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Research report

Neuroprotective effect of CPDT on THA-induced cortical motor neuron death in an organotypic culture model

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ABSTRACT

Brain stroke, trauma, and motor neuron disease each can result in cortical motoneuron (CMN) death or impairment. Glutamate excitotoxicity induces motor neuron damage in both acute motor neuron loss and chronic motor neuron degeneration. It is necessary to find effective strategies to protect CMNs from excitotoxicity in a variety of pathological conditions. 5,6-Dihydrocyclopenta-1,2-dithiole-3-thione (CPDT) is one of the phase II enzyme inducers. In our previous report, CPDT was shown to have neuroprotective effects on the spinal cord, by activating the Nrf2/ARE pathway to increase antioxidative capacity. In this study, in order to figure out whether CPDT can prevent CMN's from THA-induced death, we set up an organotypic brain slice culture system. Threo-hydroxyaspartate (THA), a glutamate transport inhibitor, was added to the culture medium to induce CMN death by glutamate excitotoxicity. Brain slices were pretreated with CPDT for 48 h, then treated with CPDT and THA simultaneously for 3 weeks. We found that pretreatment with CPDT significantly increased CMN survival. Glutamate concentration in the culture medium was significantly greater following THA treatment, whereas no significant decrease was found in the CPDT pretreatment group. However, both Nrf2 and HO-1 protein expression was significantly elevated in the CPDT pretreatment group, and Nrf2 protein translocated to the nucleus after CPDT stimulation. These findings suggest that CPDT can protect CMNs from THA-induced motor neuron death by activating the Nrf2 pathway and increasing HO-1 protein expression. Therefore, increasing antioxidative defense capacity should benefit to upper motor neuron survival following a glutamate excitotoxicity insult.

1. Introduction

Cortical motoneurons (CMNs) are found in layer 5 of motor cortex. Descending axons of CMNs form the corticospinal or pyramidal tract, corticospinal tract directly or indirectly excites lower motor neurons, resulting in contraction of specific muscle groups. CMN damage occurs in many conditions such as brain stroke, trauma, and motor neuron disease [12].

Glutamate excitotoxicity is an important factor affecting motor neuron death following both acute motor neuron loss and in chronic degenerative diseases of motor neurons. Glutamate concentration is significantly increased in stroke, closed head injury and motor neuron disease such as amyotrophic lateral sclerosis (ALS) [24,27]. Decreased expression and abnormal splice variants of the glutamate transporter GLT-1 are two mechanisms responsible for limiting uptake of glutamate and increasing extracellular glutamate accumulation [7,8]. Many beta-lactam antibiotics such as ceftriaxone potently stimulate GLT-1 expression and enhance its biochemical and functional activity. Ceftriaxone has been shown to be neuroprotective in vitro when used in models of ischemic injury and motor neuron degeneration [19]. Recently, it is suggested that ceftriaxone-mediated neuroprotection might relate more strongly to activation of antioxidant defense systems including Nrf2 (nuclear factor erythroid 2-related factor 2) [10]. Nrf2 is a transcription factor, when activated, Nrf2 translocates to the nucleus, complexes with other nuclear factors, and binds to the antioxidant response element (ARE) [23] resulting in overexpression of antioxidant enzymes such as NQO1, HO-1, GST [26]. Many Phase II enzyme inducers such as t-BHQ, D3T, CPDT can activate Nrf2. Whether phase II enzyme inducers directly protect cortical motor neurons from excitotoxicity insult is currently unknown. In our previous study, we found that pretreatment with 15 μM and 30 μM CPDT activated Nrf2 and Nrf2 target genes in spinal cord tissues and fully protected spinal motor neurons against glutamate-induced excito-

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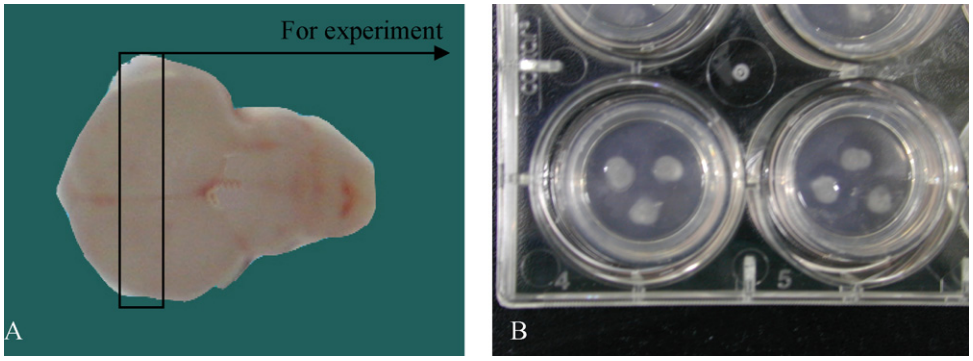

Fig. 1. (A) The brain from 1-day-old rat. (B) The brain slices in the inserts.

