VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 61, No. 4, pp. 217–220

ISSN 0015–5659
www.fm.viamedica.pl

Co-existence of apoptotic and necrotic features within one single cell as a result of menadione treatment

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[Received 7 October 2002; Revised 23 October 2002; Accepted 23 October 2002]

In the present study we examined the effects of menadione, a redox cycling agent, on structural changes of human osteosarcoma line 143B cells. It has been previously reported that menadione can cause necrotic or apoptotic cell death in a concentration- depending manner. In our experimental model, cells were treated with 100 μM menadione for 24 hours. Using electron microscopy technique cells carrying three kinds of morphological changes were detected: necrotic cells, apoptotic cells and those demonstrating a co-existence of apoptotic and necrotic features in one single cell.

key words: menadione, apoptosis, necrosis, single cell, electron microscopy

INTRODUCTION

Our recent interest has focused on apoptotic and necrotic mechanisms. In this communication we would like to report on our data presenting an interesting phenomenon, which in our opinion could be helpful for future understanding of apoptotic and necrotic mechanisms of cell death.

Menadione (MEN) is a well-known redox cyclic agent, which causes free radical generation inside cells. Several investigators have already pointed out that MEN induces both apoptosis and necrosis at different concentrations [1, 5, 8, 9]. However, the detailed mechanism by which one chemical causes both types of cell death remains unclear. Lower concentrations of MEN have been reported to be the cause of apoptosis, higher concentrations of necrosis. In the present study the concentration of 100 μM was chosen as the "border" one.

Cell death is an essential phenomenon in normal development and homeostasis, but also plays a crucial role in various pathologies. The morphological features of a cell dying either by apoptosis or by necrosis are remarkably conserved for quite different cell types derived from lower and higher organisms.

The typical features of apoptotically dying cells have been amply documented and are remarkably constant: membrane blebbing, plasma membrane asymmetry loss (exposure of phosphatidyl serine), cytosolic condensation, protein cross linking and cell shrinkage, nuclear condensation, breakdown of nuclear DNA and finally falling apart with the formation of the apoptotic bodies. On the other hand, necrosis is characterised by: cell swelling, rounding up and sudden collapse with spillage of the cell content. However, several reports have recently appeared in the literature presenting the modes of cell death which do not fit into the current view on typical necrotic and apoptotic changes [12]: certain developmental cell deaths such as autophagic cell death [2, 10, 11] cytoplasmic cell death [2, 3, 6, 7, 12]; some ischaemia-related cell death featuring cell swelling, referred to as oncosis [4].

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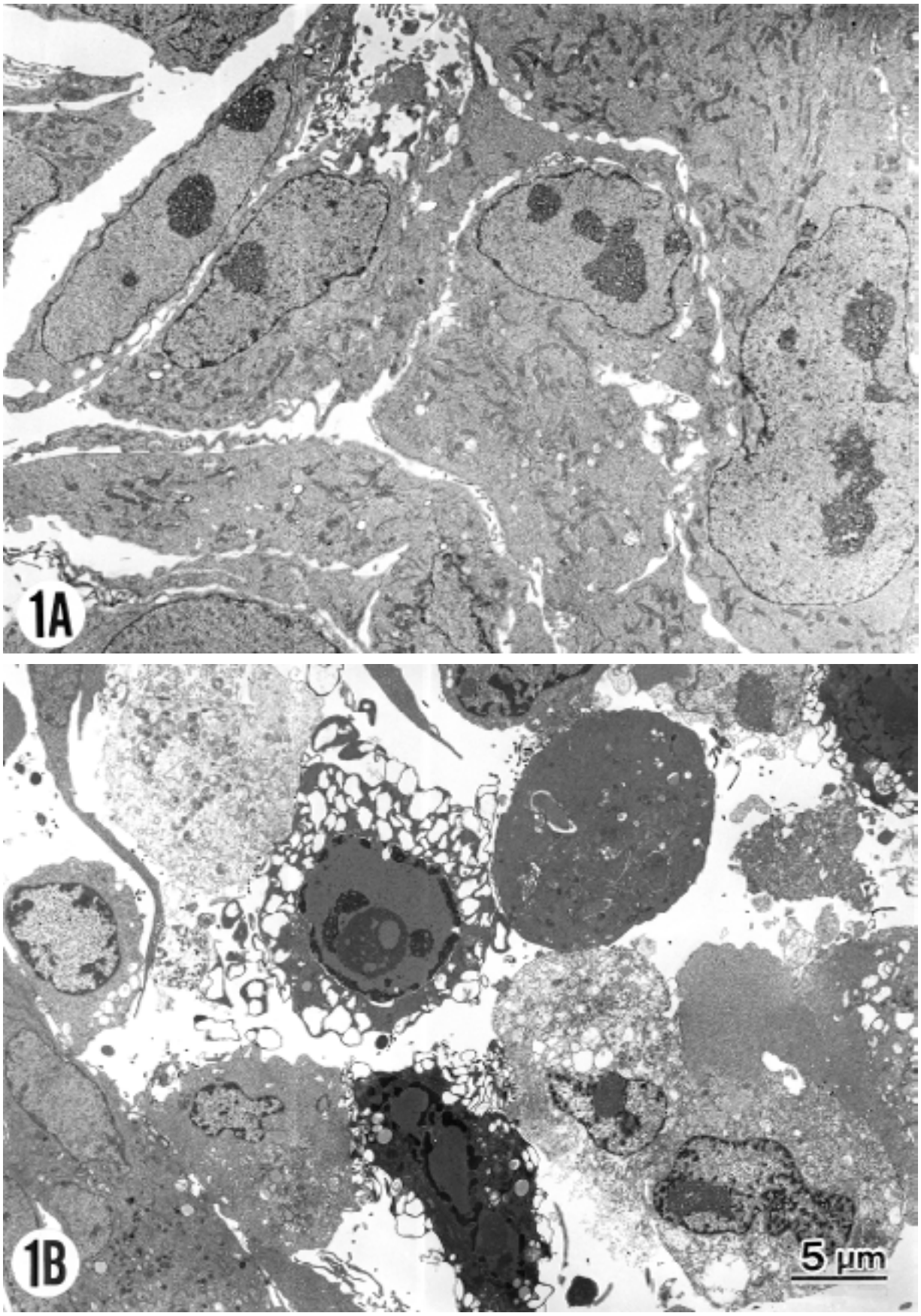

