Influence of cycloheximide on apoptosis in CHO cells, induced by ethane 1, 2-dimethanesulphonate (EDS)

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Abstract

Ethane 1, 2-dimethanesulphonate (EDS) causes apoptotic death of Leydig cells. Additionally, EDS causes damage of Chinese Hamster Ovary (CHO) cells but the occurrence of apoptosis is not such plentiful. The present study tested whether the inhibition of protein synthesis by cycloheximide (CHX) would influence apoptosis of CHO cells, induced by EDS. The study compounds induced morphological changes in CHO cell typical for apoptosis. An active form of caspase-9 and an alternation of mitochondrial transmembrane potential were also observed. In our study, a more cumulative effect of the CHX and EDS on apoptosis was observed, when both compounds were simultaneously employed. The obtained results indicated that synthesis of antiapoptotic proteins plays a very important role in the inhibition of apoptosis.

Key words: apoptosis, CHO cells, ethane 1,2-dimethanesulphonate (EDS), cycloheximide (CHX), caspase-9, mitochondrial potential.

Introduction

Cell apoptosis represents a significant phenomenon for cellular homeostasis. It results in self-destruction of cells and plays a reciprocal role to that of cell proliferation. Apoptosis is a genetically controlled death process, where the fate of the cell is determined by the relative abundance of survival and death proteins [1].

Ethane 1,2-dimethanesulphonate (EDS) is an agent that rapidly diffuses across tissues and selectively destroys Leydig cells, which are killed via apoptosis [2, 3, 4]. The influence of EDS on non steroidogenic types of cells was also studied. EDS damages Chinese Hamster Ovary (CHO) cells but the occurrence of apoptosis is not such extensive. The molecular bases of the processes are still not well understood [4]. In this study, we attempted to establish, whether inhibitions of protein synthesis by cycloheximide (CHX) would influence apoptosis of CHO, cells using EDS.

Material and Methods

The experiments were carried on *in vitro* cultured CHO tumour cells, maintained in RPMI 1640 medium, supplemented with 5% FBS, 2 mmol/l L-glutamine, 100 µg/ml streptomycin and 100 U/ml penicillin (37°C; 5% CO_2) [5]. CHO cells were exposed to EDS alone at concentrations of 5-, 10-, 20-, 50- and 100 µmol/l or at constant concentration of CHX [100mmol/l], added together with EDS. Simultaneously, the cells were treated with CHX alone and DMSO at the highest concentration used for dissolving EDS. The effects were observed after 24- and 48h intervals. Apoptotic and necrotic changes were detected by fluorescent microscopy (propidium iodide and Hoechst 33342 staining) and electron microscopy. The cells were incubated for 24h with EDS (10- and 20 mmol/l) and CHX, added at different times points, compared to the addition at EDS. The cells were also treated for 24h with EDS and CHX, added together, and for 24h with EDS, followed by CHX, added 1-, 2-, 3-, 4-, 6-and 12h later. A determination of caspase-9 activity in CHO cells was performed, according to caspase-9 inhibitor Red-LEHD-FMK staining (Oncogene Research Products™). The percentage of caspase-9 positive cells was counted after 12- and 24h incubation

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Fig.1.TheinfluenceofEDSconcentration.
Incubationtime-24h

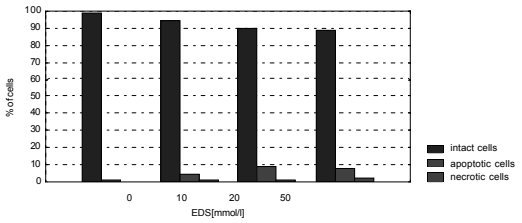


Fig.2.TheinfluenceofEDSconcentration.
Incubationtime-48h

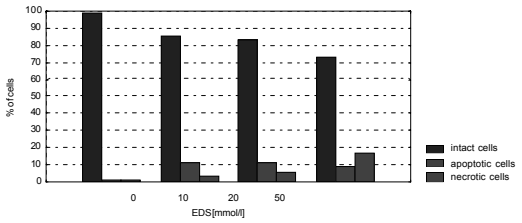


Fig.3.TheinfluenceofEDSandCHX[100umol/l]
Incubationtime-24h

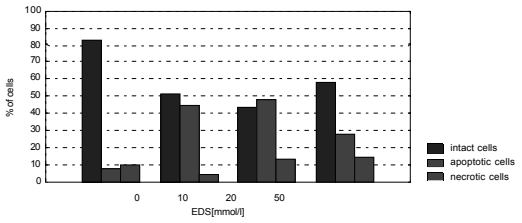


Fig.4.TheinfluenceofEDSandCHX[100umol/l]
Incubationtime-48h

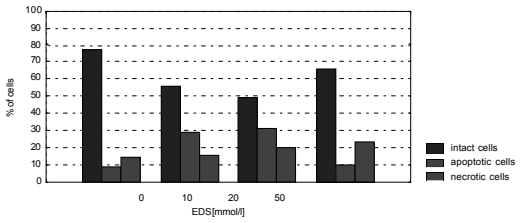


Fig.5.Theactiveformofcaspase-9incubated12h
withEDS[20mmol/l],EDS/CHXiCHX[100umol/l]

