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The role of p38 MAPK in valproic acid induced microglia apoptosis

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ABSTRACT

Valproic acid (VPA), a widely prescribed drug for seizures and bipolar disorder, induces apoptosis in microglia, but the underlying mechanism by which microglia apoptosis in response to VPA is not yet known. In this study, we found that the mitochondrial pathway played an important role in VPA-induced apoptosis in both BV-2 microglia and mouse primary microglial cells. In addition, VPA increased the level of phospho-p38 mitogen-activated protein kinase (MAPK), but had no effects on phospho-ERK and phospho-JNK MAPKs. Moreover, p38 inhibitor SB203580 strongly inhibited VPA-induced apoptosis and caspase-3 activation. Taken together, our results clearly demonstrated that VPA could induce apoptosis of microglia via p38 MAPK and mitochondrial apoptosis pathway.

Valproic acid has been found to be an inhibitor of histone deacetylase (HDAC) and is widely used for the treatment of seizures and mood disorders [20]. There have been increasing studies on the effects of VPA on nerve cells and the effects of VPA on neurogenesis and neurite sprouting have been implicated by several studies [9,12]. But there is a paucity of studies on the action of VPA on non-neuronal brain cells.

Microglial cells in central nervous system (CNS) are immunolike cells and exert a variety of functions that are usually maintained in a quiescent state, but can become activated during a variety of pathological insults [16]. Previous studies by Dragunow et al. [5] have shown that VPA induced caspase 3-mediated apoptosis in microglial cells. Based on these findings, we further explored the molecular mechanisms by which VPA induced microglia apoptosis, and tried to determine the involved signaling pathway.

MAPKs are involved in many cellular processes including development, differentiation, proliferation and apoptosis [8,21]. There are three main members of the MAPK family: c-Jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK). JNK and p38 MAPKs are involved in the pro-apoptotic signal pathway and p38 MAPK seems to sensitize cells to apoptosis by up-regulating Bax [3,17]. In contrast, ERK MAPK could be activated by mitotic stimuli and regulate cell proliferation. But the role of p38 MAPK in VPA-induced apoptosis in microglia is unknown.

In this study, we showed that VPA induced cell apoptosis in brain microglia as well as in the microglial cell line BV-2. Moreover, we reported for the first time that VPA induced microglia apoptosis through p38 MAPK signaling pathway. Thus, our results could provide a significant insight into the mechanism of microglia apoptosis in response to VPA.

All media components used in cell culture were obtained from Gibco Invitrogen Corporation (Carlsbad, CA, USA). Antibodies, including total and phospho-MKK3/6 (p-MKK3/6), total and phospho-ERK (p-ERK), total and phospho-p38 (p-p38), total and phospho-JNK (p-JNK), total and cleaved caspase-3, were purchased from Cell Signaling Technology (Beverly, MA). The antibodies of β-actin, cytochrome c, Bax, and Bcl-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). VPA and p38 inhibitor SB203580 were obtained from Sigma (St. Louis, MO, USA).

Microglial cultures: BALB/c mice were purchased from Experimental Animal Center of Shandong University (Ji'nan, China). Mice were housed, bred, and euthanized in accordance with protocols reviewed and approved by the Commission of Shandong University for ethics of experiments on animals in accordance with international standards. Mouse primary microglial cells were isolated from mixed glial cultures, as described previously [13]. Briefly, cortices were dissected from newborn BALB/c mice and dissociated by trypsinization and mechanical disruption. Primary microglial cells were co-cultivated with astrocytes in poly-D-lysine-coated 75 cm^2

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culture flasks in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. On days 10–14, microglial cells were harvested by shaking the cultures and collecting the floating cells. The cells were seeded into plastic tissue culture flasks. After incubation at 37 °C for 1 h, non-adherent cells were removed by replacing culture medium. The cells were grown in DMEM with 10% FBS and maintained at 37 °C and 5% CO_2.

BV-2 cell culture: The cells were purchased from Cell Center of Peking Union Medical College (Beijing, China) and cultured in DMEM medium with 5% FBS and 1% penicillin/streptomycin. Cultures were incubated at 37 °C and 5% CO_2 in a fully humidified incubator.

The apoptotic cells were determined by terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) assay using an in situ apoptosis detection kit (Roche Diagnostic, Indianapolis, IN) according to the manufacturer's instructions. In brief, cells were fixed for 1 h with ice cold 4% paraformaldehyde and permeabilized for 8 min with 0.1% Triton X-100 in 0.1% sodium citrate. After washing with PBS, 30 μl of TUNEL reaction mixture was added onto the cells and incubated in a humidified atmosphere for 1 h at 37 °C. 30 μl substrate solution was placed onto cells and then convert-AP incubation. Finally coverslips were washed and mounted with citiflor. The percentage of apoptotic cells was calculated by counting approximately 500 cells.