Figure 1. Apoptotic and necrotic morphologies in 100 μM menadione (24 h) — treated cells. **A.** Untreated cells (control); **B.** Mixture of cell death types after menadione treatment.

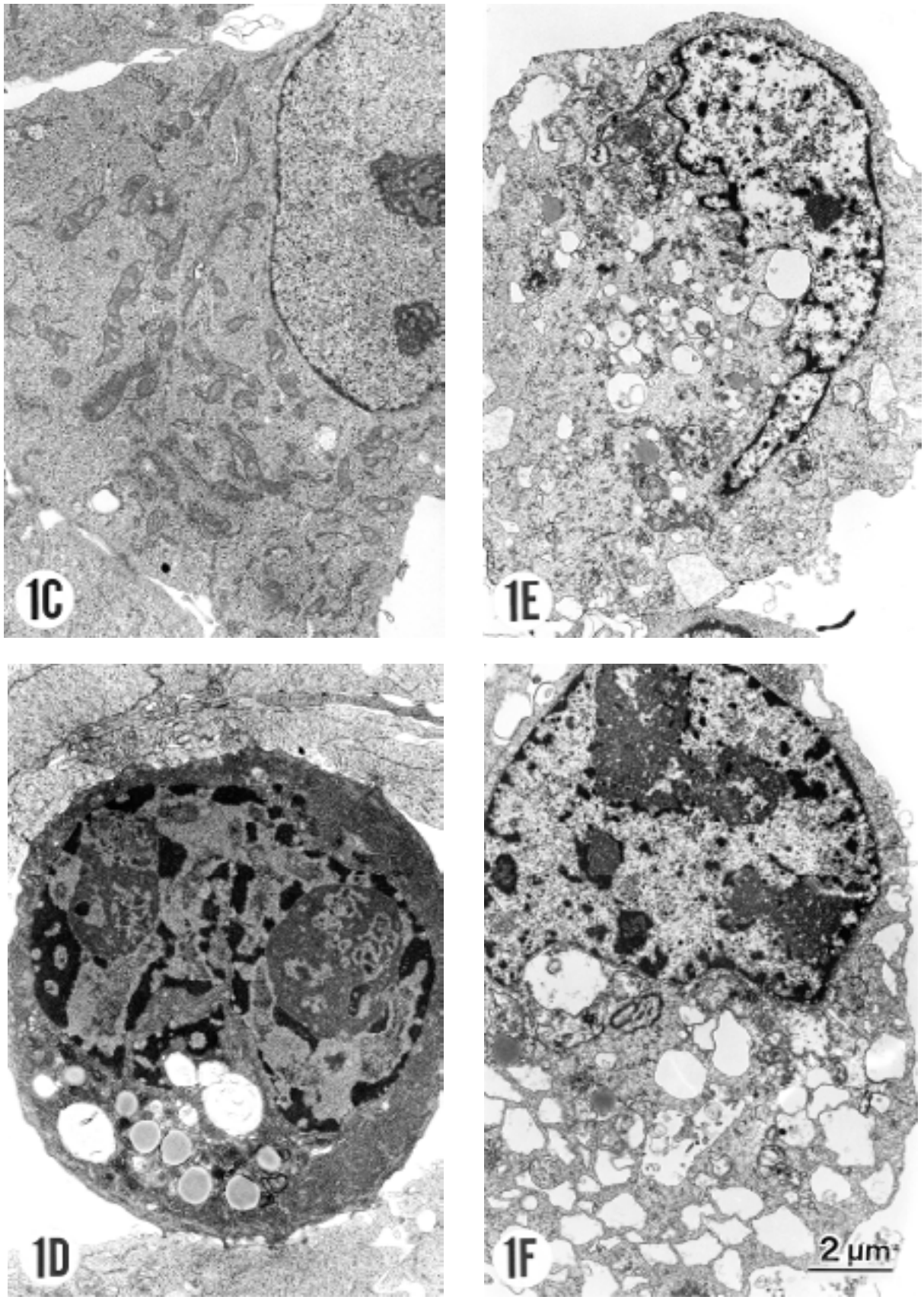

Figure 1. C. Untreated cell (control); **D.** Typical apoptotic cell; **E.** Typical necrotic cell; **F.** Cell having signs of apoptotic and necrotic changes. Magnification of electron micrographs: 1A, B: × 3,500; 1C, D: × 8,750.

Sperandio et al. [12] have proposed a form of programmed cell death that is distinct from apoptosis by the criteria of morphology, biochemistry and response to apoptosis inhibitors. They named this type of cell death "paraptosis"

In the present situation the question could arise: what is the crucial difference between apoptotic and necrotic mechanisms? We hope that our studies can shed light on a clue to this problem.

MATERIAL AND METHODS

Cell culture

Human osteosarcoma line 143B cells (ATCC-CCRL8303) were cultured at 37°C in a humidified atmosphere with 5% CO_2 in Dulbecco's modified Eagles Medium (DMEM, Nissui Co. Ltd, Tokyo, Japan), containing 1 mM pyruvate, supplemented with 10% foetal bovine serum and 50 μg/ml kanamycin. The cells were kindly provided by Dr M. Tanaka, Department of Gene Therapy, Gifu International Institute of Biotechnology, Yagi Memorial Park, Gifu, Japan.

Treatment with the chemicals

Cells were cultured in the presence of MEN (Sigma, St Louis, MO) at a final concentration of 100 μM. The reagent was prepared as 1000× concentrated stock solution dissolved in MiliQ water and stored at –20°C.

Electron microscopy

