Anti-apoptotic Effect of Bojungbangam-tang Ethanol Extract on Cisplatin-Induced Apoptosis in Rat Mesangial Cells

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Cisplatin is a anti-neoplastic agent which is commonly used for the treatment of solid tumor. Cisplatin activates multiple signal transduction pathways involved in the stress-induced apoptosis in a variety of cell types. Cytotoxicity of cisplatin was detected in rat mesangial cells and the value of IC_{50} is about 20 μM. The treatment of cisplatin to rat mesangial cells showed the apoptotic bodies and DNA fragmentation. The activation of caspase-3, -8, and -9 and proteolytic cleavage of PARP were observed in the rat mesangial cells treated time-dependently with cisplatin. The activation of ERK, p38 and JNK was also observed in the apoptosis induced by cisplatin in rat mesangial cells. The ethanol extract of Bojungbangam-tang (EBJT), a new herbal prescription composed of nine crude drugs, inhibited cisplatin-induced apoptosis in rat mesangial cells. EBJT reduced sub-G1 peak (apoptotic peak) in cisplatin-treated rat mesangial cells. The cisplatin-induced ERK and JNK activation in rat mesangial cells were blocked by EBJT, but EBJT had no effect on p38 activation. Taken together, these results can suggest that EBJT prevents cisplatin-induced apoptotic cell death in rat mesangial cells through inhibition of ERK and JNK activation.

Key words : Apoptosis, Cisplatin, Rat Mesangial Cells, Bojungbangam-tang

Introduction

Cisplatin (cis-diamminedichloroplatinum[Ⅱ]) is a potent inducer of growth arrest and/or apoptosis in most cell types and is among the most effective and widely used cheomtherapeutic agents employed for treatment of human cancers[1]. However, its clinical use is limited by its nephrotoxicity, leading to acute renal failure[2,3]. The cytotoxicity of cisplatin is primarily ascribed to its interaction with nucleophilic N7 sites of purine bases in DNA to form DNA-protein and DNA-DNA interstrand and intrastrand crosslinks[4]. The DNA adducts formed by the interaction of cisplatin with DNA activates several signal transduction pathways, including those controlled by mitogen-activated protein kinases (MAPKs), which lead to induction of the apoptotic death of tumor cells[5]. MAPKs are serine/threonine kinase involved in the regulation of various cellular responses, such as cell proliferation, differentiation and apoptosis[6,7]. Three major mammalian MAPK subfamilies have been described: the extracellular signal-regulated kinases (ERK), the c-Jun N-terminal kinases (JNK), and the p38 kinase. Each MAPKs are activated through a specific phosphorylation cascade. The ERK pathway is mainly activated by mitogens and growth factors, and plays a major role in the regulation of cell growth survival and differentiation[8,9]. In contrast, the JNK and p38 pathways are activated in response to chemicals and environmental stress and the activation is frequently associated with an induction of apoptosis[9,10].

Bojungbangam-tang, containing nine crude herbs, is partly based on the Jeongwoneum. It consists of six herbs of Jeongwoneum and another three herbs such as Agaricus mushroom as a major antitumor ingredient, Citri Pericarpium for circulating Qi and Angelicae giantis Radix for supplementing blood as auxiliary constituents. Bojungbangam-tang has been often used for effective treatment of neoplastic patients at Kyunghee Oriental Hospital in Korea.

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In previous study[11], Bojungbangam-tang showed antimetastatic and immunomodulating activities on liver metastasis. Thus, Bojungbangam-tag has been developed for protecting side effects such as indigestion, diarrhea and leukopenia induced by chemotherapy and also for preventing metastasis by the enhancement of immunological functions.

In present study, we examined the role of the MAPKs signaling pathway in regulating cisplatin-induced apoptosis in rat mesangial cells and the anti-apoptotic effect of Bojungbangam-tang on cisplatin-induced apoptosis in conection with MAPKs signaling pathway.

