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Renoprotective mechanisms of chlorogenic acid in cisplatin-induced kidney injury

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ABSTRACT

The aim of this study was to investigate the renoprotective activity of chlorogenic acid (CA) in a murine model of cisplatin (CP)-induced kidney injury. Male BALB/cN mice were gavaged daily with CA at 3, 10 and 30 mg/kg for two successive days, 48 h after intraperitoneal injection of CP (13 mg/kg). On the fifth day, serum creatinine and blood urea nitrogen (BUN) levels were significantly increased in CP-intoxicated mice, which was recovered by CA. Renal oxidative stress, evidenced by increased 4-hydroxynonenal (4-HNE) expression, was significantly reduced with CA. Simultaneously, the overexpression of heme oxygenase 1 (HO-1) and cytochrome P450 E1 (CYP2E1) was attenuated. The inhibition of inflammatory response by CA was achieved through the reduction of tumor necrosis factor-α (TNF-α) and cyclooxygenase-2 (COX-2) expression. Additionally, CA significantly suppressed p53, Bax active caspase-3, cyclin D1 and microtubule-associated protein 1 light chain 3 isoform B (LC3B) expression, suggesting the inhibition of both apoptosis and autophagy. The expression of multidrug resistance-associated proteins (Mrp1 and Mrp2) increased and organic cation transporter 2 (Oct2) decreased by CP, protecting the kidneys from nephrotoxicity by reducing the burden of tubular cells. CA dose-dependently restored Mrp1, Mrp2 and Oct2 expression. The recovery of kidney tissue form CP injury was accompanied by increased proliferating nuclear cell antigen (PCNA) expression. The results of this study suggest that CA attenuates CP-induced kidney injury through suppression of oxidative stress, inflammation, apoptosis and autophagy, with the improvement in kidney regeneration.

1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum(II), CP) is one of the most important antineoplastic drugs used in the treatment of solid tumors, including those of the head, neck, lung, breast, ovary and testis (Miller et al., 2010). CP binds to DNA and forms intrastrand crosslinks and adducts, resulting in impaired DNA replication (Chválová et al., 2007). Higher doses of CP also induce acute apoptosis through the induction of oxidative stress (Berndtsson et al., 2007). Other DNA-independent effects include inflammation, mitochondrial damage and impaired cellular transport mechanisms (Fuertes et al., 2003).

After administration, CP is taken up by renal tubular cells. Proximal tubular cells absorb the highest concentrations of the drug, acting as a primary site of CP accumulation (Yao et al., 2007). Consequently, this results in cumulative and dose-dependent nephrotoxicity, which occurs in one third of patients (Ries and Klastersky, 1986). Nephrotoxicity and a severe renal dysfunction are one of the most serious dose-limiting side effects in CP chemotherapy. The major mechanisms of CP-induced nephrotoxicity include necrosis, oxidative stress, inflammation and apoptosis (Miller et al., 2010). Natural compounds that counteract these processes may exhibit a significant renoprotective activity (Sahu et al., 2011, 2013; Pan et al., 2009; Chirino et al., 2008). Recently, we showed protective activity of rosmarinic acid and berberine in murine model of acute kidney injury induced by CP (Domitrović et al., 2013, 2014).

Chlorogenic acid (CA) is a phenolic compound widely distributed in fruits and vegetables, including apples, pears, carrots, tomatoes and sweet potatoes, with the highest concentration in coffee and tea (Upadhyay and Mohan Rao, 2013). Previous studies demonstrated free radical scavenging and antioxidant activity of CA in vitro (Xiang and Ning, 2008). In biological studies, CA prevented chemically induced damage in liver and primary cortical neurons

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Table 1
Body weight change, relative kidney weight and serum markers of kidney damage.

	Body weight change (%)	Relative kidney weight	Creatinine (μmol/L)	BUN (mmol/L)
Control	$+1.4 \pm 0.8^{a}$	7.8 ± 0.6^{a}	30.2 ± 1.5^{a}	15.6 ± 2.1^{a}
CA 30 mg/kg	$+0.9 \pm 1.4^{a}$	7.9 ± 0.4^{a}	30.6 ± 1.9^{a}	15.3 ± 1.8^{a}
CP	-19.4 ± 4.7^{b}	9.3 ± 0.7^{b}	147.8 ± 16.2^{b}	82.7 ± 7.4^{b}
CP + CA 3 mg/kg	-16.8 ± 2.8^{b}	8.9 ± 0.3^{b}	126.4 ± 10.7^{c}	64.3 ± 4.9^{c}
CP + CA 10 mg/kg	-12.6 ± 2.1^{c}	$8.4 \pm 0.4^{a,b}$	55.9 ± 7.5^{d}	26.8 ± 3.2^{d}
CP + CA 30 mg/kg	-9.5 ± 1.6^{d}	8.1 ± 0.2^{a}	32.2 ± 2.7^{a}	15.8 ± 1.8^{a}

CA was administered orally once daily for two consecutive days, 48 h after intraperitoneal injection of cisplatin (CP, 13 mg/kg). Mice were sacrificed for 4 days after CP injection. Relative kidney weight is expressed as [(kidney weight/body weight) × 1000]. Serum creatinine and BUN (blood urea nitrogen) were measured as described in Section 2. Each value represents the mean ± SD for 5 mice. Means within columns sharing the same letter are not significantly different from each other ($P < 0.05$).

by reducing oxidative damage and apoptosis (Ji et al., 2013; Kim et al., 2012a). The anti-inflammatory activity of CA has been related to the inhibition of nuclear factor-kappaB (NF-κB) activation and the release of pro-inflammatory cytokines in both cell cultures and mice liver (Hwang et al., 2014; Shi et al., 2013). Previously we showed that CA was, at least in part, responsible for the hepatoprotective effects of dandelion (*Taraxacum officinale* L.) root extract in mice (Domitrović et al., 2010).

