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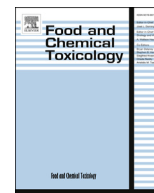

Isoliquiritigenin pretreatment attenuates cisplatin induced proximal tubular cells (LLC-PK1) death and enhances the toxicity induced by this drug in bladder cancer T24 cell line

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ABSTRACT

Cisplatin is an effective antineoplastic agent widely used in the treatment of solid tumors, however, it induces nephrotoxicity. Cisplatin-induced nephrotoxicity is associated with increased reactive oxygen species (ROS) production and decreased antioxidant system defense in kidney. Isoliquiritigenin (IsoLQ) is a chalcone, which is characterized by its antioxidant and antiinflammatory properties. Herein, we investigated the protective effect of IsoLQ on LLC-PK1 proximal tubular cells against cisplatin-induced death and its effect on the antineoplastic activity of cisplatin on bladder cancer T24 cell line. It was found that pretreatment with IsoLQ attenuates cisplatin-induced cell death, ROS production, and activation of caspase-3. IsoLQ also induced heme oxygenase-1 (HO-1) expression that may be associated with nuclear factor (erythroid-derived 2)-like 2 (Nrf2) nuclear translocation. The protective effect of IsoLQ was abrogated by tin mesoporphyrin (SnMP), an HO inhibitor. Further, bilirubin and carbon monoxide releasing molecule-2 also showed a protective effect against cisplatin-induced cell death. In addition, IsoLQ induced in a dose-dependent way, death of T24 cells and exacerbated cisplatin-induced cell death. These results suggest that IsoLQ has a protective effect on cisplatin-induced toxicity in LLC-PK1 cells, in part through induction of HO-1, without interfering with the antineoplastic activity of this agent in T24 cells.

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1. Introduction

Cisplatin (*cis*-diaminodichloroplatinum) is an inorganic coordination compound widely used for its anticancer effect, which was approved by the United States Food and Drug Administration in 1978 (Kelland, 2007). It has been used alone or in combination with other types of therapies, such as surgery and radiotherapy in the treatment of patients with solid tumors (Curran, 2002; Hanigan and Devarajan 2003, Langerak and Dreisbach, 2001). However, its use has been limited due to its side effects, which include suppression of the bone marrow, as well as neurotoxic, anaphylactic, ototoxic and nephrotoxic effects (Arany and Safirstein, 2003; Lebwohl and Canetta, 1998; Ozkok and Edelstein, 2014; Williams and Whitehouse, 1979). The kidney is the main organ responsible for its elimination and cisplatin concentration becomes 5 times higher in this organ than in blood serum due to accumulation processes in the renal cortex (Bajorin et al., 1986; Kuhlmann et al., 1997; Rosenberg, 1985). Between 20 and 35% of patients treated with a single dose of cisplatin show renal dysfunction (Ozkok and Edelstein, 2014). Cisplatin induces

Abbreviations: ANOVA, analysis of variance; Bak, Bcl-2 homologous antagonist killer; Bax, Bcl-2 associated X protein; Bcl-2, B-cell lymphoma; Bcl-xL, B-cell lymphoma-extra-large; CAT, catalase; CI, combination index; CM-H2DCFDA, chloromethyl dichlorodihydrofluorescein diacetate; CO, carbon monoxide; CORM-2, carbon monoxide-releasing molecule-2; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; FBS, fetal bovine serum; FDA, fluorescein diacetate; GSH, glutathione; GR, glutathione reductase; HCl, hydrochloric acid; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; HO-1, heme oxygenase-1; IC_{50}, half inhibitory concentration; IsoLQ, isoliquiritigenin; KCl, potassium chloride; LLC-PK1, proximal tubular cell line derived from normal pig kidney; $MgCl_2$, magnesium chloride; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; NaCl, sodium chloride; NADPH, nicotinamide adenine dinucleotide; NaF, sodium fluoride; $Na_4P_2O_7$, sodium pyrophosphate; Na_3VO_4, sodium orthovanadate; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NP-40, 4-nonylphenyl-polyethylene glycol; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; RIPA, radioimmunoprecipitation assay; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; SEM, standard error of the mean; SnMP, tin-mesoporphyrin IX dichloride; SnPP, tin-protoporphyrin; SOD, superoxide dismutase; T24, urinary bladder cell line derived from transitional human cell carcinoma; TBST, Tris-buffered saline with Tween-20.

