Protective Effect of Puerariae radix Against Ethanol-induced Apoptosis on Human Neuroblastoma Cell Line SK-N-MC

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To investigate whether Puerariae radix (PR) possesses protective effect against ethanol (EtOH)-induced apoptosis in the central nervous system, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, flow cytometric analysis, DNA fragmentation assay, and reverse transcription-polymerase chain reaction (RT-PCR) were performed on human neuroblastoma cell line SK-N-MC. Morphological and biochemical analyses demonstrated that SK-N-MC cells treated with EtOH exhibit classical apoptotic features. On the other hand, cells pre-treated with PR prior to EtOH exposure showed decreased occurrence of classical apoptotic features. In addition, it was shown that PR pre-treatment inhibits EtOH-induced increases in the levels of mRNA expression of bax and caspase-3, while it further enhances the level of bcl-2 expression. These results suggest that PR may exert protective effects against EtOH-induced apoptosis in human neuroblastoma cells.

Key words : Puerariae radix, ethanol, apoptosis, neuroblastoma

Introduction

Puerariae radix (PR) has been used as an antipyretic, antidiarrhetic, diaphoretic, and antiemetic agent. In Oriental medicine, medications based on PR were found to be useful in the treatment of ethanol (EtOH)-related problems, both as an amethystic (anti-intoxication) agent, and as an antidipsotropic (anti-drinking) agent[1], and in the treatment of various liver diseases caused by alcohol abuse[2]. PR contains daidzin, puerarin, daidzein, geinstein, and biochanin[3]; of these, daidzin and daidzein, the major active constituents of PR extracts, are known to suppress EtOH intake and to selectively inhibit human aldehyde dehydrogenase[4,5]. The central nervous system (CNS) is one of the major targets of the toxic effects of EtOH, and EtOH consumption during the period of neural development leads to substantial neuronal loss in several regions of the brain[6]. However, the exact mechanism behind the indicator of brain damage by EtOH is still elusive. In recent years, the role of EtOH as an inducer of apoptosis has been described in astroglia[7], neuroblastoma[8], hepatocytes[9], and thymocytes[10]. In addition, Ikonomidou et al[11] have reported that EtOH induces apoptotic neurodegeneration in the developing rat brain. Apoptosis, also known as programmed cell death, has an important role in neural homeostasis and is form of death in which the dying cells regulate the process by controlling the expression of certain genes. Two important families of genes involved in apoptotic cell death are bcl genes[12] and a class of cysteine proteases known as caspases[13]. The bcl family of genes can be separated into two functionally distinct groups, anti-and pro-apoptotic genes. Bcl-2, an anti-apoptotic gene, was the first of the family to be cloned; it is expressed in the CNS, and protects cell death in the developing brain[14]. A pro-apoptotic gene of the family, bax, is expressed abundantly and selectively in neurons and promotes cell death, while bcl-2 blocks cell death[15]. The caspases stand in a crucial step of the apoptotic process and are expressed in many mammalian cells[16]. Of particular interest is caspase-3, the most widely studied member of the caspase family[16] and one of the key executioners of apoptosis, being responsible either partially or totally for the proteolytic cleavage of many proteins[17].

Because of the involvement of apoptosis in the process of EtOH-induced cell death in the CNS, it appears logical that a drug which inhibits apoptosis may be of use in reducing EtOH-induced CNS damage. However, it has not yet been reported whether PR possesses such protective effects against EtOH-induced apoptosis in the CNS.

In this study, the inhibitory effects of the aqueous extracts of PR on EtOH-induced death of cells of the neuroblastoma cell line SK-N-MC were investigated via using

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MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, flow cytometric analysis, DNA fragmentation assay, and reverse transcription-polymerase chain reaction (RT-PCR).

Materials and Methods

1. Drugs and reagents

Puerariae radix(PR) was obtained from Kyung-Dong Market place (Seoul, Korea). After washing, the PR was immersed in cold water for 12 hr, and aqueous extracts from the PR was made using a rotatory evaporator. Ethanol was purchased from Merck (Darmstadt, Germany). Propidium iodide (PI) and paraformaldehyde (PFA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The MTT assay kit was purchased from Boehringer Mannheim GmBH (Mannheim, Germany). The DNA fragmentation assay kit was obtained from TaKaRa (Shiga, Japan).

