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NITRIC OXIDE PROTECTS MOUSE THYMOCYTES FROM APOPTOSIS INDUCED BY γ-IRRADIATION

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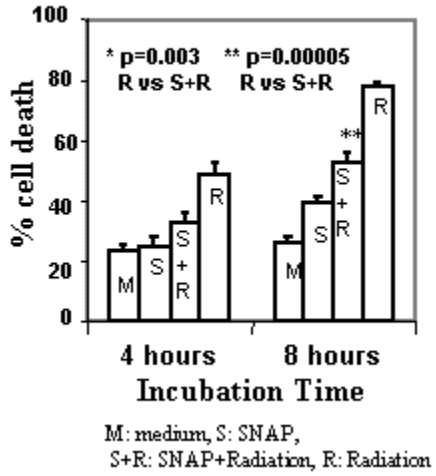
INTRODUCTION. Nitric Oxide (NO) and its reactive products can either promote or prevent apoptosis depending upon the cell systems and conditions involved (1). We have previously reported that NO-induced mouse thymocyte apoptosis in vitro involves p53 upregulation and caspase-1 activation (2, 3). To further dissect the relationship between NO and thymocyte apoptosis, we investigated the effect of NO on Balb/c thymocytes exposed to γ-irradiation. We found that NO partially protects γ-irradiated thymocytes from apoptosis by preserving mitochondrial integrity and inhibiting caspase activity.

METHODS. A single cell suspension was prepared from freshly isolated Balb/c thymus. Thymocytes were exposed to γ-irradiation in the presence or absence of an exogenous NO donor, S-nitroso-N-acetyl penicillamine (SNAP), for various time intervals. Thymocyte apoptosis was detected by Annexin V/PI staining followed by flow cytometry. Mitochondrial transmembrane potential was measured using the fluorescent dye, DiOC6, and flow cytometry. Western Blot was performed on thymocyte lysates. Caspase activity in cell lysates was measured by a colorimetric assay using specific substrates.

RESULTS. The percentage of apoptotic thymocytes at 4 and 8 hours following γ-irradiation (5Gy) was significantly decreased in the presence of 1 mM SNAP, compared to γ-irradiation alone (see Fig. 1). γ-irradiation induced release of cytochrome c and reduction in mitochondrial transmembrane potential, which were inhibited by SNAP (see Fig. 2). The increased activities of caspase 3, 6, 8 and 9 at 6 hours following γ-irradiation were diminished by SNAP (see Fig. 3). The cleavage of caspase 3, 6, and PARP in γ-irradiated cells was inhibited by co-incubation with SNAP at 6 hour post γ-irradiation (see Fig. 4).

Fig. 1



M: medium, S: SNAP,
S+R: SNAP+Radiation, R: Radiation

Fig. 2

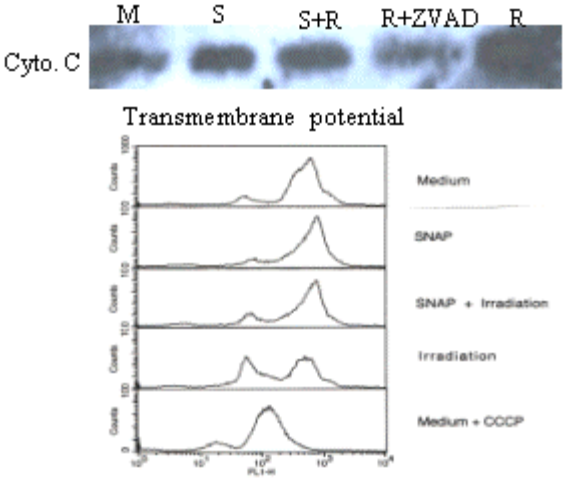


Fig. 3

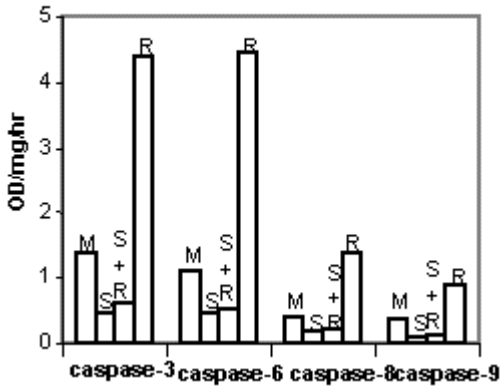
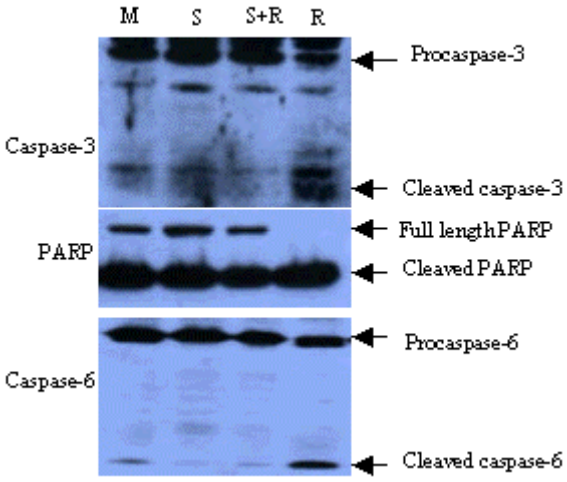


Fig. 4



DISCUSSION. γ-irradiation induces rapid apoptosis in Balb/c thymocytes by activating apoptotic signaling pathways (4). The anti-apoptotic activity of NO has been documented in certain cell types (1). Our results show that NO partially inhibits γ-irradiation-induced thymocyte apoptosis by decreasing mitochondrial damage and suppressing caspase activity. Western Blot showed that the cleavage of caspase-3 and caspase-6 was inhibited by SNAP. The data suggest that NO may act on upstream molecules in the apoptotic pathway to inhibit apoptosis. The detailed mechanism of this protective effect is under intense investigation at present time.

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