An equal volume of fixative containing 4% glutaraldehyde, 4% formaldehyde and 0.2 M Na-cacodylate was added to the culture medium to fix detached and attached cells. After fixation with the aldehyde solution, samples were processed for electron microscopy, as described before [13]. Thin sections were stained with lead citrate and examined in a Hitachi 7000 electron microscope operated at 75 kV.

RESULTS AND DISCUSSION

It is a well-known fact that the switch from apoptosis to necrosis takes place when intracellular levels of ATP become decreased, intracellular levels of reactive oxygen species become too high or caspases are inactivated.

When the cells treated for 24 hours with 100 μM MEN were examined by low magnification electron microscopy, most of them seemed to have undergone apoptotic or necrotic changes (Fig. 1B) compared to the control cells (Fig. 1A, C). Closer examination of these cells by higher magnification electron microscopy detected three different types of cells depending on their structural changes: apoptotic cells with condensed nuclei and cytoplasm (Fig. 1D), necrotic cells with pale nuclei and swollen cytoplasm (Fig. 1E); and those with condensed nuclei and swollen cytoplasm (Fig. 1F). Namely, both apoptotic and necrotic changes co-existed in one single cell in the third type of the cells.

We believe that the present result is the first in literature to give direct evidence that the switch from apoptosis to necrosis does occur inside the cell in the case of menadione treatment. We are now carrying out experiments to clarify detailed switch mechanism and the data will be reported soon.

ACKNOWLEDGEMENTS

The authors thank Mr M. Masaoka for his skilled technical assistance.

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