2. Cell culture

The human neuroblastoma cell line SK-N-MC was purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL) at 37℃ in 5% CO_2, 95% O_2 in a humidified cell incubator, and the medium was changed every 2 days.

3. MTT cytotoxicity assay

Cell viability was determined using an MTT assay kit as per the manufacturer's protocol. In order to determine the cytotoxicity of EtOH, cells were treated with EtOH at concentrations of 10 mM, 50 mM, 100 mM, and 500 mM for 3 hr. To observe the effect of PR on cells, cells were treated with PR at concentrations of 0.1 mg/ml, 1 mg/ml, and 10 mg/ml for 24 hr. For analysis of the protective effects of PR against cell death induced by EtOH, cells were pre-treated with PR at a concentration 1 mg/ml for 24 hr. After cells were treated with PR, EtOH was applied at a concentration of 100 mM for 3 hr, The control group was left untreated. Ten $\mu\ell$ of the MTT labeling reagent was added to each well, and the plates were incubated for 4 hr. The cells were then incubated in 100 $\mu\ell$ of the solubilization solution for 12 hr, and the absorbance was measured with a microtiter plate reader (Bio-Tek, Winooski, VT, USA) at a test wavelength of 595 nmwith a reference wavelength of 690 nm. The optical density (O.D.) was calculated as the difference between the absorbance at the reference wavelength and that at the test wavelength. Percent viability was calculated as (O.D. of drug-treated sample/control O.D.) x 100.

4. TUNEL assay

For in situ detection of apoptotic cells, TUNEL assay was performed using ApoTag®peroxidase in situ apoptosis detection kit. TM3 cells were cultured on 4-chamber slides (Nalge Nunc International, Naperville, IL, USA) at a density of 2 x 10^4 cells/chamber. After treatment with PR and EtOH, cells were washed with PBS and fixed in 4% paraformaldehyde for 10 min at 4℃. The fixed cells were incubated with digoxigenin-conjugated dUTP in a terminal deoxynucleotidyl transferase-catalyzed reaction for 1 hr at 37℃ in a humidified atmosphere and were then immersed in stop/wash buffer for 10 min at room temperature. The cells were then incubated with an anti-digoxigenin antibody conjugated with peroxidase for 30 min. The DNA fragments were stained using 3,3'-diaminobenzidine (Sigma Chemical Co., St. Louis, MO, USA) as the substrate for the peroxidase.

5. DAPI staining

In order to determine whether ethanol induces apoptosis, DAPI staining was performed. Cells were first cultured on 4-chamber slides. After treatment with PR and EtOH, the cells were washed twice with PBS and fixed by incubation in 4% paraformaldehyde for 30 min. Following a second washing in phosphate-buffered saline, cells were incubated in 1 μg/ml DAPI solution for 30 min in the dark. The cells were then observed through a fluorescence microscope (Zeiss, Oberkchen, Germany).

6. Flow cytometric analysis

Flow cytometric analysis was performed as previously described[18]. Briefly, after treatment with PR and EtOH, cells were collected, washed twice with PBS, and fixed with 75% ethanol in PBS at -20℃ for 1 hr. After washing twice with PBS, the cells were incubated with 100 μg/ml RNase (Sigma) and stained with 20 μg/ml PI in PBS. The stained cells were incubated for 30 min at 37℃ and were analyzed using FACScan (Becton Dickinson, San Jose, CA, USA).

7. DNA fragmentation

For detection of apoptotic DNA cleavage, DNA fragmentation assay was performed using ApopLadder EXTMDNA fragmentation assay kit (TaKaRa, Shiga, Japan). In brief, after treatment with PR and EtOH, cells were lysed with 100 $\mu\ell$ of lysis buffer. The lysate was incubated with 10 $\mu\ell$ of 10 % SDS solution containing 10 $\mu\ell$ of Enzyme A at 56℃ for 1 hr followed by treatment with 10 $\mu\ell$ of Enzyme B at 37℃ for 1 hr. This mixture was then centrifuged for 15 min after adding 70 $\mu\ell$ of precipitant and 500 $\mu\ell$of ethanol. The DNA was extracted by washing the resultant pellet in ethanol and

resuspending it in TE (Tris-EDTA) buffer. DNA fragmentation was visualized by electrophoresis in a 2% agarose gel containing ethidium bromide.