Materials and Methods

1. Reagents and antibodies

Cisplatin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromid (MTT), 4,6-diamidino-2-phenylindole (DAPI), propidium iodine (PI), Ribonuclease A (RNase A), and protease inhibitor cocktail were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). N-Acetyl-Asp-Glu-Val-Asp-pNA (Ac-DEVD-pNA), N-Acetyl-Ile-Glu-Thr-Asp-pNA (Ac-IETD-pNA), N-Acetyl-Leu-Glu-His-Asp-pNA (Ac-LEHD-pNA), PD98059, SB203580, and SP600125 were purchased from Calbiochem (California, USA). Anti-procaspase-3 antibody was purchased from Santa Cruz Biotechnology, INC. (Santa Cruz, CA, USA). Anti-PARP, anti-ERK, anti-phospho-ERK, anti-p38, anti-phospho-p38, anti-JNK, and anti-phospho-JNK antibodies were purchased from Cell Signaling Technology INC. (Beverly, MA, USA).

2. Preparation of Bojungbangam-tang

Bojungbangam-tang is composed of nine crude drugs as shown in Tabel 1. The ethanol extract (EBJT) was prepared as follows: The nine dried and pulverized medicinal herbs were mixed together and each 360g-batch was soaked with ethanol (3 liters x 3 changes) at room temperature for 3 days. The extract was evaporated and lyophilized to produce 44 g of powder (yield, 12.2%). Lyophilized extract was dissolved in DMSO at a concentration 200 ㎎/㎖ and stored at -20℃ and diluted in cell culture medium before use.

3. Cell culture

Immortalized rat mesangial cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in Dulbecco's Modified Eagle's medium (Invitrogen, Burlington, ON, Canada) supplemented with 0.4 ㎎/㎖ G418, 15% fetal bovine serum (FBS, Invitrogen, Burlington, ON, Canada), 100 U/㎖ penicillin, 100 ㎍/㎖ streptomycin at 37℃ in humidified 95% air/5% CO_2 incubator. Cells were propagated for at least five passages in medium supplemented with G418 to verify stable growth characteristics and morphological features, the subsequent cells were cultured on culture plates in DMEM containing 15% FBS, 100 U/㎖ penicillin, 100 ㎍/㎖. When the cells reached confluence, subcultures were prepared using 0.25% trypsin-0.03% EDTA solution. Cells were plated in cell culture dishes (or plates) 1 day prior to cisplatin treatment.

4. Determination of cell viability

Cell viability was determined using the MTT. The MTT assay is based on the reduction of MTT to formazan by the mitochondrial enzyme succinate dehydrogenase. Exponentially growing cells were seeded into a 12 well plate at 5×10^4 cells/well. After preincubation for 24 h, cells were treated with increasing concentrations of cisplatin or/and EBJT for 24 h or 48 h. After the incubation period, culture medium was removed and 500 ㎕ of MTT (0.5 ㎎/㎖ in PBS) solution was added to each wells and the cells were incubated for 4 h at 37℃. The supernatant was removed and 500 ㎕ of DMSO was added to each well to solubilize the water-insoluble purple formazan crystals. The amount of formazan was determined at 570 ㎚ using SpectraMAX 250 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). Percent of the cell viability was calculated using the equation: (mean OD of treated cells/mean OD of control cells) × 100.

5. DNA fragmentation analysis

DNA was purified with the Wizard®Genomic DNA Purification Kit (Promega, Madison, USA). Briefly, rat mesangial cells (5×10^5 cells/dish) in 100-㎜ culture dishes were treated with cisplatin (20 μM) for the indicated times, then harvested using cell scraper and washed 2× with ice-cold PBS, and DNA was purified according to some modified manufacturer's protocol and rehydrated in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) by incubating overnight at 4℃. DNA was analyzed after separation by gel electrophoresis (1.2% agarose gel contained 0.5 ㎍/㎖ of ethidium bromide at 100 V for 2 h). DNA bands were visualized under UV transilluminator.

6. DAPI staining

The rat mesangial cells were cultured in 35-㎜ culture dishes at 1.5×10^4 cells/dish, then treated with cisplatin (20 μM) for 24 or 48 h. Cells were washed with DAPI-methanol (1 ㎍/㎖), and then stained with DAPI-methanol for 15 min at 37℃. After, stained cells were washed once with methanol and observed under a fluorescence microscope. Apoptotic cells were identified by features characteristic of apoptosis (e.g. nuclear condensation,

formation of membrane blebs and apoptotic bodies)

7. Caspase activity assay

Cells were resuspended in lysis buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 1mM DTT, 0.1 mM EDTA) and lysed by freezing/thawing. Cells lysates were obtained after centrifugation (10,000 rpm) for 1 min at 4℃. Caspase activity assay was performed following the manuufacturer's instructions. In brief, the whole reaction contained 10 ㎕ cell lysates (30 ㎍ total protein), 88 ㎕ reaction buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 10mM DTT, 0.1 mM EDTA, 10% glycerol), and 2 ㎕ fluorogenic Ac-DEVD-pNA (caspase-3), Ac-IETD-pNA (caspase-8) or Ac-LEHD-pNA (caspase-9) substrate (200 ㎛M final concentration). Samples were incubated for 3 h at 37℃ and enzyme-catalyzed release of p-nitroanilide was monitored at 405 ㎚ in SpectraMAX 250 microplate spectrophotometer.

