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D609 blocks cell survival and induces apoptosis in neural stem cells

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Abstract—In order to investigate the effects of tricyclodecane-9-yl-xanthogenate (D609) on the survival of neural stem cells (NSCs), which were isolated from rat forebrain, we treated the NSCs with D609 in the presence of basic fibroblast growth factor (bFGF). We found that when NSCs were exposed to 18.76–56.29 μM D609, the viability of the cells remarkably declined and apoptosis occurred. At the same time, the ROS level in NSCs was depressed. The data suggested that D609 was a powerful growth inhibitor and apoptosis inducer in NSCs.

D609 (tricyclodecane-9-yl-xanthogenate), a tricyclodecanol derivative of xanthic acid, has been reported to have antiviral, antitumor, anti-inflammatory, and anti-apoptosis properties.[1,2] Most of these activities have been largely attributed to the characterized competitive inhibitory effect of D609 on phosphatidylcholine-specific phospholipase C (PC-PLC).[3] However, as a xanthate derivative that can dissociate in solution to xanthate anions and/or xanthic acid with a free thiol group, D609 was a potent antioxidant. It has been reported that D609 has the ability to inhibit ionizing radiation (IR)-induced cellular oxidative stress and protects the mice from IR-induced lethality.[4] Recently, a zinc-independent PtdCho-PLC was isolated from *Pseudomonas aeruginosa*. This enzyme was insensitive to D609.[5] These data suggested that D609 had extensive effects on cellular processes, but the mechanism of its function needs to be clarified.

In the previous studies, we found that D609 was involved in the proliferation, apoptosis, and differentiation of human umbilical vascular endothelial cells (HUVECs), and human marrow stromal cells (hMSCs).[6–9] In addition, we discovered that 7.5–30 μM D609 blocked the survival of mouse neural cells under normal condition.[10] So we are interested in the effect of D609 on neural stem cells (NSCs), which are defined as progenitor cells in central nervous system (CNS), and are capable of both self-renewal and differentiation into neurons and glial cells. Moreover, these cells can be cultured in vitro and may provide an unlimited source of cells for grafting into patients with Parkinson's disease, Huntington's disease, and multiple sclerosis. Although, D609 was widely studied due to its wide variety of functions, there is no report about the effect of D609 on NSCs. Therefore, in this research, we investigated the roles of D609 in the survival and apoptosis of rat NSCs under normal condition.

NSCs that we used were isolated from rat forebrain and identified by the monoclonal antibody against nestin. Moreover, the neurons derived from the cells exhibited intensive positive neuron-specific enolase (NSE), neurofilament-L (NF-L), and synapsin (Fig. 1). These results ensured that the cells obtained were multi-potential NSCs consistent with previous report.[10] When rat NSCs were exposed to 18.76–56.29 μM D609 (these concentrations are similar to that used to inhibit PC-PLC in other cells[1,3,10]) in the presence of basic fibroblast growth factor (bFGF), the viability of the cells dramatically declined within 72 h ($P < 0.05$

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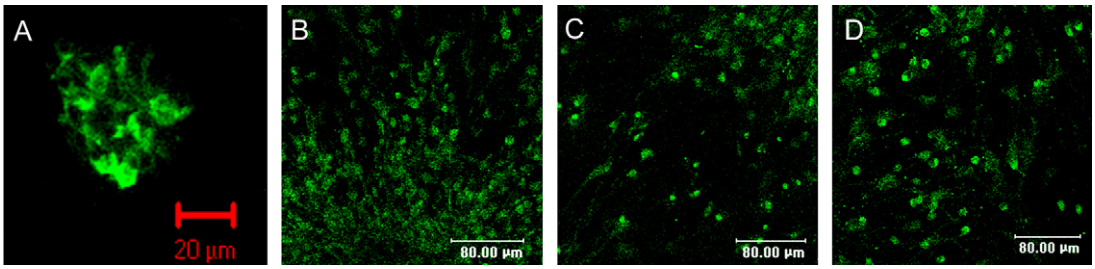

Figure 1. Identification of NSCs. (A) The result from the immunocytochemical staining showed positive expression of nestin. (B, C, and D) The neurons derived from NSCs exhibited, respectively, intensive positive NSE, NF-L, and synapsin.