8. RNA isolation and RT-PCR

Total RNA was isolated from SK-N-MC cells using RNAzolTMB (TEL-TEST, Friendswood, TX, USA) as per the manufacturer's instructions. Two μg of RNA and 2 $\mu\ell$ of random hexamers (Promega, Madison, WI, USA) were added together, and the mixture was heated at 65℃ for 10 min. One $\mu\ell$ of AMV reverse transcriptase (Promega), 5 $\mu\ell$ of 10 mM dNTP (Promega), 1 $\mu\ell$ of RNasin (Promega), and 5 $\mu\ell$ of 10 x AMV RT buffer (Promega)were then added to the mixture, and the final volume was brought up to 50 $\mu\ell$with dimethyl pyrocarbonate (DEPC)-treated water. The reaction mixture was then incubated at 42℃for 1 hr. The primer sequences for bax, bcl-2, and caspase-3as used in the study were as reported by Sawa et al[14], Zhang et al[19] and Wang et al[20], respectively. For human bax, the primer sequences were 5'-GTGCACCAAGGTGCCGGAAC-3' (a 20-mer sense oligonucleotide starting at position 375) and 5'-TCAGCCCATCTTCTTCCAGA-3' (a 20-mer anti-sense oligonucleotide starting at position 560). For human bcl-2, the primer sequences were 5'-CGACGACTTCTCCCGCCGCTACCGC-3' (a 25-mer sense oligonucleotide starting at position 334) and 5'-AGATCATCTCTGCCTGAGTATCTT-3' (a 25-mer anti-sense oligonucleotide starting at position 628). For caspase-3, the primer sequences were 5'-CTCGGTCTGGTACAGATGTCGATG-3' (a 24-mer sense oligonucleotide starting at position 412) and 5'-GGTTAACCCGGGTAAGAATGTGCA-3'(a 24-mer anti-sense oligonucleotide starting at position 922). For cyclophilin, the internal control used in the study, the primer sequences were 5'-ACCCCACCGTGTTCTTCGAC-3'(a 20-mer sense oligonucleotide starting at position 52) and 5'-CATTTGCCATGGACAAGATG-3' (a 20-mer anti-sense oligonucleotide starting at position 332). The expected sizes of the PCR products were 205 bp (for bax), 318 bp (for bcl-2), 533 bp (for caspase-3) and 299 bp (for cyclophilin). PCR amplification was performed in a reaction volume of 40 $\mu\ell$ containing 1 $\mu\ell$ of the appropriate cDNA, 1 $\mu\ell$ of each set of primers at a concentration of 10 pM, 4 $\mu\ell$ of 10 x RT buffer, 1 $\mu\ell$ of 2.5 mM dNTP, and 2 units of Taq DNA polymerase (TaKaRa, Shiga, Japan). For bax, bcl-2 and caspase-3, the PCR procedure was carried out using a GeneAmp 9600 PCR system (Perkin Elmer, Norwalk, CT, USA) under the following conditions: initial denaturation at 94C for 5 min, followed by 40 amplication cycles, each consisting of denaturation at 94C for 30 sec, annealing at 58C for 30 sec, and extension at 72C for 30 sec, with an additional extension step at the end of the procedure at 72C for 5 min. For cyclophilin, the PCR procedure was carried out under identical conditions except that 25 amplification cycles were executed. The final amount of RT-PCR product for each of the mRNA species as calculated densitometrically using Molecular AnalystTM software version 1.4.1 (Bio-Rad, Hercules, CA, USA).

9. Statistical analysis

Statistical analysis was performed using Student's t-test and results were expressed as mean ± standard error mean (S.E.M). Differences were considered significant for $p < 0.05$.