8. Western blot analysis

Cells were washed with ice-cold phosphate-buffered saline (PBS) and gently resuspend in ice-cold lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% Sodium deoxycholate, 0.1% SDS, 1 mM Na_3VO_4) with freshly added 1% protease inhibitor cocktail, incubated on ice for 30 min. Cell lysates were centrifuged at 14,000 ×g for 15 min at 4℃, and the protein concentration was determined using a Bradford assay. Samples containing 30 ㎍ of total protein were resolved by a 7.5%, 10% or 12% SDS-PAGE gel, and transferred onto a nitorcellulose membrane for 3 hr at 40V. The membranes were blocked with Tris-buffered saline with Tween-20 (20 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween-20) containing 5% milk and probred with different antibodies. Immunoreactivity was detected using either anti-rabbit or anti-mouse peroxidase-conjugated secondary immunoglobulin G antibody followed by SuperSignal West Pico Chemiluminescent (Pierce, Rockford, IL, USA).

9. Flow cytometric analysis

Cells were preincubated in 100-㎜ culture dishes and treated with cisplatin or/and EBJT. After 48 h, attached and floating cells were pooled, pelleted by centrifugation, washed in PBS, and fixed with ice-cold 70% ethanol at 4℃ overnight. Cells were washed with PBS, resuspended in 1 ㎖ of PI (10 ㎍/㎖) solution containg 100 ㎍/㎖ RNase A, and incubated for 1 h at 37℃ in the dark. DNA contents were analyzed using fluorescence-activated cell sorting (FACS) Calibur (BD Biosciences, CA, USA). Apoptotic cells were quantified on the PI histogram as a hypodiploid peak (Sub-G1). Cell cycle analysis was determined DNA content from fixed cells stained with PI. The percent of cells in sub-G1 was quantified using the CellQuest Pro (BD Biosciences, CA, USA) software.

Table 1. The botanical origins and ration of crude herbs of Bojungbangam-tang

Crude herbs	Botancial origin	Place	Weight (g)	Specimen No.
Agricus mushroom	Agaricus blazei Murill	Korea	40	GSM011
Atractylodis Macrocephalae Rhizoma	Atractylodes macrocephala Koidz	China	12	GSM012
Ginseng Radix	Panax ginseng C.A. Meyer	Korea	20	GSM013
Polyporus	Polypours umbellatus (PERS.) Fries	China	12	GSM014
Citri Pericarpium	Citrus unshiu Markovich	Korea	8	GSM015
Astragali Radix	Astragalus membranaceus Bunge	Korea	8	GSM016
Dioscoreae Rhizoma	Dioscorea japonica Thunb	Korea	8	GSM017
Angelicae giantis Radix	Angelica gigas Nakai	Korea	8	GSM018
Glycyrrhizae Radix	Glycyrrhiza uralensis Fisch	China	4	GSM019

Results

1. Induction of apoptotic cell death in rat mesangial cells treated with cisplatin

To evaluate the effects of cisplatin on the cell viability of rat mesangial cells, the cells were treated with series of concentrations of cisplatin from 20-100 μM for 24 h and 48 h. Cell viability was evaluated using the MTT assay. As shown in Fig. 1A, cisplatin decreased the viability of rat mesangial cells in dose-dependent manner. After 48 h of treatment, at 20 μM, cisplatin caused nearly a 50% inhibition of cell growth compared to the control. This concentration was used in all further experiments.