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oxidative stress, mitochondrial dysfunction and activation of proinflammatory proteins, which finally triggers damage and cell death (dos Santos et al., 2012; Ozkok and Edelstein, 2014; Siddik, 2003).

Heme oxygenase-1 (HO-1) is an enzyme that catalyzes the degradation of the heme group into biliverdin, ferrous ion (Fe^{2+}) and carbon monoxide (CO); biliverdin is reduced subsequently to bilirubin by the action of the enzyme biliverdin reductase. These products have been suggested as the main mediators of the cytoprotective effect of HO-1 in several *in vitro* and *in vivo* models of cell and tissue damage (Prawan et al., 2005; Gozzelino et al., 2010). HO-1 expression is induced by a large number of physiological and pathological stimuli, including signals of cellular stress, cytokines and growth factors (Sikorski et al., 2004; Hill-Kapturczak et al., 2007). Chemical inhibition of HO-1 activity by meso and protoporphyrins in proximal tubular cells and genetic knockout of HO-1 in mice caused severe cellular damage in response to treatment with cisplatin, whereas gene overexpression and chemical induction reduce its nephrotoxic effect (Schaaf et al., 2002; Jung et al., 2014; Tayem et al., 2014; Shiraishi et al., 2000; Bolisetty et al., 2016).

Several compounds of edible plants with different structures have gained considerable attention by their effects as reactive oxygen species (ROS) scavengers and inductors of cytoprotective proteins, like antioxidant enzymes. These compounds are an alternative for decreasing cisplatin-induced nephrotoxicity, however, it has been argued that its effect could interfere with the antineoplastic activity of cisplatin by stabilizing ROS, which has been considered one of the mediators of the cytotoxic mechanism of this drug, and/or by forming complexes with this drug and reducing, in this way, its biological activity (dos Santos et al., 2012).

Isoliquiritigenin (IsoLQ) is a chalcone isolated from roots and stems of *Glycyrrhiza glabra*, *Glycyrrhiza uralensis* and *Glycyrrhiza inflata* (Wang et al., 2013a). IsoLQ has been studied for its potential as a cytoprotective agent, in *in vitro* and *in vivo* models (Peng et al., 2015). IsoLQ inhibits carbon tetrachloride (CCl_4)-induced expression of proinflammatory mediators, as tumor necrosis factor alpha and cyclooxygenase 2, and prevents the decrease in glutathione (GSH) levels induced by this compound (Zhao et al., 2015). Cao et al. (2016) reported that IsoLQ decreases ROS and enhances intracellular GSH levels and expression of HO-1 and nicotinamide adenine dinucleotide (NADPH) quinone oxidoreductase 1 in HepG2 cells treated with triptolide, an hepatotoxic agent. IsoLQ also has antiinflammatory activity, mediated by HO-1, in RAW 264.7 macrophages stimulated with lipopolysaccharide (Lee et al., 2009). Lee et al. (2008) reported that IsoLQ exerts nephroprotective and hepatoprotective effects against cisplatin-induced toxicity in a model of xenotransplant of tumoral cells of colon in BALB/c mice, without interfering with the antineoplastic activity of cisplatin. However, the mechanisms involved in IsoLQ nephroprotective effect have not been described yet.

The aim of the present study was to evaluate some possible mechanisms that may be involved in the protective effect of IsoLQ on cisplatin-induced death in LLC-PK1 cells as well as to evaluate the effect of IsoLQ on the antineoplastic activity of cisplatin on bladder cancer T24 cell line. It was evaluated the potential role of the transcriptional factors nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB); the antioxidant enzymes HO-1, glutathione reductase (GR), catalase (CAT) and superoxide dismutase- 1 (SOD-1); and the proteins involved in the apoptotic process Bcl-2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2), and cleaved caspase-3.

