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Protective effect of cepharanthin on cisplatin-induced renal toxicity through metallothionein expression

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ABSTRACT

Aims: Cisplatin (CDDP) is a potent anticancer agent, but severe renal toxicity can limit its use. We investigated the protective effect of cepharanthin (CE), a biscoclaurin alkaloid, on the renal toxicity of CDDP.
Main methods: Mice were given CDDP along with CE. Effects of CE on CDDP toxicity were investigated by assaying markers of renal toxicity together with MT expression, and by histopathological examination of the kidney. MT-null mice were also examined.
Key findings: CE induced expression of metallothionein (MT). Pre-administration of CE attenuated an increase in blood urea nitrogen (BUN) concentrations after the CDDP injection. A histochemical analysis demonstrated protection against CDDP-induced necrocytosis of kidney tissues by CE. The protective effect of CE did not occur in the MT-null mice.
Significance: Pretreatment with CE may reduce the renal toxicity of CDDP through expression of MT.

Introduction

Cancer chemotherapy contributes to the improvement of not only disease states but also quality of life for patients. However, it frequently has adverse effects, making it difficult to achieve a complete cure, and sometimes being a dose-limiting factor for its use. Cisplatin (cisplatinum or cis-diamminedichloroplatinum (II), CDDP) is a highly effective anti-cancer agent, which was accidentally discovered in the 1960's (Rosenberg et al., 1969). While it has severe adverse effects including ototoxicity, neurotoxicity, myelosuppression, allergic reactions and emetogenicity (Wang and Lippard, 2005), the main side effect of CDDP as a dose-limiting factor is nephrotoxicity (Arany and Safirstein, 2003; dos Santos et al., 2012). Therefore, management of these problems, especially renal toxicity, is important for cancer therapy with CDDP. Although potent antagonists of the serotonin 5-HT_3 receptor and NK1 receptor were introduced recently, resulting in a reduction of CDDP-induced vomiting (Navari, 2003), renal toxicity remains a major problem in CDDP treatment (dos Santos et al., 2012).

Metallothionein (MT) is a cysteine-rich, low molecular-weight protein with high affinity for metals, such as cadmium (Cd), mercury (Hg), bismuth (Bi) and zinc (Zn) (Vasak and Meloni, 2011). MT is thus considered to have a protective role against heavy metal-induced toxicity and other related disorders (Coyle et al., 2002; Namdarghanbari et al., 2011). MT is produced in various organs, including the liver and kidney, in response to various heavy metals. Therefore, it was suggested that MT production in the kidney contributed to the reduction in CDDP-induced renal toxicity. Regarding this, there is a line of evidence demonstrating that treatment with MT inducers including bismuth reduced the renal toxicity of CDDP (Naganuma et al., 1985, 1987; Satoh et al., 1997; Petering et al., 2006).

MT production is induced not only by heavy metals but also by medicines, physical stress and chemical mediators such as cytokines (Coyle et al., 2002). It was also reported that some herbal medicines induced MT expression in the liver and small intestine (Anjiki et al., 2005) and in the kidney (Shibayama et al., 2007). Cepharanthin® (CE), a biscoclaurin alkaloid prepared from extracts of *Stephania cepharantha HAYATA*, has four active agents; cepharantine, isotetrandrine, berbamine and cycleanine. It has been widely used in Japan for more than 40 years to treat a variety of acute and chronic diseases (Furusawa and Wu, 2007; Rogosnitzky and Danks, 2011). Since CE has an anti-cancer effect and acts as an antioxidant, we speculated that it induces production of MT in the kidney, thereby reducing CDDP-induced renal toxicity.

We initially found that CE did induce the expression of MT mRNA in cultured cells derived from kidney and of the MT protein in mouse kidney. Based on these findings, we further investigated the protective effects of CE on the renal toxicity of CDDP.

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Materials and methods

Cell culture

COS-7 (RCB0539) and MDCK (RCB0995) cells were obtained from RIKEN BioResource Center (Tsukuba, Japan). Both cell lines were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 units/ml penicillin-G, 100 μg/ml streptomycin and 2.5 μg/ml fungizone at 37 °C under 5% CO_2/95% air.

Animal experiments

All animal experiments were performed according to the guidelines for the care and use of laboratory animals approved by Okayama University and the Japanese Pharmacological Society.

Mice (8–9 weeks old, male) used in this study were the ddY and C57BL/6J strains, and MT-I/II null mice, purchased from Japan SLC Co., Ltd. (Shizuoka, Japan), CLEA Japan, Inc. (Tokyo, Japan) and The Jackson Laboratory (Maine, U.S.A.), respectively. The ddY and C57BL/6J mice were used after at least a one-week acclimation period in the Animal Quarters of the Dental School. The MT-null mice were bred in the Animal Quarters by ourselves. The MT-null and C57BL/6J mice were kept in an isolation rack and supplied sterilized food and water, and filtered air.