Results

1. MTT assay for cell viability

1) Effect of EtOH on SK-N-MC cell viability

In order to find out the effects of EtOH on the SK-N-MC cell line with respect to concentration, cells were cultured with EtOH at final concentrations of 10 mM, 50 mM, 100 mM, and 500 mM for 3 hr, and MTT assay was carried out, with cells cultured in EtOH-free media as the control. The viabilities of cells incubated with EtOH at concentrations of 10 mM, 50 mM, 100 mM, and 500 mM were 71.72 ± 3.09%, 49.31 ± 2.35%, 42.76 ± 1.90% and 33.56 ± 2.32% of the control value, respectively. A trend of decreasing viability with increasing EtOH concentration was observed.

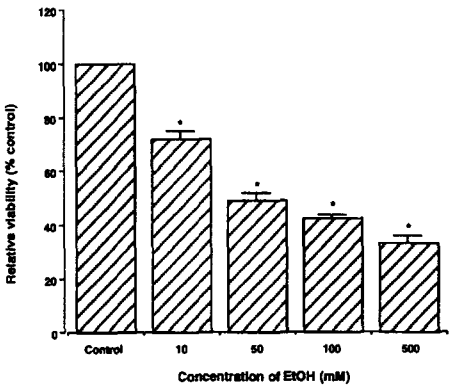

Fig. 1. Cytotoxic effects of ethanol (EtOH). SK-N-MC cells were incubated with EtOH at various concentrations (10 mM, 50 mM, 100 mM, and 500 mM) for 3 hr prior to the determination of cellular viability through MTT assay. Relative viability is shown as the percentage absorbance of the sample with respect to that of the control (% control), i.e. the untreated cells. Results are represented as mean ± standard error mean. * represents $p < 0.05$.

2) Effect of PR on SK-N-MC cell viability

To observe the cytotoxic effects of PR on the SK-N-MC cell line with respect to concentration, cells were cultured with PR at final concentrations of 0.1 mg/ml, 1 mg/ml, and 10 mg/ml, for 24 hr. As shown in figure 2, the viability of cells incubated with PR at concentrations of 0.1 mg/ml, 1 mg/ml, and 10 mg/ml were 96.53 ± 1.77%, 97.60 ± 2.43%, and 101.15 ± 2.77% of the control value, respectively.

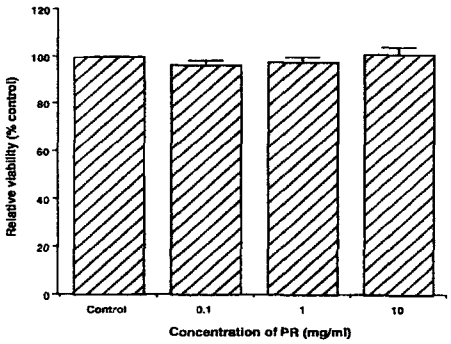

Fig. 2. Cytotoxic effects of Puerariae radix (PR). SK-N-MC cells were incubated with PR at various concentrations (0.1 mg/ml, 1 mg/ml, and 10 mg/ml) for 24 hr. Relative viability (% control) is shown as the percentage absorbance of the sample with respect to that of the control (without PR). Results are represented as mean ± standard error mean. * represents $p < 0.05$ compared with the control.

3) Protective effects of PR on EtOH-induced cytotoxicity

The viability of SK-N-MC cells pre-treated with PR before exposure to EtOH is shown in figure 3. The viability of cells treated with PR only at a concentration of 1 mg/ml for 24 hr was 95.23 ± 3.09% of the control value. The viability of cells exposed to EtOH without PR pre-treatment was 41.94 ± 1.50% of the control value. The viability of cells pre-treated with PR at a concentration of 1 mg/ml before exposure to EtOH was increased to 70.89 ± 2.56%.

