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THE ROLES OF P38MAPK AND CASPASE-3 IN DADS-INDUCED APOPTOSIS IN HUMAN HEPG2 CELLS

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Abstract - The roles of p38MAPK and caspase-3 in DADS-induced apoptosis in human HepG2 cells were investigated. After the human HepG2 cells were treated with DADS, the cell viability, apoptosis and the activity changes of p38MAPK and caspase-3 were measured. The results indicated that DADS can activate p38MAPK and caspase-3. The results showed that p38MAPK and caspase-3 are involved in the process of DADS-induced apoptosis in human HepG2 cells and interact with each other.

Keywords: DADS, p38MAPK, caspase-3, inhibitor, apoptosis

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INTRODUCTION

The MAPK (mitogen-activated protein kinase) system is a cluster of serine/threonine protein kinases in cells. Activated MAPKs participate in a variety of cellular responses, including gene transcription, induction of apoptosis, maintenance of cells and regulation of the cell cycle [1-3]. p38MAPK is a key member of the MAPK family. A large number of studies have shown that the activity of p38MAPK is necessary in the apoptotic process induced by various anti-cancer drugs. Caspases play a very important role in the early stage of apoptosis as its central effectors.

Dialyl disulfide (DADS) is an oil-soluble sulfur organic compound, and a potential broad-spectrum anti-cancer drug. Studies have shown that DADS can inhibit the growth of human tumor cells, including those originating from the colon, lung, skin, breast and liver [4-8]. In this report we investigated the roles of p38MAPK and caspase-3 in the apoptotic process induced by DADS. The results showed that p38MAPK and caspase-3 are involved in DADS-induced apoptosis in human HepG2 cells and that they interact with each other.

MATERIALS AND METHODS

Materials and chemicals

DADS (80% purity) was purchased from Fluka Co. Dulbecco's modified Eagle medium (DMEM) medium, BSA and SB203580 were obtained from Sigma. Z-DEVD-FMK was purchased from CALBIOCHEM (USA) and goat horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibodies were purchased from Santa Cruz Biotech. Antibodies to p38, phospho-p38 (p-p38), caspase-3 were purchased from Cell Signaling.

Cell culture

HepG2 cells, a human hepatoma cell line, were cultured in DMEM with 10% heat-inactivated fetal bovine serum (FBS), benzylpenicillin (100kU/L) and streptomycin (100mg/L) at 37°C in an incubator containing humidified air with 5% CO_2.

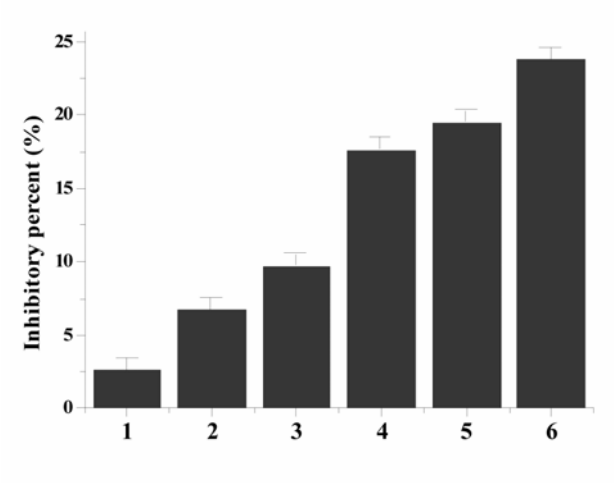


Figure 1.

Cell viability assay

Cells were seeded into 96-well plates at 1×10^4 cells per well 24 h before treatment. The cultures were then rinsed in phenol-free DMEM medium and incubated with respective test substances in phenol-free and serum-free DMEM for 24 h. In the inhibition test, the cells were treated with DADS. At the end of this time interval, 20 μl (5 mg/ml) MTT [3-(4,5dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] was added to each well, and after incubation at 37°C for 4 h the MTT solution was removed and 200 μl of dimethylsulfoxide (DMSO) was added to dissolve the crystals. The absorbance of each well at 570 nm was measured.

Flow cytometry analysis

Cells were seeded into 100-ml cell culture bottles at a density of 12×10^6 cells 24 h before treatment. The cells were treated according to the aforementioned method and incubated for 24 h. The cells were collected, adjusted to a single cell suspension and centrifuged at 800 ×g for 5 min. After discarding the supernatant, the cells were washed three times with cold PBS and fixed for 24 h with alcohol at 4°C. 1 ml cell suspension (10^6/ml), washed three times with cold PBS, treated with RNase for 30 min at 37°C, stained with PI for 30 min at 37°C in the dark, and taken for flow cytometry analysis.