In the current study, we evaluated the renoprotective efficacy of CA against CP-induced kidney injury. Possible molecular mechanisms for the therapeutic effects of CA were investigated, including antioxidant, anti-inflammatory, antiapoptotic and antiautophagic activity. The impairment of renal cellular transport mechanisms and the regenerative capability of the kidneys were also investigated.

2. Materials and methods

2.1. Chemicals

Chlorogenic acid, *cis*-diamineplatinum(II) dichloride, dimethyl sulphoxide (DMSO), sodium dodecyl sulfate (SDS) and tris (hydroxymethyl) aminomethane (Tris) were purchased from Sigma–Aldrich (Steinheim, Germany). Anesthetic and analgesic

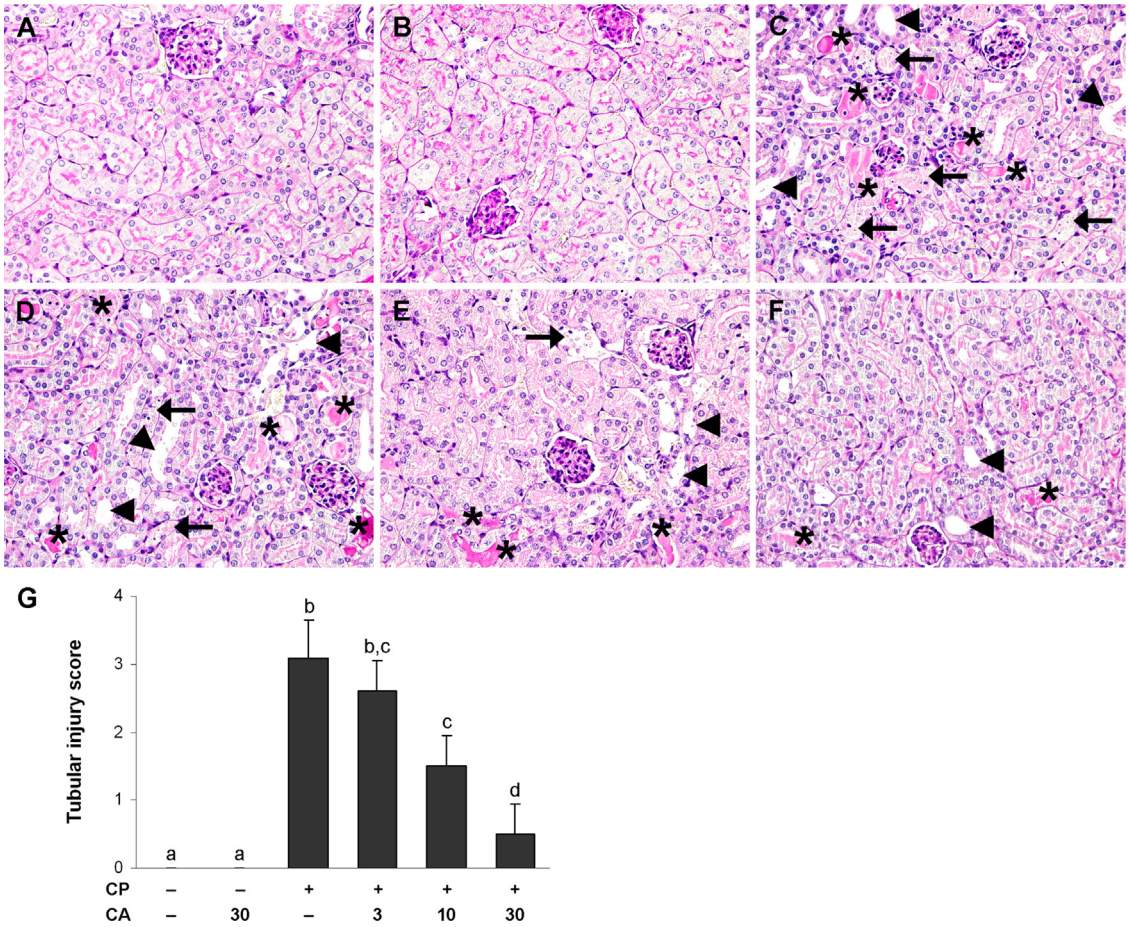

Fig. 1. Representative histopathological changes in the corticomedullary junction of mice kidneys. Mice were treated with vehicle (A), CA 30 mg/kg (B), CP (C), CP + CA 3 mg/kg (D), CP + CA 10 mg/kg (E) and CP + CA 30 mg/kg (F). Treatment with CP resulted in necrosis (arrows), tubular dilatation (arrowheads), cast formation (stars), which was dose-dependently ameliorated with CA. Representative results from 5 similarly treated mice. PAS staining. Original magnification ×400. Tubular injury score (G). Each value represents the mean ± SD for 5 mice. Different letters indicate statistical differences between groups ($P < 0.05$).