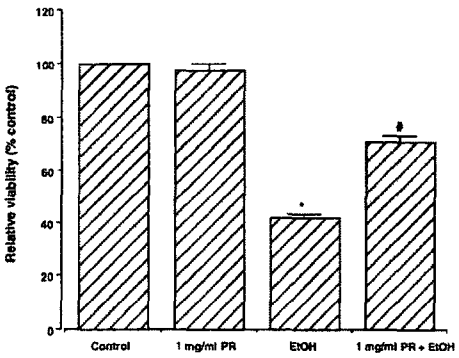

Fig. 3. Effects of Puerariae radix (PR) on cell viability. Cells were treated with PR at a concentration of 1 mg/ml for 24 hr, followed by exposure to ethanol (EtOH) at a concentration of 100 mM for 3 hr. In cultures, which were exposed to EtOH after PR pre-treatment, PR was shown to exert a protective effect against EtOH-induced apoptosis. Results are represented as mean ± standard error (bars) for two independent experiments, each with a minimum of three cultures. * represents $p < 0.05$ compared to the control, # represents $p < 0.05$ compared to the EtOH-treated group.

2. Protective effect of PR on EtOH-induced apoptosis

To observe the protective effects of PR against EtOH-induced apoptotic changes in cell morphology, cells were examined by phase-contrast microscopy. As shown in figure 4, cells treated with EtOH only were seen to have detached from the dish, with cell rounding, cytoplasmic blebbing, and irregularity in shape, while cells pre-treated with PR prior to EtOH exposure were morphologically similar to the control.

Through flow cytometric analysis, the protective effects of PR against EtOH-induced apoptosis were determined. As shown in figure 4, the population of cells in the sub-G1 phase in the EtOH group was increased from 32.21% to 60.93%, while the population was decreased with PR pre-treatment to 31.31%, a value similar to that of the control. Taken together, PR was shown to exert protective effects against EtOH-induced apoptosis.

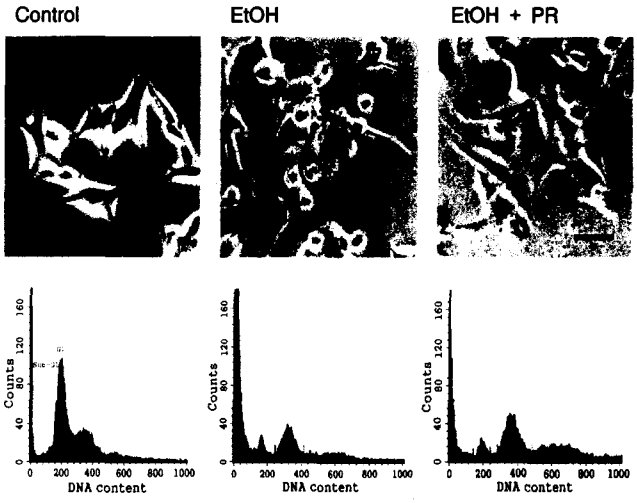

Fig. 4. Characterization of the effects of Puerariae radix (PR) on the changes in SK-N-MC cells induced by ethanol (EtOH). Cells were treated with PR at a concentration of 1 mg/ml for 24 hr, followed by exposure to 100 mM EtOH for 3 hr. Above: Phase-contrast microscopy showed cell shrinkage, irregularity in shape, and cellular detachment in the EtOH-treated cultures. these morphological characteristics were not observed in the control (untreated), and although present, with a far lower intensity in the PR pre-treated group. Scale bars represent 100 m. Below: Results of flow cytometric analysis. The fraction of cells in the sub-G1 phase was increased in the EtOH-treated cells compared to the control, but was reduced again in the PR pre-treated group, to a level comparable to that seen in the control.

3. Characterization of apoptosis via examination of DNA fragmentation

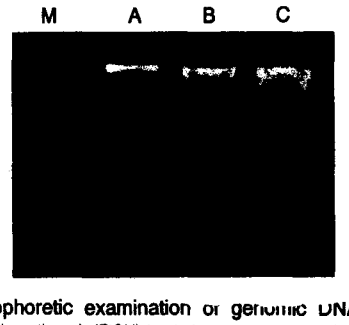

Fig. 5. Electrophoretic examination of genomic DNA of SK-N-MC cells. Cells of the ethanol (EtOH)-treated group were incubated with 100 mM EtOH for 3 hr, while Puerariae radix (PR) pre-treated cells received a 24 hr treatment with PR prior to incubation in EtOH. Genomic DNA was extracted and analyzed via electrophoresis on a 2% agarose gel containing ethidium bromide. Cells of both groups exhibited the ladder pattern characteristic of apoptosis, although this pattern was of lower intensity in the case of the PR pre-treated group. M: Marker, A: Control, B: EtOH-treated group, C: PR pre-treated group.