2. Material and methods

2.1. Chemicals

T24 cell line, a urinary bladder cell line derived from transitional human cell carcinoma, and LLC-PK1 cells, a proximal tubular cell line derived from normal pig kidney, were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Cisplatin (*cis*-Diamminedichloroplatinum II), sodium dodecyl sulfate (SDS), fluorescein diacetate (FDA), mouse anti-α tubulin antibody, 3-[4,5-dimethylthiazol-|2-yl)]-2,5-diphenyl-tetrazolium bromide (MTT), Tris(hydroxymethyl)aminomethane, sodium deoxycholate, 4-nonylphenyl-polyethylene glycol (NP-40), sodium fluoride (NaF), magnesium chloride ($MgCl_2$), sodium pyrophosphate ($Na_4P_2O_7$), sodium orthovanadate (Na_3VO_4), glycerophosphate, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), Triton X-100, dithiothreitol (DTT), Tween 20, glycerol, trypsin-ethylenediaminetetraacetic acid (EDTA), dimethyl sulfoxide (DMSO), and CO-releasing molecule (CORM-2, tricarbonyldichlororuthenium II dimer) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Biowest (Riverside, MO, USA). Isopropyl alcohol and EDTA disodium salt, sodium chloride (NaCl), and hydrochloric acid (HCl) were purchased from J.T. Baker (Xalostoc, Edo. Mex, Mexico). Potassium chloride (KCl) was purchased from Mallinckrodt plc. (St. Louis, MO, USA). IsoLQ (4, 2′, 4′-trihydroxychalcone) was purchased from AK Scientific (Union City, NJ, USA). Sn (IV) mesoporphyrin IX dichloride (SnMP) and bilirubin alpha were purchased from Frontier Scientific (Logan, UT, USA). Chloromethyl dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) was purchased from Molecular Probes (Eugene, OR, USA). Hoechst 33258 stain was purchased by Fluka (Schnelldorf, Germany). Rabbit anti-HO-1 antibody and rabbit anti-Nrf2 antibody were purchased from Enzo Life Sciences, Inc. (Farmingdale, NY, USA) and Santa Cruz Biotech, Inc. (Dallas, TX, USA), respectively. Rabbit anti-NFκB antibody was purchased from Invitrogen (Carlsbad, CA, USA). Rabbit anti-Bax antibody was purchased from GeneTex (Irvine, CA, USA). Mouse anti-Bcl-2 antibody was purchased from eBioscience (Waltham, MA, USA). Rabbit anti-cleaved caspase-3 antibody was purchased from Cell Signaling Technology (Danver, MA, USA) and rabbit anti-lamin B1 was purchased from Abcam (Cambridge, MA, USA). Fluorescent secondary antibodies anti-rabbit 800CW and anti-mouse 680RD were purchased from LI-COR Biosciences (Lincoln, NE, USA).

2.2. Cell culture and viability

LLC-PK1 and T24 cells were grown to 90% confluence with DMEM, supplemented with 10% FBS, 100 U/ml penicillin and 50 U/ml streptomycin in a humidified incubator with 5% CO_2 atmosphere at 37 °C. LLC-PK1 cells were within passages 13–23 while T24 cells were within passages 10–20. After 24 h, LLC-PK1 cells were exposed to different concentrations of cisplatin (0–60 μM) or IsoLQ (0–25 μM) for 24 h to establish the half inhibitory concentration (IC_{50}) value of cisplatin and cytotoxicity of IsoLQ in these cells. Cell viability was assessed by reduction of MTT to formazan or by deacetylation of FDA. Cells were washed with phosphate buffered saline (PBS) followed by MTT addition (0.3 mg/mL) and incubation for 4 h at 37 °C or by FDA addition (5 μg/mL) and incubation for 5 min at 37 °C in the darkness. For the MTT assay, the medium was discarded and formazan crystals were dissolved in HCl (0.04 M)-isopropanol, then the absorbance was measured at 570 nm using a

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