Melatonin prevents H_2O_2-induced apoptosis in U937 cells

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Keywords: H_2O_2, apoptosis, melatonin, U937, MTT

The pineal hormone melatonin (MEL) is implicated in protection against cell death resulting from oxidant treatments, chemotherapy and radiation action in various cell types [1, 2, 3, 4]. UVB-radiations induce an apoptotic effect in U937, a human leukemia monocytic cell line, antagonized by MEL addition [2]. Both UVB and H_2O_2 lead to an increased free radical production, responsible for cell death induction. These free radicals progressively generate intracellular H_2O_2, that is a relatively stable compound during UVB treatment, no more dangerous for the cell [5]. In fact, the antioxidant catalase and glutathione monoester inhibit apoptosis only when induced by H_2O_2 and not by UVB [6], so confirming the different H_2O_2 and UVB mechanisms of action.

The aims of the present study are: 1) to analyse the apoptotic cell death induced in U937 cells exposed to 500 μM H_2O_2, the concentration known to induce apoptosis [7] and 2) to study MEL effect before and after H_2O_2 exposure.

MTT assay [8] evidences a 100% cell viability in the control. Differently, H_2O_2 alone treatment and MEL incubation after H_2O_2 exposure reduce U937 cell viability to 75.7 % and 74%, respectively. On the other hand, MEL pre-treatment prevents cell viability loss, and the percentage of living cells is 83.9% (A).

Morphological analyses at light (LM) and electron microscopy (TEM) show that control U937 reveal a good cell morphology (B, C), with well preserved nucleus and organellar component (D).

After 500 μM H_2O_2 exposure, they undergo apoptosis: morphological analyses reveal indeed chromatin margination and condensation, micronuclei, cell vacuolisation and secondary necrosis (E, F). Figure G shows the typical cup-shaped chromatin patches. MEL pre-treatment prevents apoptosis: in fact, microscopy reveals a decreased number of apoptotic cells, however in all apoptotic stages (H, I). Figure L evidences an apoptotic body, containing several nuclei with clumped chromatin. On the contrary, MEL post-incubation reveals morphological patterns comparable to those observed after H_2O_2 treatment alone: in particular, the presence of a larger amount of micronuclei and cells in secondary necrosis appears at LM and TEM observations (M, N). Cytoplasmic vacuolisation is, again, observable in figure O.

In conclusion, these preliminary results demonstrate that H_2O_2, at a proper concentration, can act as an apoptotic trigger in U937 cells. MEL anti-apoptotic role is also revealed, but only in pre-incubated U937 cells. Further studies are in progress to better characterize this mechanism and, in particular, MEL behavior in H_2O_2-induced apoptosis in comparison to that induced by UVB.

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-263, © Verlag der TU Graz 2009

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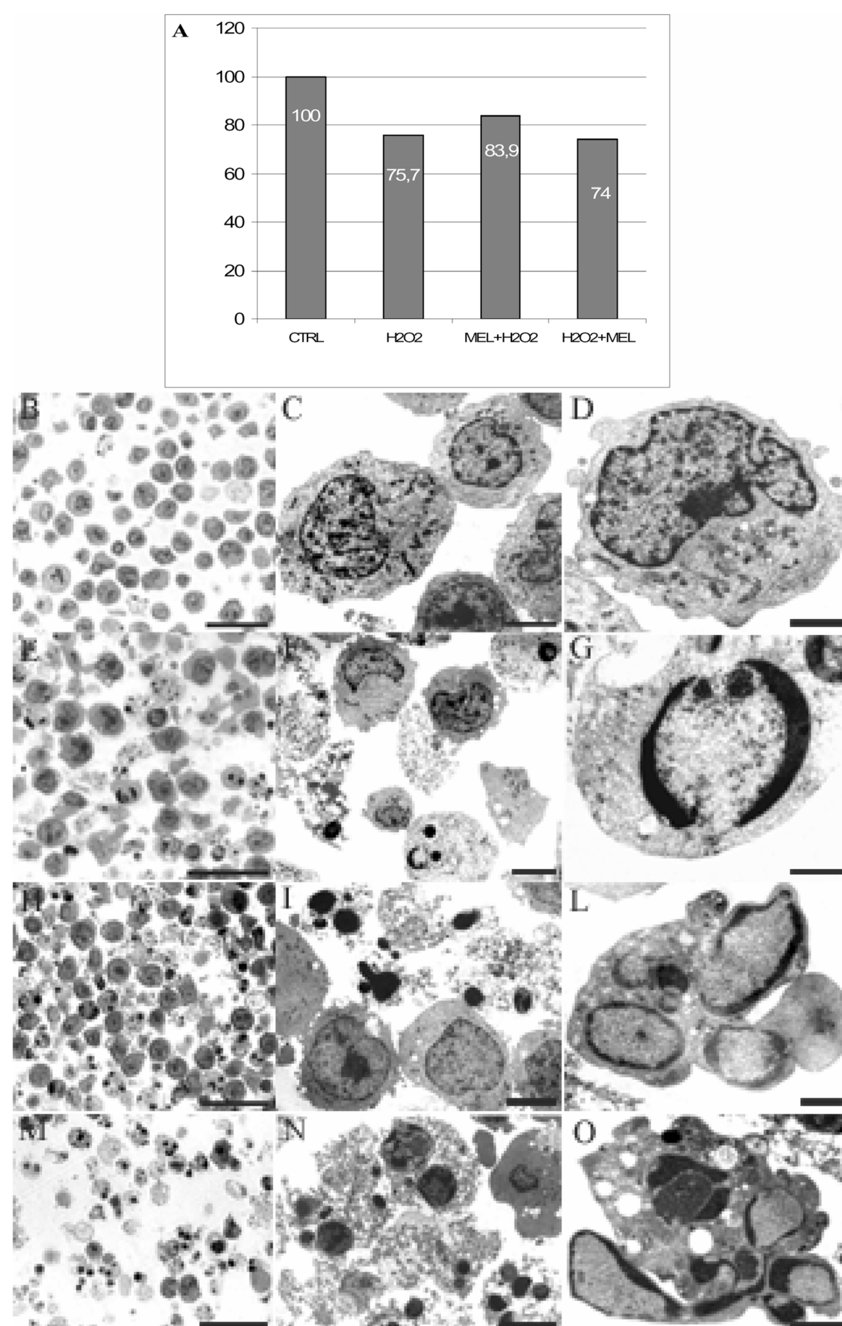

Figure 1. MTT assay (A). U937 LM and TEM in control condition (B, C, D), after H_2O_2 exposure (E, F, G), with MEL pre-incubation (H, I, L) or MEL post-incubation (M, N, O) after H_2O_2 exposure. Bar = 20µm for B, E, H, M; bar = 5 µm for C, F, I, N; bar = 2µm for D, G, L, O.

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