Western-blotting

The cells were harvested during the logarithmic growth phase, treated as described above and incubated for 24 h. After lysis on ice for 20 min, the lysates were centrifuged at 15,000 ×g for 10 min at 4°C, the protein was collected, quantitated by the BCA method. Proteins were separated by SDS-PAGE (10%), electrotransferred to NC filters Protein expression of the cells was assessed by western blotting/ECL. Protein bands were quantified using Quantyone software.

Statistics

The data are expressed as mean ± S.D of three independent experiments and were evaluated by one-way analysis of variance (ANOVA). Significant differences were established at $P<0.05$.

RESULTS AND DISCUSSION

Cell activity

Fig. 1 shows the inhibitory effect of DADS on HepG2 cells. As shown in Fig. 1, after 24h incubation, inhibition obtained by 100 μmol/L DADS on HepG2 cells was 23.83%. After treatment with 10 μmol/L SB203580 for 30 min, the inhibition obtained by 100 μmol/L DADS was 19.45%. When treated with 10 μmol/L Z-DEVD-FMK for 30 min, the inhibition rate of 100 μmol/L DADS was 17.64% (after 24h incubation). These results suggested that the inhibitors of P38MAPK and caspase-3 both obviously decrease the inhibitory effect of DADS on HepG2 cells.

Cell apoptosis

The results from flow cytometry analysis showed that the apoptosis rates of the SB203580-DADS group and the Z-DEVD-FMK-DADS group were 18.98% and 17.45%, respectively. However, the cell apoptosis rate of DADS was 25.23% (Fig. 2). These results suggested that inhibitors of P38MAPK and caspase-3 both had the obvious effect of inhibiting cell apoptosis.

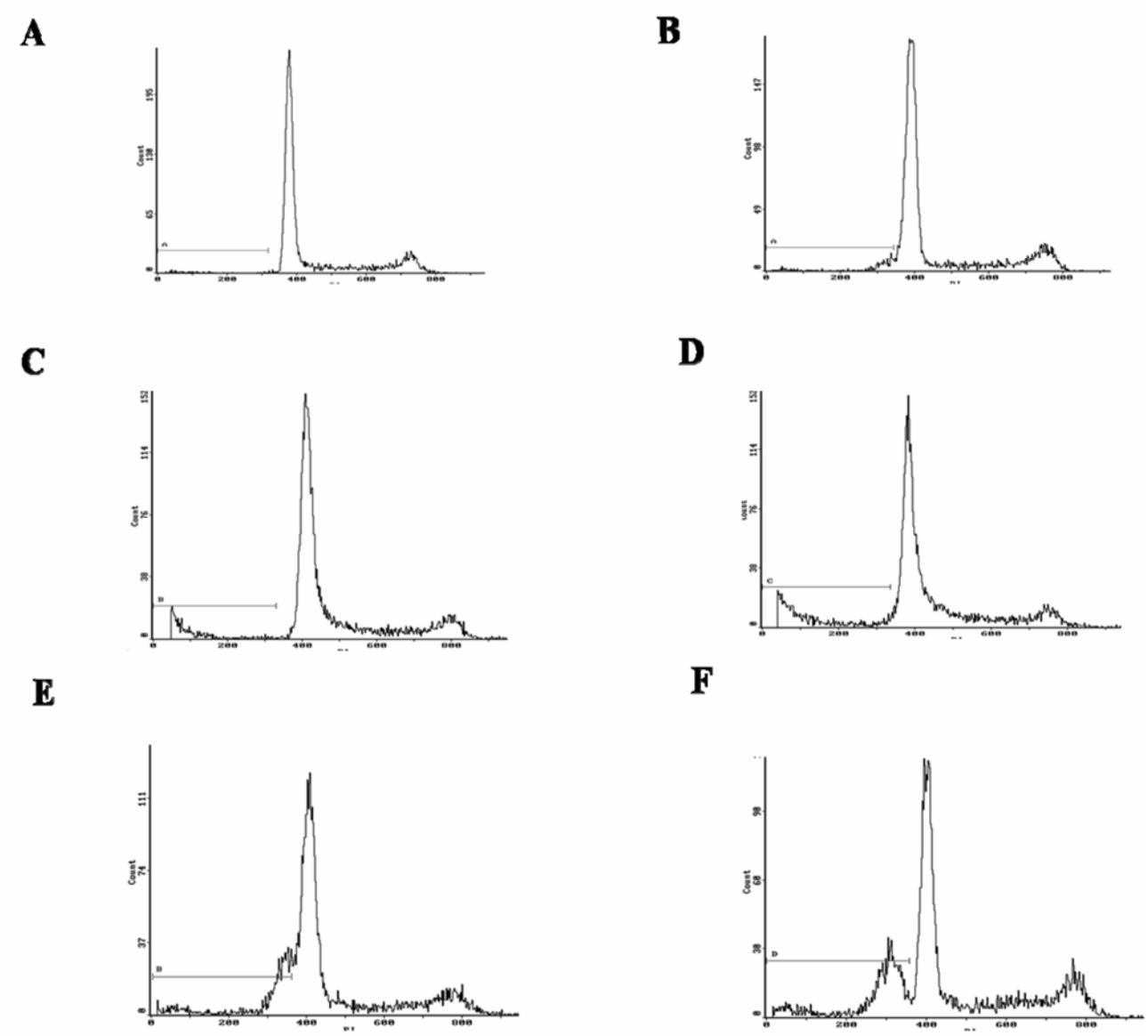
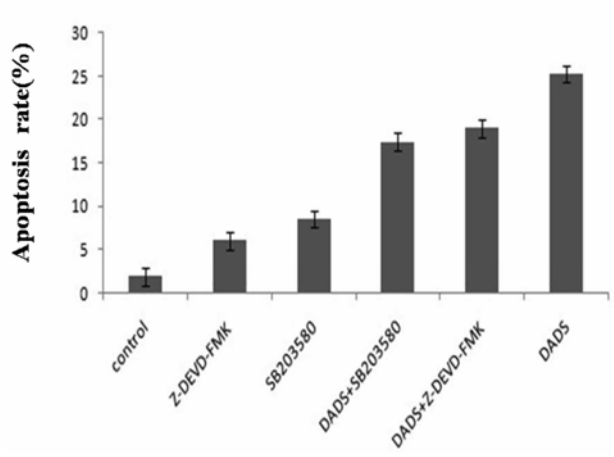