CE was administered by intra-peritoneal (i.p.) injection for 7 consecutive days. For the control, 0.9% NaCl (pH 5.0) was injected instead of CE. Four active ingredients of CE, cepharanthine, isotetrandrine, berbamine and cycleanine, were dissolved in 0.1 N HCl and the pH adjusted to 5.0 with 0.1 N NaOH. Twenty-four hours after the last injection, kidney and liver were removed for MT and other assays. CDDP (15 mg/kg) was administered by subcutaneous (s.c.) injection 24 h after the last injection of CE or 0.9% NaCl. Then kidney (for MT content assay and histochemical study) and serum (for BUN assay) were collected 4 days after the CDDP injection.

Measurement of MT mRNA expression

Cells were inoculated on to 6 cm dishes at a density of 2×10^5 cells/dish (COS-7) and 4.5×10^5 cells/dish (MDCK), and treated with $CdCl_2$ (5 μM) or CE (5, 25, 50 μg/ml) for 3 days. Total RNA was extracted after the indicated intervals by the acid-phenol method using TRIzol Reagent and treated with DNase I.

The expression of MT mRNA was investigated by the RT-PCR method, as described previously (Sogawa et al., 2000) using an RNA PCR Kit (TaKaRa). First strand cDNA from 0.2 μg of total RNA was synthesized with random hexamer primers and AMV reverse transcriptase. PCR was performed by initial denaturation at 95 °C for 2 min, followed by 20 or 25 cycles of 94 °C for 1 min, 65 °C for 1 min and 72 °C for 2 min with a final extension at 72 °C for 7 min. A primer set for MT mRNA was newly synthesized with reference to MT-I and -II cDNA sequences for the mouse and rat, and to MT cDNA sequence for the monkey (5′-GGTACCCCAACTGCTCCTGC/5′-AAGCTTTGCAGAC(A/G)CAGCCC). Each PCR amplicon was electrophoresed on 2% agarose gel and stained with ethidium bromide.

Measurement of MT protein

The amount of MT protein was evaluated using the cadmium-binding assay of Onosaka et al. (1983) with minor modifications (Sogawa et al., 2001). Livers and kidneys were homogenized with a 5-fold volume of 0.25 M sucrose solution containing 2 μg/ml antipain and 2 mM 1,4-dithiothreitol. The homogenate underwent heat treatment in boiling water, and was then centrifuged at 12,000×rpm for 10 min. The supernatants were used for the Cd-binding assay. The hemoglobin solution (20 mg/ml Tris–HCl buffer, pH 8.0) was used instead of rat red-blood-cell hemolysate. Cd was measured using an atomic absorption spectrophotometer (Z-8000, Hitachi Ltd., Tokyo, Japan), and concentrations of MT were calculated.

Measurement of BUN

Urea nitrogen (BUN) concentrations in serum were determined by the urease-indophenol method using Urea NB-Test Wako. Absorbance was measured with a double-beam spectrophotometer (UV-180, Shimadzu, Kyoto, Japan).

Histochemistry of renal tissues treated with CDDP

Mice were anesthetized with 50 mg/kg pentobarbital and perfused via the left ventricle with saline and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). The kidney was removed and fixed in the same fixative for 2 h at 4 °C. The post-fixation was performed by using a microwave oven. Dehydration was carried out with a series of ethanol and toluene solutions, and the tissue then embedded in paraffin. The kidney block was cut into 5-μm sections, and stained with periodic acid-Schiff reagents and hematoxylin.

Statistics

Data were expressed as the mean ± SEM. Statistic analyses were performed by ANOVA following Fisher's post-hoc test or Student's *t*-test. Significance was defined at P values <0.05.

Chemicals

CDDP (Randa®) was purchased from Nippon Kayaku Co., Ltd. (Tokyo, Japan). CE (Lot No. K022196, 6006) was provided by Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan). Cepharanthine, isotetrandrine, berbamine and cycleanine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Periodic acid-Schiff reagents were purchased from Muto Pure Chemicals Co., Ltd. (Tokyo, Japan). Cadmium (Cd) chloride was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Dulbecco's modified Eagle's medium (DMEM) was from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum (FBS) was purchased from JRH Biosciences (Lenexa, KS). TaKaRa RNA PCR Kit (AMV) Ver.2.1 and Ver.3.0, and DNase I were from Takara Bio Inc (Shiga, Japan). TRIzol Reagent was obtained from Invitrogen (Carlsbad, CA, USA). The glyceraldehyde 3-phosphate dehydrogenase (G3PDH) control amplimer set was obtained from BD Biosciences Clontech (Palo Alto, CA). Other chemicals were purchased from Nacalai Tesque Inc. and Sigma Chemical Co.

Results

Expression of MT mRNA induced by CE in COS-7 and MDCK cells

There are four isoforms of MT, MT-I, -II, -III and -IV, in mammals. The MT-I and -II genes are expressed in many tissues including brain, liver and kidney tissues, while MT-III and MT-IV are expressed mainly in the central nervous system and squamous epithelia (Coyle et al., 2002). Therefore, we investigated the expression of MT-I/II mRNA, considered the main isoforms in kidney, in cells derived from the kidney after the administration of CE.

MT mRNA was detected 3 h after the administration of $CdCl_2$ and CE in the COS-7 and MDCK cell lines, and continued to be expressed for at least 12 h (Fig. 1). The expression was more pronounced in the COS-7 cells than in MDCK cells. The induction of MT mRNA expression by CE in COS-7 cells was dose-dependent (data not shown).