DAPI staining was used to detect changes characteristic to the apoptotic nuclear morphology. The dye is a specific DNA-binding fluorochrome and under the fluorescence microscope it exhibits a blue fluorescence. The bright blue apoptotic nuclei were readily identified by their condensed chromatin and apoptotic bodies. rat mesangial cells were treated for 24 or 48 h with concentration of 20 μM cisplatin. The apoptotic cells were detected after 24 h of incubation with cisplatin. As shown in Fig. 1B, along with the appearance of elongated cells, disintegrated cells, as evidenced by apoptotic bodies, and cells with condensed nuclear chromatin appeared in response to cisplatin treatment. The biochemical hallmark of apoptosis in degradation of DNA by endogenous DNase, which cut the internucleosomal regions into double-stranded DNA fragments of 180~200 base pairs. We examined the effects of cisplatin on the internucleosomal DNA fragmentation in rat mesangial cells treated for 12, 24, 36, or 48 h with 20 μM cisplatin by agarose gel electrophoresis. A ladder pattern of

internucleosomal DNA fragmentation was observed time-dependent when cisplatin was applied to the rat mesangial cells. As shown in Fig. 1C, rat mesangial induced DNA fragmentation after treatment for 24 h. Efficient induction for apoptosis was observed 24 h after the treatment.

Caspase family plays key roles in the execution of apoptotic cell death. The caspase-3, -8 and -9 are the most important cell executioners for apoptosis. We observed the proteolytic activation of procaspase-3, -8 and -9 induced by the cisplatin. Caspase activity assay showed that cisplatin-treatment induced time-dependent activation of caspase-3, -8 and -9. First significant activation of caspases could be detected 24 h after treatment (Fig. 2A). To confirm that the proteolytic-cleavage and activation of procaspase-3, procaspase-3 and PARP have been western blot analysis. Cisplatin induced time-dependent cleavage of procaspase-3 (Fig. 2B). Activation of caspase-3 lead to the cleavage of a number of proteins, one of which is poly(ADP-ribose) polymerase (PARP). Although PARP is not essential for cell viability, the cleavage of PARP is another hallmark of apoptosis. As shown in Fig. 2B, treatment of HL-60 cells with various times of cisplatin induced and time-dependent proteolytic cleavage of 116 kDa PARP with accumulation of the 89 kDa cleaved products. These results indicate that cisplatin-induced apoptotic cell death in rat mesangial cells involves activaiton of caspase-3, caspase-8 and caspase-9.

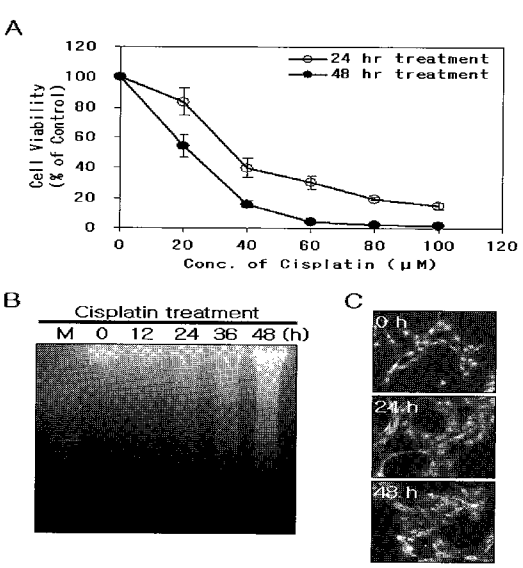

Fig. 1. Effect of cisplatin on cytotoxicity, DNA fragmentation, and morphology in rat mesangial cells. (A) The cells were treated with various concentrations of cisplatin for 24 h or 48 h and the cells were tested for viability by MTT assay. Value are means ± SD, N = 3 (B) The cells were treated with 20 μM of cisplatin for 0, 12, 24, and 36 h. DNA was extracted, then separated by agarose gel (contained EtBr) electrophoresis, and visualized under UV light. M, 100 bp DNA ladder marker. (C) The cells were treated with 20 μM of cisplatin for 0, 24, and 48 h. After, cells were stained with DAPI-MeOH and observed under a fluorescence microscope.