Figure 2.

Protein expression

After the treatments with various drugs for 24 h, the band corresponding to caspase-3-DADS (100 μmol/L) became thinner compared with the control, suggesting that DADS enhances the activity of caspase-3. After treatment with SB203580 (10 μmol/L) and Z-DEVD-FMK (10 μmol/L), the bands corresponding to caspase-3 became thicker compared with those treated with DADS (100 μmol/L) (Fig. 4).

Similarly, SB203580 (10 μmol/L) and Z-DEVD-FMK(10 μmol/L) had an inhibitory effect on p38 MAPK. When SB203580 (or Z-DEVD-FMK) was added to the HepG2 cells for 30 min before the DADS treatment or only SB203580 (or Z-DEVD-FMK) was added to HepG2 hepatoma cells, the P-



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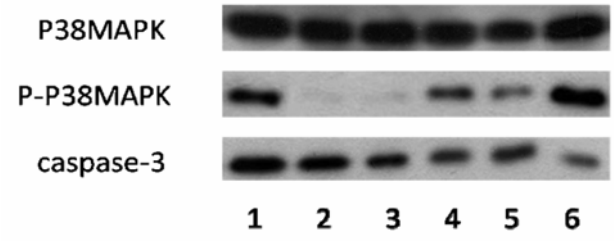

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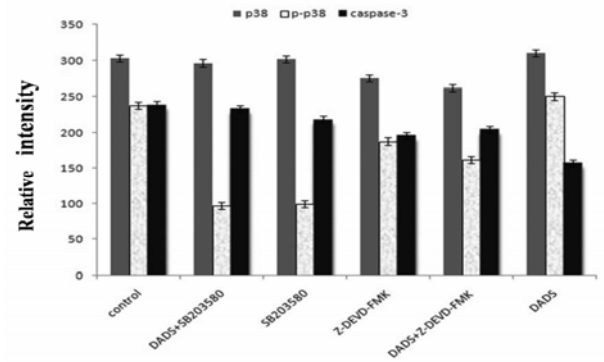

Figure 5.

p38 MAPK was markedly decreased. DADS induced activations of P-p38 MAPK, compared to DADS-treatment alone or no treatment (Fig. 4).

These results confirmed that SB203580 and Z-DEVD-FMK inhibit the activity of p38 MAPK and caspase-3, and that the inhibition of SB203580 was comparatively stronger than that of Z-DEVD-FMK (Fig. 5).

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