In order to ascertain the protective effects of PR against EtOH-induced cell death, DNA fragmentation, reflecting the endonuclease activity characteristic of apoptosis, was analyzed. As seen in figure 5, EtOH treatment resulted in the formation of

definite fragments which could be seen via electrophoresis as a characteristic ladder pattern; PR pre-treatment resulted in a significantly decreased intensity of EtOH-induced DNA laddering.

4. Effects of EtOH and PR on mRNA expression of bax, bcl-2 and caspase-3

RT-PCR analysis of the mRNA levels of bax, bcl-2, and caspase-3were performed in order to provide an estimation of the relative levels of expression of these genes. Table 1 shows the levels of mRNA expression of cyclophilin, bax, bcl-2, and caspase-3 in the SK-N-MC cells. Expression of bax and caspase-3 mRNA was increased in EtOH-treated groups, and it was reduced following PR pre-treatment. In the case of bcl-2, the indication of expression by EtOH was enhanced profoundly by PR pre-treatment. The results showed that the mRNA expression of bcl-2, bax, and caspase-3 was increased in the EtOH-treated group while that of bax and caspase-3 was decreased, and that of bcl-2 profoundly elevated in the PR pre-treated group.

Table 1. Effect of Puerariae radix (PR) on ethanol (EtOH)-induced changes in expression of bax, bcl-2 and caspase-3 genes.

Gene	control	100 mM EtOH	1 mg/ml PR +100 mM EtOH	10 mg/ml PR +100 mM EtOH
bax	1	2.67±0.67*	0.50±0.05#	0.59±0.20#
bcl-2	1	2.71±0.39*	5.193±0.79#	4.33±0.09#
caspase-3	1	14.79±3.35*	8.44±3.76#	5.37±1.23#

The mRNA levels of all three genes were measured by RT-PCR. The level of expression of bcl-2 in cells pre-treated with PR was higher than that in cells exposed to EtOH only, while the levels of expression of bax and caspase-3 were significantly lower. As the internal control, cyclophilin mRNA was also reversetranscribed and amplified. * represents $p < 0.05$ compared to the control group # represents $p < 0.05$ compared to the EtOH-only group.

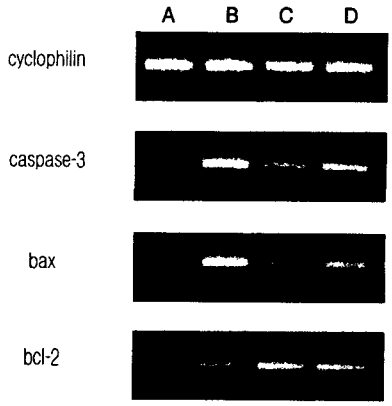

Fig. 6. Results of RT-PCR analysis of the mRNA levels of bax, bcl-2, and caspase-3. The level of expression of bcl-2 in cells treated with Puerariae radix (PR) was higher than that in the cells exposed to ethanol (EtOH), while the levels of expression of bax and caspase-3were significantly lower. As the internal control, cyclophilin mRNA was also reversetranscribed and amplified. A: Control, B: EtOH-treated group, C: PR pre-treated group.