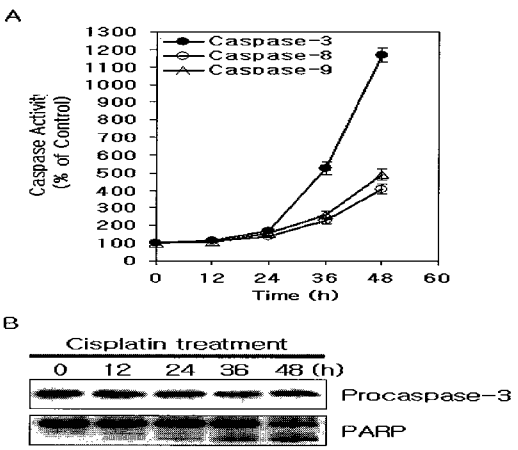

Fig. 2. Effect of cisplatin on activation of caspase-3, -8 and -9, proteolytic cleavage of PARP in rat mesangial cells. The cells were treated with Cisplatin (20 μM) for 12, 24, 36 and 48 h. (A) Caspase-3, -8, and -9 activities were measured with AC-DEVD-pNA, AC-IETD-pNA, and AC-LEHD-pNA respectively. The activation was measured as the increase in activity (%) with respect to control cells (100%). Value are means ± SD, N = 3. (B) Whole cell lysates were subjected to SDS-PAGE followed by Western blot analysis with an anti-caspase-3 and anti-PARP antibodies.

2. Activation of MAPKs in cisplatin-induced apoptosis in rat mesangial cells

MAPK signaling pathways have been shown to play an important role in the regulation of apoptosis. To clarify the involvement of MAPKs in cisplatin-induced apoptotic cell death of rat mesangial cells, we examined the effects of cisplatin on the activation of ERK, p38 and JNK. ERK, p38 and JNK were activated time-dependently in rat mesangial cells after cisplatin treatment (Fig. 3). The activation of ERK, p38 and JNK were markedly increased 24 h after cisplatin treatment.

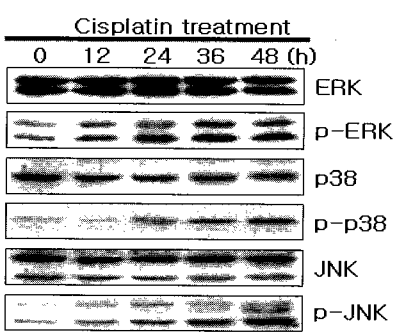

Fig. 3. Effect of cisplatin on activation of ERK, p38 and JNK in rat mesangial cells. The cells were treated with cisplatin (20 μM) for 12, 24, 36 and 48 h. Cells were the lysed, and the supernatants were subjected to Western blot analysis using anti-ERK, anti-p-ERK, anti-p38, anti-p-p38, anti-JNK and anti-p-JNK antibodies.

To confirm whether activation of ERK, p38 and JNK are involved in the cisplatin-induced apoptotic cell death, we examined the effect of MAPKs inhibitors on cisplatin-induced apoptosis in rat mesangial cells through caspase-3 activity. Rat mesangial cells were incubated with cisplatin after pretreated

with MAPKs inhibitors. PD98059 (ERK inhibitor), SB203580 (p38 inhibitor) and SP600125 (JNK inhibitor) decreased the caspase-3 activity (Fig. 4) in cisplatin-treated cells. In particular, inhibition of ERK (40 μM PD98059) and p38 (20 μM SB203580) were markedly decreased caspase-3 activity (339.3±23.38% and 235.1±24.28%, respectively versus cisplatin alone). These results indicate that ERK, p38 and JNK may contribute to cisplatin-induced apoptotic cell death in rat mesangial cells.

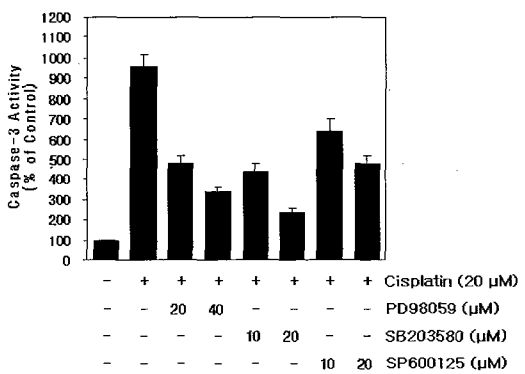

Fig. 4. Effect of ERK, p38 and JNK inhibitors on cisplatin-induced apoptosis in rat mesangial cells. The cells were preincubated with each inhibitor (ERK inhibitor, PD98059; p38 inhibitor, SB203580; JNK inhibitor, SP60125) for 3 hr, and then treated with 20 μM cisplatin for 48 hr. The caspase-3 activity was measured as the increase in activity (%) with respect to control cells (100%). Value are means ± SD, N = 3.