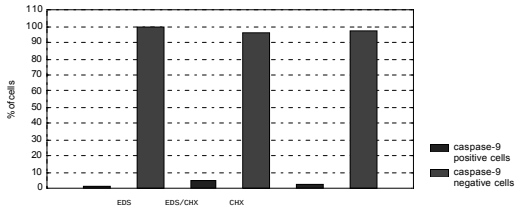
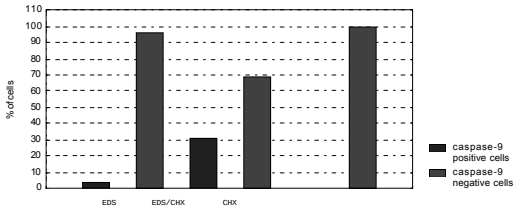


Fig.6.Theactiveformofcaspase-9incubated24h
whitEDS[20mmol/l],EDS/CHX,CHX[100umol/l]



with EDS alone, EDS with CHX, and CHX alone, using an LSM 510 (Zeiss) confocal microscope.

Mitochondrial potential was assessed, using the fluorescent dye JC-1. Fluorescence was measured at 490nm (excitation) and 530nm (emission) for the JC-1 monomer (green colour) and 590nm for the JC-1 aggregate (orange colour) [6, 7].

Results and Discussion

It was established that EDS could induce apoptosis in CHO cells, however, the level of apoptotic cells was lower, when compared with the degree of apoptosis induced by EDS and CHX combined together. The number of apoptotic cells depended on the concentration of EDS and on the incubation time. The percentage of cells showed apoptotic features. When they were incubated with 10- and 20mmol/l, EDS was 10%, whereas the number of necrotic cells did not exceed 5% (Fig. 1 and 2). The highest level of apoptosis, about 50%, was observed for the cells, exposed to the action of both compounds: 10- and 20 mmol/l EDS and 100µmol/l CHX for 24h. The level of necrotic cells in that case was about 10%. At higher doses of EDS [50-, 100 mmol/l] apoptosis was inhibited, whereas necrosis increased to 15% (Fig. 3 and 4). Incubation with DMSO did not influence the cells, whereas incubation with CHX [100 µmol/l] alone for 24h caused apoptosis in about 5% of studied cells. After 24h incubation with EDS [10-, 20 mol/l], followed by addition of CHX (6h later than EDS), the percentage of apoptotic cells increased to 50%. The number of apoptotic cells was similar to that for the cells, incubated for 24h with EDS and CHX added together. The active form of caspase-9 was observed only after 12- and 24h incubation of cells with [20 mol/l] EDS and CHX. The highest percentage - 30% - of caspase-9 positive cells was observed after 24h incubation with EDS and CHX (Fig. 6). After 12h incubation, the level of cells with active form caspse-9 was low and did not exceed 5% (Fig. 5). During JC-1 assay, changes of cell mitochondria colours, from green to orange, were observed, corresponding to the changes of mitochondrial potential. The ultrastructure of cells, incubated with 20 mmol/l of EDS, showed typical apoptotic changes, like chromatin condensation and fragmentation of the nuclei or many vesicles, accumulating in the cytoplasm. In mitochondria, the cristae exhibited a loss of concavity and convexity and became more linear.

This study demonstrated that apoptosis of CHO cells, induced by EDS, required some expression of a new protein, synthesized already after the initiation of the process. CHX, which is capable to block protein synthesis, when combined with EDS, increased the number of apoptotic cells. Thus, the antiapoptotic proteins play an important role in apoptosis of CHO cells. The amount of apoptotic cells depended on the time of action of both EDS and CHX. When added together, those two compounds were able to induce apoptosis in more than 50% of the studied cells. It seems that a simultaneous addition of EDS and CHX causes a loss of pro- and antiapoptotic factors balance, leading to cell death. The results are consistent with the current view that apoptosis depends on the expression of specific genes [8, 9] and the amount of apoptot-

ic cells is related to the balance between anti- and proapoptotic proteins in the cell [10]. Further studies of the apoptotic pathway would be helpful in finding out the successful methods to regulate the process and to use it as an effective tool, interrupting the progressive course of many diseases.

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