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Chemical-induced hemopoietic cell death: the protective action of melatonin

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Melatonin is a methoxyindole synthesized by the pineal gland. The hormone is secreted during the night and appears to play multiple roles in the human organism.

Melatonin is a powerful anti-oxidant in tissues [1] as well as within cells [2], with a fundamental role in ameliorating homeostasis in a number of specific pathologies. It acts both as a direct radical scavenger and by stimulating production/activity of intracellular anti-oxidant enzymes, i.e. superoxide dismutase and glutathione peroxidase as well as up-regulating their expression. Its function consists in the reduction of oxidative stress, i.e. molecular damage produced by reactive oxygen and nitrogen species [3].

A survey of literature shows that in leukocytes melatonin mainly exerts an anti-apoptotic role, as also demonstrated in an our previous work exposing hemopoietic cells to UVB rays [4].

In this work, some chemical triggers (hydrogen peroxide, etoposide and staurosporine), with different mechanism of action and able to increase radical oxygen species levels [5], have been chosen to induce cell death in U937 hemopoietic cell line. Cells were pre-treated with different melatonin concentrations (1 or 0.1 mM) and then exposed to chemical agents.

Potential melatonin anti-oxidant and anti-apoptotic effects were evaluated through morphological and statistical analyses, the latter by means of Tali Image-Based Cytometer, able to monitor cell viability as well as apoptosis presence. Samples, observed at scanning (SEM) and transmission electron microscopy (TEM), revealed a preserved morphology in control (Figure 1A-B) and melatonin alone (Figure 1C-D) conditions. After all chemical treatments typical apoptotic features appeared. Cells showed membrane blebs, nuclear cup-shaped masses, micronuclei, altered and swollen mitochondria, cytoplasmic vacuolisation and secondary necrosis (Figure 1E, F, I, L, O, P). A cell death decrease was observed in all experimental conditions pre-treated with melatonin (Figure 1G, H, M, N, Q).

In addition, in cells pre-incubated with melatonin (in both concentrations) and then exposed to chemicals, autophagic vacuoles appeared at TEM (Figure 1R).

Melatonin anti-apoptotic activity was also demonstrated by quantitative analyses. Supravital propidium iodide [6], used to evaluate cell viability, evidenced that melatonin administration significantly prevents cell death (Table 1). In particular, melatonin 100 µM showed an elevated protection against apoptosis induced by hydrogen peroxide (H_2O_2), staurosporine and etoposide. Melatonin 1mM has moderate anti-oxidant and anti-apoptotic effects *vs* chemical treatments.

In conclusion, we found that in U937 cell line melatonin can be considered a powerful molecule in preventing chemical apoptotic cell death.

1. G. Baydas, S.T. Koz, M. Tuzcu, E. Etem and V.S. Nedzvetsky. J Pineal Res. 43 (2007), p.225.
2. F. Luchetti,B. Canonico, F. Mannello, C. Masoni, A. D'Emilio, M. Battistelli, S. Papa and E. Falcieri. Toxicol In Vitro. 21 (2007), p. 293..
3. R.J. Reiter, D.X. Tan, L.C. Manchester and W. Qi. Cell Biochem Physiol 34 (2001), p, 237.
4. F. Luchetti, B. Canonico, R. Curci, M. Battistelli, F. Mannello, S. Papa, G. Tarzia and E. Falcieri. J Pineal Res. 40 (2006), p.158.
5. S. Salucci, M. Battistelli, S. Burattini, C. Squillace, B. Canonico, P. Gobbi, S. Papa and E. Falcieri. Micron. 41(2010), p.966.
6. L. Zamai, B. Canonico, F.Luchetti, P. Ferri, E. Melloni, L. Guidotti, A. Cappellini, G.Cutroneo, M.Vitale, S. Papa. Cytometry. 44 (2001), p.57.