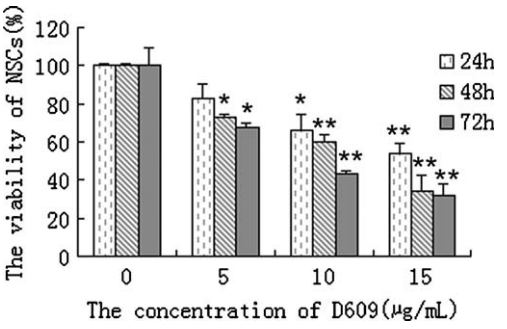

Figure 2. The viability of NSCs treated with 0, 18.76, 37.53, and 56.29 μM D609 for 24, 48, and 72 h, respectively (*$P < 0.05$, **$P < 0.01$, $n = 3$).

to $P < 0.01$) (Fig. 2). The results showed that 18.76–56.29 μM D609 inhibited NSC growth in concentration- and time-dependent manners.

As shown in Figures 3A and B, the specific morphological changes of apoptosis could be observed under a phase contrast microscope. Consistent with cell morphological changes, nuclear condensation, DNA fragmentation, and apoptotic body formation were observed clearly (Figs. 3C and D). These results showed that the death of NSCs belonged to a typical kind of apoptosis. TUNEL assay further confirmed D609 could induce apoptosis in NSCs (Fig. 4A). Compared with the 09 treatment group showed an overwhelming majority of apoptotic NSCs (Fig. 4B) ($P < 0.01$).

To understand whether D609 causes NSC necrosis, we measured the lactate dehydrogenase (LDH) activity in the cell culture medium. As shown in Figure 5, there was no significant difference ($P > 0.05$) in LDH release between control group and D609 treatment group. The data suggested that 56.29 μM D609 did not induce NSC necrosis within 72 h.

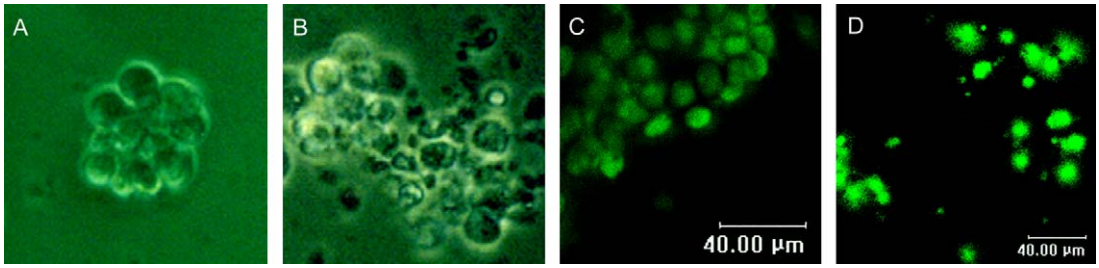

Figure 3. The morphological changes and nuclear fragmentation of NSCs at 24 h. A and B are the morphological micrographs obtained under phase contrast microscope. (A) Cells in control group were cultured in the medium with bFGF. (B) Cells in D609 treatment group were cultured in the medium with bFGF and 37.53 μM D609 (400×). C and D are the fluorescent micrographs which show nuclear fragmentation stained with acridine orange. (C) Cells in control group. (D) Cells in D609 treatment group.

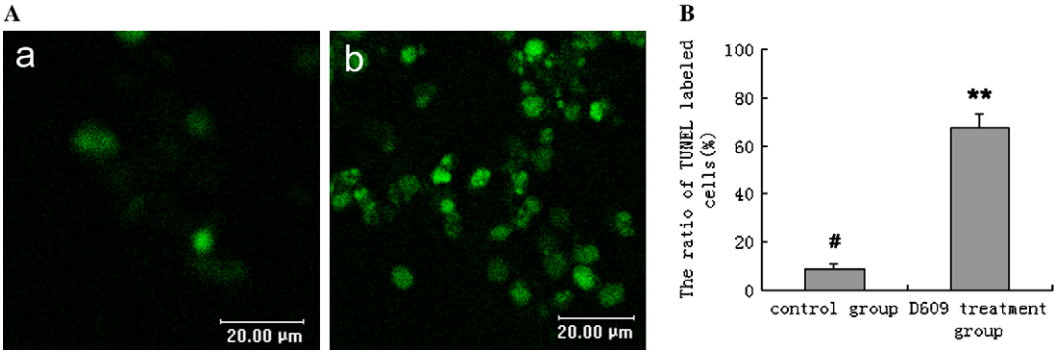

Figure 4. Quantification of apoptotic cells by TUNEL assay. (A) Fluorescent micrographs show the TUNEL staining of NSCs. (a) Cells in control group. (b) Cells in D609 treatment group. (B) The quantity of apoptotic cells. (**$P < 0.01$, $n = 3$).