THE INFLUENCE OF CYCLOHEXIMIDE ON THE INDUCTION OF APOPTOSIS IN CHO CELLS BY EDS

URSZULA POLAK and JERZY B. WARCHOŁ
Department of Radiobiology and Cell Biology, K. Marcinkowski University of Medical Sciences in Poznań, Święcickiego 6, 60-781 Poznań, Poland

Ethane 1,2-dimethanesulphonate (EDS) is a glutathione-dependent alkylating agent which induces apoptosis and necrosis of Chinese Hamster Ovary (CHO) cells. However, the molecular basis of these processes are not well understood. In this study, we attempted to establish whether the inhibition of protein synthesis by cycloheximide (CHX) will initiate apoptosis of CHO cells using EDS. The experiments were performed on CHO cells, growing in an RPMI 1640 medium, supplemented with 5% FBS (37°C; 5% CO₂). The CHO cells were exposed to EDS alone at concentrations of 5-, 10-, 20-, 50-, 100 mmol/l, or EDS with a constant concentration of CHX of 100 µmol/l. Simultaneously, the cells were treated with CHX alone, and DMSO (at the highest concentration used for dissolving EDS). The effects were monitored after 24 and 48h intervals. The apoptotic and necrotic cells were detected with propidium iodide and Hoechst 33342. It was established that EDS alone can induce a lower degree of apoptosis than the degree of apoptosis induced by EDS and CHX combined. The EDS with CHX showed a maximum of apoptotic cells at 10-20 mmol/l EDS (24h), and the apoptotic and necrotic changes began to recede at 48h. The obtained results indicated that synthesis of anti-apoptotic proteins plays a very important role in the inhibition of apoptosis. Additionally, the activation of apoptosis probably involved active gene expression.