, and LDH activity (Figure 3d). The reduction in the renal total GSH content was inhibited only by ED-catalase ($P<0.05$; Figure 3e). The increase in the renal MDA level was also significantly ($P<0.01$) inhibited only by ED-catalase (Figure 3f). For all parameters measured, ED-catalase produced greater changes than catalase, and the differences were significant for BUN ($P<0.01$) and renal MDA levels ($P<0.05$). These results indicate that ED-catalase is more effective than catalase in protecting against high-dose cisplatin-induced nephrotoxicity, and that the co-administration of ED-catalase allows an increase in the cisplatin dose for anticancer therapy. The administration of cisplatin (20 mg kg^{-1}, i.p.) to mice resulted in severe necrosis in the proximal tubules, with extensive epithelial vacuolization, swelling, and tubular dilatation (Figure 3h), which was markedly inhibited in catalase- or ED-catalase-treated mice (Figure 3i and j). The glomeruli appeared normal in all the groups studied. The graded histological changes are summarized in Table 1. Compared with the saline-treated mice, the catalase- and ED-catalase-treated groups exhibited much less significant changes. The platinum content in the kidneys of the saline-treated mice was 1.01 ± 0.13 μg kidney^{-1}, which was not significantly ($P=0.1$) different from that of the ED-catalase-treated mice (0.77 ± 0.18 μg kidney^{-1}).