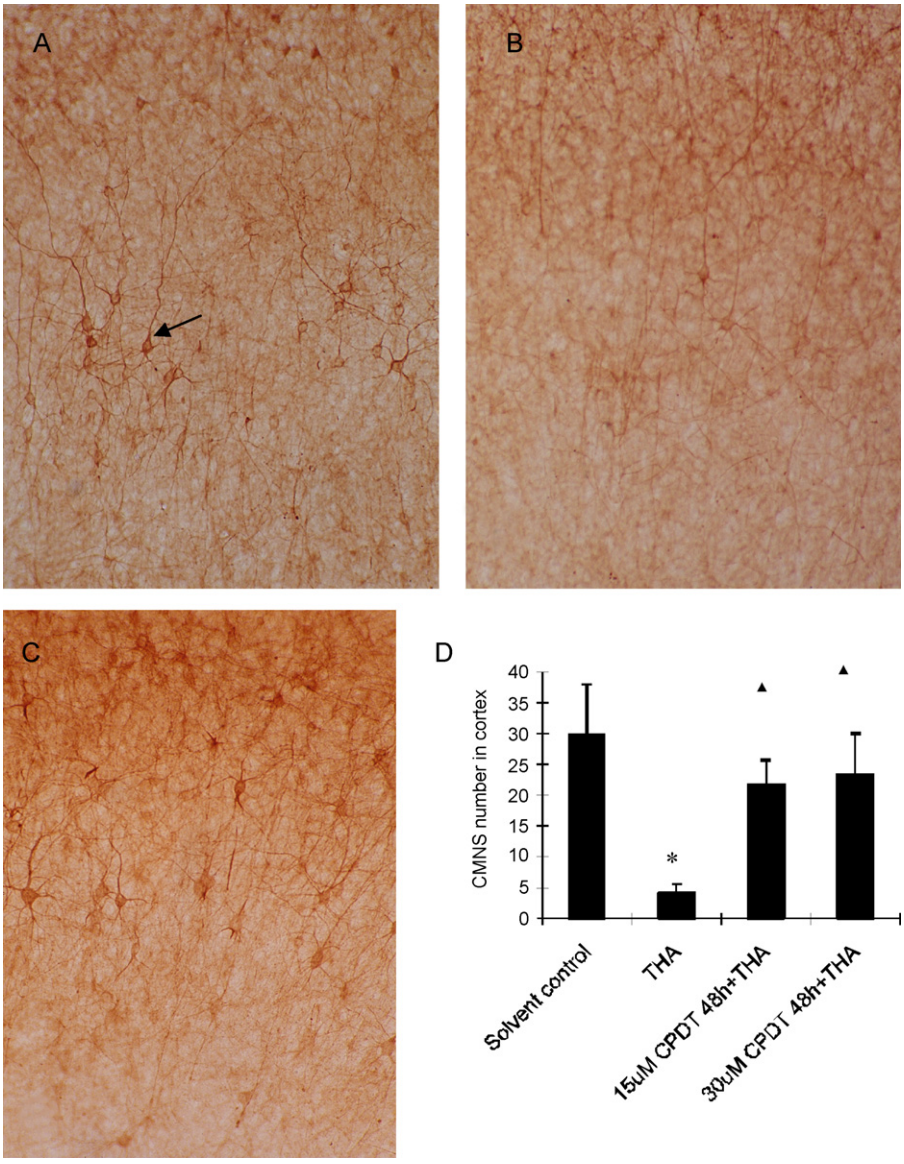

Fig. 2. Neuroprotection of CPDT on THA-induced CMNs death. CMNs in control slices were characterized by SMI-32 immunostaining (A, arrow). A chronic 3-weeks treatment with 100 μm THA resulted in decreasing the number of CMNs in the cortical explants (B and D, Compared with control group $^{*}P < 0.001$). The number of survival CMNs was elevated by CPDT pre-treatment (C and D, Compared with THA group $^{\blacktriangle}P < 0.001$) (15 μM, and 30 μM CPDT pretreatment for 48 h, and then added the same concentration CPDT with 100 μM THA simultaneously for 3 weeks). No significant difference was found between 15 μM CPDT and 30 μM CPDT pretreatment group ($P > 0.05$).