3. Anti-apoptotic effect of EBJT through inhibition of ERK and JNK activation on cisplatin-induced apoptosis in rat mesangial cells

Cisplatin is frequently used for the treatment of solid tumors, but nephrotoxicity for renal tubular or glomerular cells in the most common adverse effect limiting its clinical use. To evaluate the protective effect of EBJT against cisplatin-induced nephrotoxicity, we examined the effect of EBJT on cisplatin-induced apoptotic cell death in rat mesangial cells. Rat mesangial cells were incubated with cisplatin after pretreated with various concentration of EBJT. EBJT did not reduce cisplatin-induced cytotoxicity in rat mesangial cells (Fig. 5A). EBJT was also exhibited the little cytotoxicity in rat mesangial cells. We then examined the degree of reduced apoptotic cells in rat mesangial cells treated with combination of cisplatin and EBJT. Apoptotic cell was determined by measuring the sub-G1 peak (apoptotic peak) of PI stained cells with flow cytometry. Fig. 5B shows that the percent of apoptotic cells reduced to 28.1±2.60%, 24.2±2.76%, 23.3±2.23%, and 19.6±2.00% after treatment with combination cisplatin and 50, 100, 150 and 200 μg/mℓ EBJT, respectively, versus 31.1±2.35% for cisplatin alone. Cisplatin induces G2/M phase arrest in cell cycle progression. However, EBJT did not prevent cisplatin-induced G2/M phase arrest (Fig 5B), which may be cause of cell proliferation inhibition in rat mesangial cells treated with combination of cisplatin and EBJT.

Next, we examined the effect of EBJT on cisplatin-induced activation of three MAPKs, ERK, p38 and JNK in rat mesangial cells. Cisplatin-induced ERK and JNK activation were markedly reduced by EBJT in dose-dependent manner, but p38 activation was not changed (Fig. 5C). These resluts indicate that anti-apoptotic effect of EBJT on cisplatin-induced apoptosis in rat mesangial cells mediates through inactivation of ERK and JNK, but cell proliferation not increase because cisplatin-induced G2/M phase arrest.

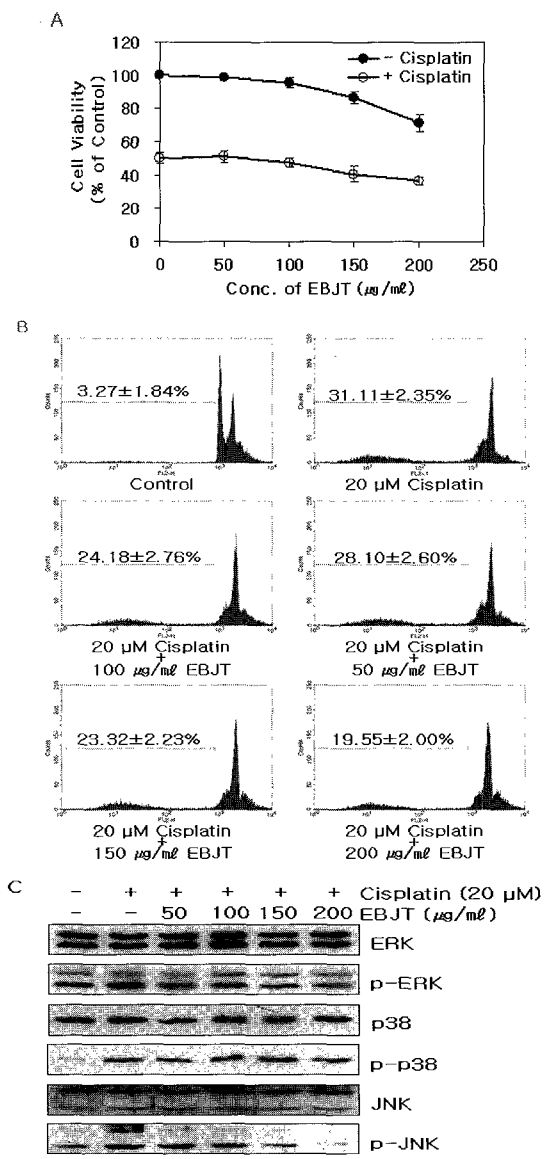

Fig. 5. Effect of EBJT on Cisplatin-induced apoptosis in rat mesangial cells. The cells were preincubated with 50, 100, 150 and 200 μg/ml of EBJT for 3 hr, and then treated with 20 μM cisplatin for 48 hr. (A) cell viability was determined by MTT assay. Value are means ± SD, N = 3. (B) The cells were fixed and stained with PI and the DNA content was analyzed by flow cytometry. (C) MAPKs activation was determined by Western blot analysis using anti-p-ERK, anti-p-p38 and anti-p-JNK antibodies.

