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Rhus verniciflua Stokes prevents cisplatin-induced cytotoxicity and reactive oxygen species production in MDCK-I renal cells and intact mice

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Abstract

Cisplatin-induced oxidative stress can cause liver and kidney damage, thus limiting therapeutic efficacy. Thus, in the present study, since *Rhus verniciflua* Stokes (RVS) containing flavonoids has antioxidant effects, we investigated whether it can protect cisplatin-induced toxicity *in vitro* and *in vivo*, The *in vitro* effects of RVS on the cell viability and reactive oxygen species (ROS) production were investigated using cisplatin-treated Madin–Darby Canine kidney (MDCK)-I renal cells. Its *in vivo* effects were also studied in BALB/c mice inoculated with CT-26 colon adenocarcinoma cells and treated with cisplatin with or without RVS. Liver and renal functions were assessed together with indices of tissue oxidation. RVS prevented cisplatin-induced cytotoxicity and ROS release against MDCK-I cells. RVS alone exerted modest antitumor activity against CT-26 cells. When used concurrently with cisplatin, RVS prevented the increases in serum blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and NO, while reducing liver and kidney tissue MDA content, and increasing catalase, glutathione (GSH), and superoxide dismutase (SOD) activities. Moreover, the antitumor efficacy of cisplatin was not altered by concurrent administration of RVS. These findings demonstrate that RVS prevents cisplatin-induced toxicity *in vitro* and *in vivo* via an antioxidant activity without hurting its antitumor effectiveness, suggesting that RVS can be usefully applied to the neoplastic patients as a combined chemopreventive agent with cisplatin.

Keywords: *Rhus verniciflua* Stokes; Cisplatin; MDCK-I cells; Nephrotoxicity; Hepatotoxicity; Antioxidant activity

Introduction

Toxic side effects limit the potential utility of many chemotherapeutic agents. Drug-induced liver injury is the most frequent reason for the withdrawal of an approved drug from the market, and it is also responsible for more than 50% of cases of acute liver failure in the United States (Lee, 2003). Recently, many plant extracts have been found to protect

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against chemotherapeutic agent-induced toxicity. One of the most effective antineoplastic drugs is cisplatin, often used for the treatment of ovarian, testicular, and head and neck cancers (Loehrer and Einhorn, 1984). Amongst its potential adverse effects are renal dysfunction, myelosuppression, ototoxicity, hepatotoxicity, and nephrotoxicity (Cavalli et al., 1978; Pollera et al., 1987; Waszkiewicz, 2001). Among these nephrotoxicity and hepatotoxicity are the most frequent.

Evidence suggests that cisplatin can cause the death of normal cells by inactivating enzymes such as glutathione and metallothioneins (Timmer-Bosscha et al., 1992). Glutathione depletion can induce accumulation of reactive oxygen species (ROS) leading to cell death, whereas the precursor of glutathione (GSH) protects against cisplatin cytotoxicity (Lu et al., 2004). Cisplatin also induces cytotoxicity through production of ROS (Hannemann and Baumann, 1988; Masuda et al., 1994; McGinness et al., 1978; Sodhi and Gupta, 1986; Sugihara and Gemba, 1986). Reports also indicate that cytochrome P450 2E1 (CYP2E1), an active producer of ROS, can enhance cisplatin-induced cytotoxicity (Lu and Cederbaum, 2006), and administration of free radical scavengers or antioxidants inhibits the cisplatin-induced nephrotoxicity in animal models (Hannemann and Baumann, 1988; McGinness et al., 1978; Sugihara and Gemba, 1986).

Rhus verniciflua Strokes (RVS) has been used for the treatment of blood stasis and cancer in Asian countries as a folk remedy (Kitts and Lim, 2001; Lim et al., 2001). RVS contains phenolic compounds such as *p*-coumaric acid, fustin, kaempferol-3-*O*-glucoside, sulfuretin, butein and kaempferol, and so was reported to exert antioxidant (Lee et al., 2002; Lim et al., 2001) and antitumorigenic (Kitts and Lim, 2001; Lee et al., 2003a; Lim et al., 2003) activities. Also, butein derived from RVS exhibits antifibrogenic activity (Lee et al., 2003b) and inhibits cell proliferation (Jang et al., 2005; Samoszuk et al., 2005), whereas the glycoprotein fustin inhibits apoptosis in glucose/glucose oxidase (G/GO)-induced BNL CL.2 cells (Lee et al., 2005) and protects against 6-hydroxydopamine-induced neuronal cell death (Park et al., 2007). Recently Sohn et al. (2008) reported that herbal extracts such as macelignan from *Myristica fragrans* Houtt can prevent cisplatin-induced rises in hepatic ALT and AST enzymatic activities. However, the protective effects of RVS against hepatotoxicity and nephrotoxicity have not yet been tested.

Thus, the current study was designed to elucidate the potential protective mechanisms of RVS against cisplatin-induced side effects, using cultured renal cells (MDCK-I line) and an intact mouse tumor model (BALB/c mice inoculated with CT-26 colon adenocarcinoma cells).

Materials and methods

Reagents and chemicals

RPMI 1640 medium, fetal bovine serum, penicillin, and streptomycin were purchased from GIBCO (Grand Island, NY). Cisplatin, 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT), phenylmethylsulfonyl fluoride (PMSF), sodium nitrite, 1,1,3,3-tetramethoxypropane, glutathione (GSH), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), glutathione reductase, nicotinamide adenine dinucleotide phosphate (NADPH), superoxide dismutase (from bovine erythrocytes; SOD), xanthine oxidase, α-naphthylamine, sulfanilic acid, xanthine, catalase, ascorbic acid, and 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald®) were purchased from Sigma-Aldrich Chemical. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin–EDTA, and phosphate-buffered saline (PBS) were obtained from Gibco BRL.

Preparation of RVS

Rhus verniciflua Stokes (RVS) was purchased from Jeil Herbal drug store (Seoul, South Korea). A voucher specimen (KHU 07-10) was deposited at CPMDRC, Kyunghee University, South Korea. Two hundred grams of RVS was boiled twice for 3 h with 21 of water at 95 °C according to Korean patent (No. 0504160). The extract was concentrated in a vacuum evaporator and lyophilized. The yield was 13.5%.

Cell culture

Renal epithelial cells of the Madin–Darby Canine kidney (MDCK) line, type I, derived from the distal nephron were a generous gift of Carl Verkoelen (Erasmus University, Rotterdam, The Netherlands). The cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 25 mmol/l glucose at 37 °C in a humidified atmosphere containing 5% CO_2. The CT-26 mouse colon carcinoma cells were cultured in DMEM supplemented with 10% FBS and grown at 37 °C in a humidified atmosphere containing 5% CO_2. The DMEM was supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin.

Cytotoxicity assay

Cell respiration (an indicator of cell viability) was determined by measuring the mitochondrial-dependent reduction of MTT to formazan as previously described (Gerlier and Thomasset, 1986). Briefly, cells (3×10^3) were seeded onto the wells containing 100 μl of DMEM in 96-well plates and stabilized for 24 h. To evaluate the