Cell viability was assessed by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). In brief, 20 μl MTT (5 mg/ml, Sigma–Aldrich) was added to each well, and plates were incubated at 37 °C for 4 h and then quantifying the color formation by the way of an ELISA plate reader (Dynotech Instruments) at 570 nm wavelength using 200 μl MTT solubilization solution.

To obtain cytosolic and mitochondrial protein extracts, the cells were subfractionized in homogenization buffer. The cytosolic and mitochondrial fractions were separately isolated by centrifugation as described previously [10]. Twenty micrograms of protein were separated by SDS–polyacrylamide gel electrophoresis and

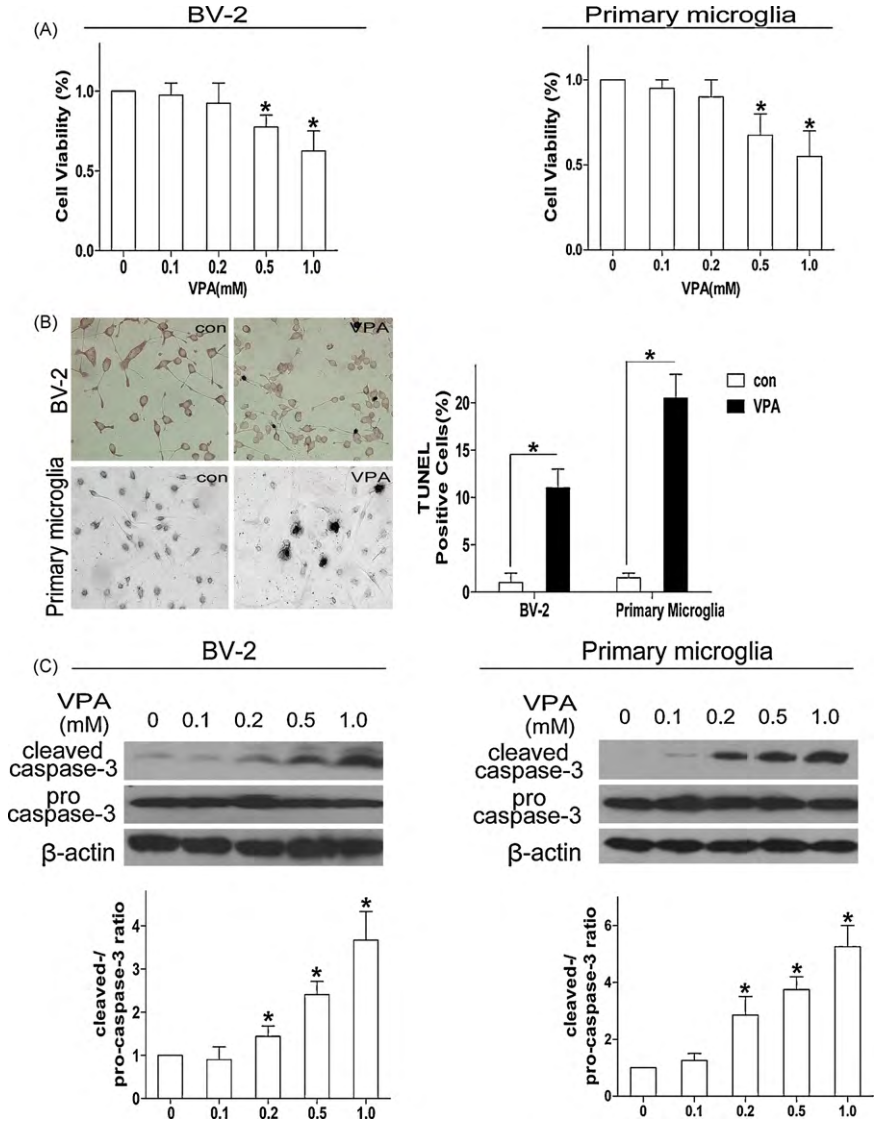

Fig. 1. VPA induces apoptosis in microglia. (A) BV-2 and primary microglial cells were treated with VPA at indicated concentrations for 24 h and cell viability was examined by the MTT analysis. *$p < 0.01$ compared with control. (B) BV-2 and primary microglial cells were treated with 1 mM VPA for 24 h and apoptotic cells (dark cells) were determined by TUNEL assay. Photographs of representative TUNEL-stained cells are shown at the left. Magnification 40×. The bar graph at the right shows the percentage of apoptotic cells. (C) BV-2 and primary microglial cells were treated with VPA at indicated concentrations for 24 h and cleaved caspase-3 was determined by Western blot. All data are representative of three independent experiments. *$p < 0.01$ compared with control.