Discussion

Apoptosis is mainly brought about by activation of caspases, a protease family with unique substrate selectivity. It is known that caspase-3, which is the main executioner caspase, can be activated by caspase-8 and/or caspase-9[12]. It depends upon the apoptosis-inducing stimulus whether caspase-3 is activiated by both/either caspase. As shown in Fig. 2, cisplatin have increased the caspase-3, caspase-8, and caspase-9, but how cisplatin activates caspase-3 is not clear. Because activation of caspase-3 mediated by two pathways consisting of the initiator caspases, caspase-8 or caspase-9. Caspase-8 can activate apoptotic pathways involving effector caspases through mitochondria-dependent or-independent pathways. In the mitochondria-independent pathway, caspase-8 can directly activate caspase-3, but in the mithochondria-dependent, which involves the release of cytochrome c from mitochondria, activate caspase-3 through induction of caspase-9[13,14]. Apoptotic pathway of rat mesangial cells by cisplatin is not yet clear. We are need of further studies that examine the pathway of apoptotic cell death by cisplatin.

The importance of MAPK signaling pathways in regulating apoptosis during condition of stress has been widely investigated. The activation of ERK pathway is thought to mediate survival signals that counteract pro-apoptotic effects associated with JNK and p389[15,16]. However, in present study, we observed that the ERK activation mediates cell death, not cell survival in cisplatin-treated rat mesangial cells (Fig. 3). The cisplatin-induced apoptosis was also prevented by PD98059, inhibitor of ERK (Fig. 4). These results are at variance with studies on ovarian cancer cells in which the cisplatin-induced ERK activation increased cells in which the cisplatin-induced ERK activation increased cell survival, not cell death[17-19]. Recent studies on renal epithelial cells have shown that cisplatin induces apoptosis through a mechanism independent of ERK1/2 activation[20,21]. However, other studies have reported that the ERK1/2 activation in involved in the cisplatin induction of apoptosis in primary cultured renal proximal tubular, OK renal epithelial, Hela, A549 cells[20,22,23]. The role of ERK pathway in cisplatin-induced apoptosis may vary with cell types, drug concentration and the extent of ERK activity[24]. Several studies have provided evidence indicating that the p38 and JNK pathways contributes to cisplatin-induced apoptosis[21,25-28]. We also showed that the activation of p38 and JNK were markedly increased in cisplatin-treated rat mesangial cells (Fig. 3 and 4). Thus, the activation of all three MAPKs (ERK, p38 and JNK) play an important role in the cisplatin-induced apoptosis.

Herbal prescriptions have been recognized as potentially valid by the scientific medical establishment, and their use has been increasing. Since tradition herbal prescriptions are generally prepared from combinations of many crude drugs on the basis of oriental prescriptions and herbalogy[11]. Bojungbangam-tag has been developed for protecting side effects by cancer chemotherapy and also for preventing metastasis and angiogenesis. In the present study, we showed that anti-apoptotic effect of EBJT on cisplatin-induced apoptosis in rat mesangial cells is mediated by inhibition of ERK and JNK activation, but not of p38 activation (Fig. 5C). ERK and p38 participate in the regulation of the G2/M transition of the cell cycle. ERK have been shown to be required for normal G2/M progression and DNA damage-induced G2/M arrest[29,30]. p38 has also been shown to be involved in the G2/M DNA damage cell cycle checkpoint[31,32]. EBJT did not increase cell proliferation, because it did not reduce p38 activation related to cisplatin-induced G2/M phase arrest.

In conclusion, cisplatin-induced apoptotic cell death in rat mesangial cells was associated with caspase-3, caspase-8, caspase-9, and activation of ERK, p38 and JNK. EBJT prevented cisplatin-induced apoptotic cell death in rat mesangial cells through inhibition of ERK and JNK activation, but did not prevent cisplatin-induced G2/M phase arrest because activation of p38 by cisplatin. Taken together, EBJT showed anti-apoptotic effect through inhibition of ERK and JNK activation in cisplatin-treated rat mesangial cells. However, cell proliferation did not increase because EBJT did not reduce p38 activation related to cisplatin-induced G2/M phase arrest.

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