Discussion

The purpose of the present study was to find out whether PR exerts protective effects against EtOH-induced apoptosis of the neuroblastoma cell line SK-N-MC. EtOH intake is associated with various CNS impairments; in particular, EtOH consumption during pregnancy is associated with various teratogenic effects on the fetus, one of which is deficiencies in CNS development which can lead to a constellation of behavioral and anatomical defects collectively termed fetal alcohol syndrome (FAS)[8]. However, the mechanism behind the induction of brain damage by EtOH is poorly understood. Recently, it has been demonstrated that EtOH induces apoptotic neurodegeneration in the developing rat brain[11]. Assessment of cell viability in the present study via MTT assay confirmed that EtOH has its cytotoxic effects in a dose dependent manner and the administration of PR has a protective effect against the cytotoxic actions of EtOH. From flow cytometric analysis of DNA contents using the DNA-specific dye PI, an increase in the fraction in the sub-G1 phase, which could be seen as a peak positioned close to the sub-G1 phase, was observed in the EtOH-treated group ; this observation was similar to the results reported by Holownia et al[7] from their study involving cultured rat astroglia. This increase in the sub-G1 phase fraction was reduced, to a level comparable to that seen in the control group, in the PR pre-treated group. It has been known that apoptosis involves the activation of endonucleases and that this activation results in the cleavage of genomic DNA into well-defined fragments, which appear as a characteristic ladder pattern upon agarose gel electrophoresis[21].To provide evidence supporting the involvement of a poptosis in EtOH-induced cytotoxicityand the protective action of PR against this effect, DNA fragmentation assay was performed. EtOH-treated group revealed the distinctive ladder pattern characteristic of apoptosis, similar to the results observed by Holownia et al[7] in cultured rat astroglia and those presented by Oberdoerster and Rabin[22] in their study involving rat cerebellar granule cells. On the other hand, the PR pre-treated group showed a noticeable decreases in the intensity of EtOH-induced DNA laddering. In addition, EtOH was shown to cause characteristic changes in the morphology of SK-N-MC cells and PR was seen to have a noticeable protective effect against these alterations. Under the phase-contrast microscope, cells of the PR pre-treated group appeared morphologically similar to those of the control group, but changes in appearance, including cell shrinkage, cytoplasmic condensation, and irregularity in shape, were seen in cells of the EtOH-treated group. EtOH has been reported to induce neuronal apoptosis[7-11], and understanding the molecular events triggering apoptosis is an important step toward the development of effective treatment strategies for such neurological diseases[23]. Members of the bcl-2 family of

proteins are characterized by their ability to form a complex combination of heterodimers with bax, and homodimers with itself[24], and are regulators of neuronal apoptosis[25]. When bax, the first proapoptotic homologue to be identified was overexpressed in cells, apoptotic death in response to a death signal was accelerated resulting in its designation as a death agonist. When bcl-2 was overexpressed, it heterodimerized with bax, and cell death was repressed. Presumable, the ratio of bax to bcl-2 serves to determine the susceptibility of cells apoptosis[12]. Recent studies have provided considerable new information concerning the mechanisms of bcl-2 activity within the apoptotic network. In a previous study, bcl-2 overexpression was shown to protect cells from a variety of adverse conditions, including ischemia, hypoglycemia, and oxidative stress[26], and it may be of significance that these same conditions have often been hypothesized to be mechanisms contributing to the detrimental effects of EtOH on CNS development[27]. The present results are consistent with an earlier report in which bcl-2gene expression was shown to produce increased resistance to the effects of EtOH in neuroblastoma cells, because cells of the PR pre-treated group remarkably overexpressed the gene compared to cells of the EtOH-treated group. In addition, the present data has revealed an up-regulation of bax mRNA level in cells exposed to EtOH only, while a remarkable decrease in bax mRNA level was observed in PR pre-treated cells.

One set of enzymes that appears to be modulated by the presence of bax is the caspase family[28]. Caspases, a family of cysteine proteases, are integral parts of the apoptotic pathway [17]; in particular, caspase-3 when activated, has many cellular targets that, when served and/or activated, produce the morphologic features of apoptosis. Recent reports indicate that caspases may play a role in neuronal cell death during development as well as after neuronal injury[29] Deaciuc et al[30] have reported that caspase-3 activity is significantly increased in EtOH-treated rats in vivo. The present results also showed an increase in caspase-3 mRNA level in EtOH-treated cells, while cells pre-treated with PR showed a remarkable decrease. Thus, it was shown that PR inhibits EtOH-induced overexpression of the apoptotic pathway genes, bax and caspase-3.

In the present study, it was investigated whether PR was an inhibitory influence on EtOH-induced cell death in cells of the neuroblastoma cell line SK-N-MC. Based on the results, it is suggested that PR possesses protective effects against EtOH-induced apoptosis in neuroblastoma cells.

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