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Original Article

Nephroprotective effect of electrolyzed reduced water against cisplatin-induced kidney toxicity and oxidative damage in mice

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Abstract

Background: Cisplatin is a potent chemotherapeutic drug for cancer therapy, but it has serious side effects in clinical treatment, particularly nephrotoxicity. The purpose of this study was to evaluate the protective effect of electrolyzed reduced water (ERW) on renal injury caused by cisplatin.

Methods: Animals were divided into four groups as follows: normal control group, cisplatin control group, ERW control group and ERW + cisplatin group. Each group comprised 10 animals, which were orally treated with normal saline or ERW daily companion by administration of one dose of cisplatin for 28 days. Animals in the cisplatin group received an intraperitoneal single-dose injection of cisplatin (20 mg/kg body weight) as a single i.p. dose on the 25th day of the experiment. We determined the hydration state in urine and the level of serum markers of kidney function, the levels of glutathione (GSH) and thiobarbituric acid-reactive substances (TBARS) levels and the activities of glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxidase dismutase (SOD) in kidney and histopathological changes.

Results: After administration of ERW, the reduced urinary osmolality was increased and elevated Na^+, K^+, Mg^{2+} and Ca^{2+} levels in urine were significantly decreased in cisplatin-induced renal injury mice. Besides, the results demonstrated that significantly decreased elevated serum levels of creatinine and blood urea nitrogen (BUN) and the levels of TBARS in the kidneys that were induced by cisplatin. Moreover, ERW treatment was also found to markedly increase ($p < 0.05$) the activities of GPx, GR, CAT and SOD, and to increase GSH content in the kidneys. Histopathology showed that ERW protects against cisplatin-induced renal injury to both the proximal and distal tubules.

Conclusion: ERW exhibits potent nephroprotective effects on cisplatin-induced kidney damage in mice, likely due to both the increase in antioxidant-defense system activity and the inhibition of lipid peroxidation.

Keywords: Cisplatin; Electrolyzed reduced water; Kidney; Nephrotoxicity; Oxidative stress

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum II) is a potent chemotherapeutic drug used in the clinical treatment of several human cancers.[1] Unfortunately, treatment with cisplatin has several side effects, including neurotoxicity, ototoxicity and nephrotoxicity, resulting in dosage limiting in cancer therapy.[2,3] Reactive oxygen species (ROS), including hydroxyl radicals, superoxide anion and hydrogen peroxide (H_2O_2), are known to play critical roles in the pathology and progression of kidney disease,[4] and ROS have been shown to be associated with cisplatin toxicity.[5] In addition, cisplatin induces injury in the renal vasculature that eventually leads to DNA damage, renal antioxidant enzyme inactivity and finally to ischemic tubular cell death, which are strongly associated with the renal toxicity of this compound.[3] In this context, many studies demonstrated that an important mechanism of nephroprotection might be related to the antioxidants' potentiality to scavenge ROS.[4,5]

Electrolyzed reduced water (ERW) is produced by electrolysis of tap water and is characterized by higher dissolved hydrogen, lower dissolved oxygen, lower oxidation-reduction potential and higher pH than any form of tap water.[6] An increasing number of people are using ERW as drinking water in Asia. However, there are limited studies concerning the functional activity of ERW have been published. ERW with ROS scavenging ability might have the potential effect of protecting DNA, RNA and cells against oxidative stress.[7] Several studies have demonstrated that ERW has anticancer effects may be due to inhibition of angiogenesis in A549 cells[8] and suppression of metastasis in melanoma-injected mice.[9] Recently, we reported that ERW has antioxidant-like activity and scavenging activity of hydroxyl radicals, superoxide anion and H_2O_2 detected by chemiluminescence. Moreover, ERW with GSH showed a significantly promoted apoptosis-inducing effect on human promyelocytic leukemia cells and did not affect the normal functioning of the normal cell or the antitumor activity by chemotherapeutic drug.[6]

In various experimental models, ERW protected pancreatic β cells against the diabetogenic agent alloxan-induced damage[10] and increased the release of circulating insulin and ameliorated the sensitivity of insulin in diabetic mice.[11,12] One clinical report showed that hemodialysis with ERW supplementation efficiently reduced oxidative damage to leukocytes and endothelial cells in end-stage renal disease patients on chronic hemodialysis.[13] Recently, we demonstrated that ERW played a protective role in the reduction of oxidative damage and maintain the hepatic antioxidant enzymatic system, including CAT, SOD and GPx.[14] Although the antioxidant activity of ERW is well known, it is still unclear what the protective effect of ERW against cisplatin-induced nephrotoxicity.

In this study, we reported the nephroprotective effects of ERW against cisplatin-induced acute renal injury *in vivo*. The animals were orally treated with ERW daily companion by administration of one dose of cisplatin for 28 days. The urinary hydration state and the levels of creatinine and BUN in the serum, as well as the levels of GSH and TBARS levels and the activities of GPx, GR, CAT and SOD in kidney, were measured to observe renal damage. The degree of cisplatin-induced renal damage was examined through histopathological examination.

2. Methods

2.1. Chemicals

Cisplatin was purchased from Sigma−Aldrich (St. Louis, MO, USA). All of the other reagents and solvents were of analytical grade.

2.2. Apparatus for producing ERW

The ERW producing system used in this study was as described in detail elsewhere.[6] It consisted of a filtration system for the purification and an electrolyzer for the electrolysis of the water. The electrolysis system maintained the pH between 8.10 and 10.1, the values of ORP between −160 mV and −607 mV, and the water flow rate between 2.0 L/min and 3.4 L/min. The instrument was bound to a water tap. After turning on the equipment, the tap water was first cleansed and then electrolyzed to generate both ERW and electrolyzed oxidized water (EOW). In each experiment, the values of ORP and pH of ERW were maintained at 9.2 ± 0.2 and −360 ± 20 mV, respectively. The characteristics of tap water, distilled-deionized (DD) water, ERW and electrolyzed-oxidized water (EOW) are shown as Table 1. The ERW had been freshly prepared and then used in this study.

2.3. Experimental animals

Eight weeks old male ICR mice were obtained from BLT (BioLASCO Taiwan CO. Ltd.). Mice were quarantined under specific pathogen-free conditions and allowed to acclimate for seven days prior to experimentation. The animals were handled in a humidity- and temperature-controlled laboratory. Water and food were available *ad libitum*. Our Institutional Animal Care and Use Committee approved the procedures for the animal research, and the animals were cared for in accordance with the institutional ethical guidelines.

2.4. Treatment

To establish an optimal animal model of cisplatin-induced renal injury, a dose−response test and a time course experiment of cisplatin's effects on renal function were conducted. For the dose−response study, the mice were divided into four groups of six mice each and given were cisplatin (0, 5, 10 and 20 mg/kg body weight, respectively) intraperitoneally (i.p.). Kidney-functional parameters, serum levels of creatinine and BUN, were evaluated 72 h later. For the time course study, the animals were divided into five groups (0 h, 24 h, 48 h, 72 h and 120 h,

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