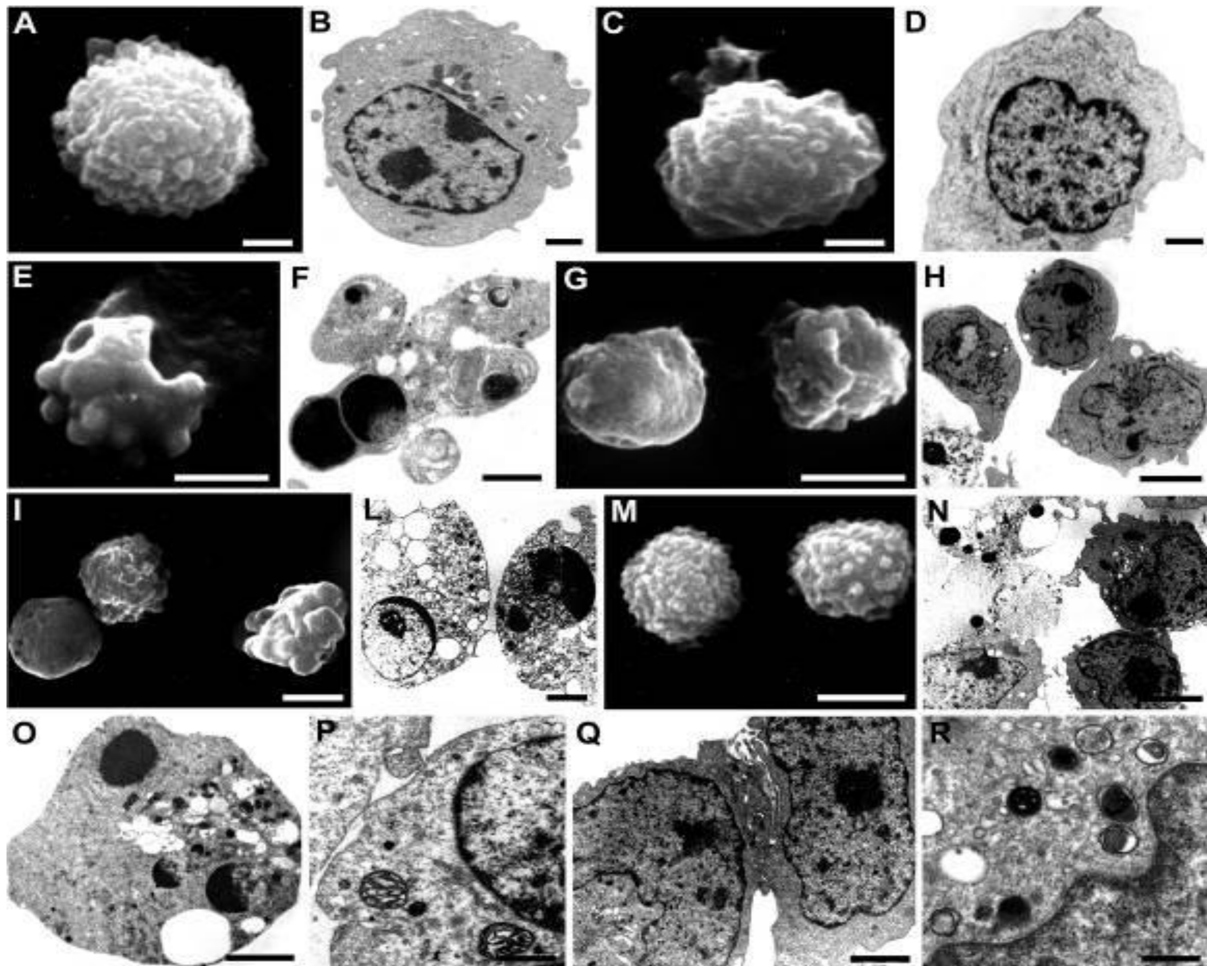

Figure 1. U937 cells at SEM (A, C, E, G, I, M) and TEM (B, D, F, H, L, N,O,P,Q,R). A preserved morphology is evident in control (A,B) and melatonin (0.1mM) alone conditions (C,D). Cells treated with H_2O_2 (E,F) etoposide (I,L) and staurosporine (O,P) reveal typical apoptotic patterns. Melatonin added to H_2O_2 (G,H) etoposide (M,N) and staurosporine (Q) treated cells prevents cell death. In R, autophagic vacuoles appeared in melatonin added to etoposide. Bars: 2µm in A-D, O,Q; 5 µm in E-N; 1 µm in P; 0.5 µm in R.

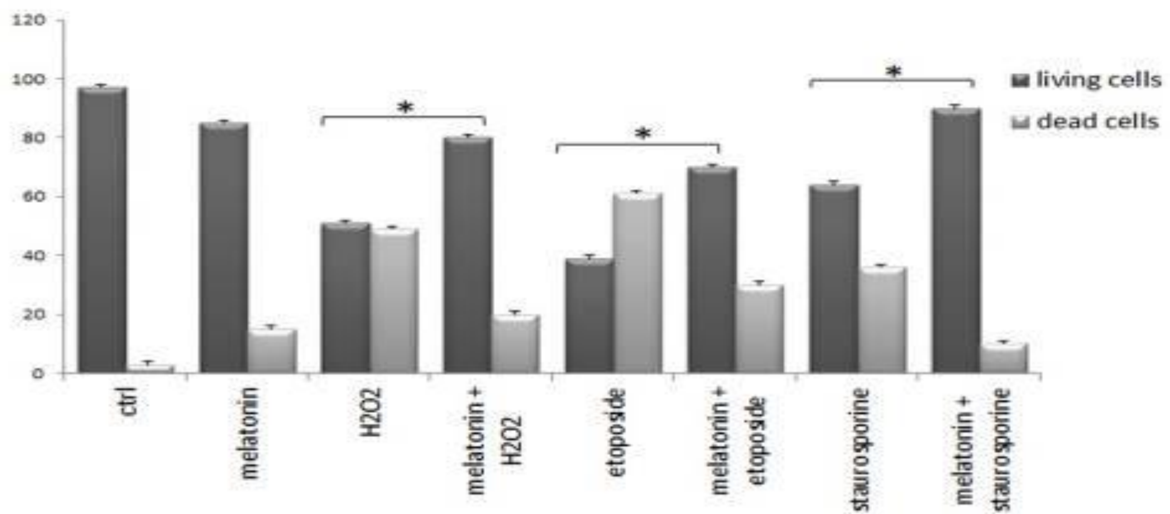

Table 1. Cell viability percentage. Data have been are verified by t-test and appeared statistically significant ($p < 0.05$).