A Novel PKC Delta (PKCδ) Inhibitor Protects Against Oxidative Stress-Induced Apoptotic Cell Death in Neurodegenerative Disease Models

Anantharam G. Kanthasamy, Calivarathan Latchoumycandane, Siddharth Kaul, Vellareddy Anantham and Arthi Kanthasamy

Parkinson’s Disorder Lab., Dept. of Biomedical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, 50011, USA

ABSTRACT
Oxidative stress and apoptosis are considered common mediators of many diseases including Alzheimer’s and Parkinson’s disease (PD). Recently, we identified that PKCδ, a member of the novel PKC isoform family, is proteolytically cleaved by caspase-3 into a 41 kDa catalytic and 38 kDa regulatory subunits, leading to a persistent activation of the kinase during oxidative insults (Kaul et al., 2003; Yang et al., 2004). Upon further characterization using dominant negative mutant or siRNA, we found that the proteolytic activation of PKCδ mediates apoptosis in neuron cell culture models. Since caspase-3 cleavages PKCδ at 324DIPD327 site to activate the kinase, we developed an irreversible and competitive peptide inhibitor Z-DIPD-fmk, for the cleavage site and tested its efficacy against oxidative stress-induced cell death in PD models. Co-treatment of z-DIPD-fmk with MPP+ or 6-OHDA dose-dependently attenuated caspase-3 activation and DNA fragmentation in dopaminergic neuronal cells. z-DIPD-fmk at as low as 3 μM effectively inhibited the 6-OHDA-induced DNA fragmentation, demonstrating the potent anti-apoptotic effect of the inhibitor. The PKCδ z-DIPD-fmk was much more potent (EC50 6 μM) than the caspase-3 inhibitor z-DEVD-fmk (EC50 18 μM). Collectively, these results demonstrate that the PKCδ cleavage site inhibitor z-DIPD-fmk effectively protects against oxidative-induced neuronal apoptosis in neurodegenerative models (Patent pending).

MATERIALS AND METHODS

INTRODUCTION
Parkinson’s disease (PD) is a major common neurodegenerative disorder affecting more than 1% of the population over the age of 60 in the US. Selective degeneration of nigral dopaminergic neurons is the primary pathology of PD. Both clinical and experimental evidence clearly demonstrates that oxidative stress and apoptosis are key cellular mechanisms that contribute to the selective nigral neuronal loss. However, the key cellular target that mediates the nigral apoptotic cell death process following oxidative insult is not completely understood. Recently, we reported that proteolytic activation of PKCδ, a member of the novel PKC isoform family, plays a key role in apoptotic cell death of dopaminergic neurons in a cell culture model of PD (MPP+) as well as oxidative stress models (Anantharam et al., 2003; Kitaizawa et al., 2002; Kaul et al., 2003; Yang et al., 2004). Latchoumycandane et al., 2005). We demonstrated that blockade of PKCδ activation by the kinase dominant negative mutant, cleavage-resistant mutant or siRNA almost completely prevented the nigral cell death (Kaul et al., 2003; Anantharam et al., 2004; Yang et al., 2004). PKCδ is proteolytically cleaved by caspase-3 at the 324DIPD327 residue, resulting in 41 kDa catalytic and 38 kDa regulatory subunits, leading to a persistent activation of the kinase (Kaul et al., 2003; Anantharam et al., 2004; Yang et al., 2004). Hence, we developed a cleavage site inhibitor, namely z-DIPD-fmk, and characterized its neuroprotective efficacy in cell culture models of PD.

MATERIALS AND METHODS

Cell culture: A rat mesencephalic dopaminergic neuronal cell line (N27 cells) and primary mesencephalic neuronal cultures were used. As described in our previous publications (Kaul et al., 2003; Yang et al., 2004), N27 cells were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 1% L-glutamine, penicillin (100 U/ml), and streptomycin (100 U/ml). The cells were maintained at 37°C in a humidified atmosphere of 5% CO2. Primary mesencephalic neuronal cells were obtained from E14.5 embryos and cultured in neurobasal medium supplemented with B2 as described in our publication (Yang et al., 2004).

REFERENCES