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TETRAHYDROPAPAVEROLINE INDUCES DNA DAMAGE AND APOPTOTIC CELL DEATH THROUGH GENERATION OF REACTIVE OXYGEN SPECIES

Mi-Hyun Shin, Jung-Hee Jang, Jeong-Sang Lee and Young-Joon Surh.
College of Pharmacy, Seoul National University, Seoul 151-742, Korea

Tetrahydropapaveroline(THP), a dopamine-derived 6,7-dihydroxy-1-(3',4'-dihydroxybenzyl)-1,2,3,4-tetrahydrosioquinoline, has been suspected as a possible dopaminergic neurotoxin to elicit Parkinsonism. Autoxidation or monoamine oxidase-mediated oxidation of THP and subsequent generation of reactive oxygen species (ROS) may contribute to the degeneration of dopaminergic neurons induced by this isoquinoline alkaloid. In the present study, we have found that THP undergoes redox cycling in the presence of Cu(II) to produce ROS capable of causing DNA strand scission. THP and Cu(II)-induced DNA damage was protected by bathocuproine disulfonic acid (copper chelator), EDTA (non-specific metal chelator), catalase and certain antioxidants. Reaction of THP with calf thymus DNA resulted in the formation of 8-hydroxydeoxyguanosine, another hallmark of oxidative DNA damage. THP also exerted cytotoxicity in cultured rat pheochromocytoma (PC12) cells. Reduced glutathione and *N*-acetyl-L-cysteine attenuated cytotoxicity induced by THP. THP treated cells exhibited increased intracellular peroxide accumulation based on 2',7'-dichlorofluorescein diacetate (DCF-DA) fluorescence. Cells exposed to THP underwent apoptosis as determined by poly(ADP-ribose) polymerase (PARP) cleavage, terminal trasferase-mediated dUTP nick end labeling (TUNEL) and positive staining with the fluorescent dye DAPI (4,6-diamidino-2-phenylindole). PC12 cells treated with THP also exhibited higher levels of Bax protein than did untreated control cells. In contrast the expression of anti-apoptotic Bcl-X_L level decreased in a time dependent manner. Furthermore, overexpression of *bcl-2* attenuated THP-induced apoptosis in PC12 cells.Exposure of PC12 cells to 30μM THP resulted in activation of ERK1/2 and p38. THP treatment also led to activation of the redox sensitive transcription factor NF-kB and subsequent induction of inducible nitric oxide synthase (iNOS). Pretreatment of PC12-cells with the iNOS inhibitor N-nitro-L-arginine methylester (L-NAME) ameliorated THP-induced cytotoxicity. Taken together, the above findings suggest that THP-induced cell death is associated with reactive oxygen and/or nitrogen species mediated apoptosis.