

Research report

CI-1010 induced opening of the mitochondrial permeability transition pore precedes oxidative stress and apoptosis in SY5Y neuroblastoma cells[☆]

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Accepted 16 October 2002

Abstract

The hetero-bifunctional nitroimidazole radiosensitizer CI-1010, *R*- α -{[(2-bromoethyl)-amino]methyl}-2-nitro-1H-imidazole-1-ethanol monohydrobromide, causes selective irreversible apoptotic loss of retinal photoreceptor cells *in vivo*. The human neuroblastoma cell line, SH-SY5Y, was used as a neuronotypic model of CI-1010-mediated retinal degeneration. Exposure to CI-1010 for 24 h induced apoptosis in neuroblastoma cells, as determined by histopathological and ultrastructural analysis and by TUNEL technique. CI-1010 causes a dose-dependent decrease in cell viability in SY5Y cells, as measured by the reduction of MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. Superoxide dismutase reduced loss of cell viability following CI-1010 treatment suggesting an oxidative stress-mediated mechanism of toxicity. The effects of CI-1010 on mitochondrial membrane potential and intracellular levels of reactive oxygen species were assessed in live SY5Y cells by confocal microscopy using the fluorescent dyes, tetramethylrhodamine methyl ester and 5,6-carboxy-2',7'-dihydrodichlorofluorescein diacetate. CI-1010 caused a rapid depolarization of mitochondria in SY5Y cells followed by an increase in ROS. Both CI-1010-induced mitochondrial depolarization and subsequent increases in ROS were prevented by pretreatment with either the permeability transition pore inhibitor, cyclosporin A (CsA), and by the antioxidant, α -tocopherol. However, CsA and α -tocopherol were unable to prevent apoptosis in CI-1010-treated cells, suggesting the influence of additional mechanism(s) of CI-1010-induced toxicity. This study evaluates intracellular oxidative stress associated with pore opening prior to apoptosis and provides evidence in support of a mitochondrial mechanism of CI-1010-induced neuronal cell death.