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Melatonin role in the prevention of myoblasts apoptosis induced by chemical triggers

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Melatonin, or N-acetyl-5-methoxytryptamine, is a hormone principally secreted by the pineal gland that regulates the sleep-wake cycle maintaining the circadian rhythm. Due to its ability to induce sleep, melatonin is used in pharmacological doses to resynchronize the internal biological clock [1]. Furthermore, melatonin regulates gonadal function, stimulates the immune response, has anti-inflammatory actions and exhibits strong antioxidant effects [2]. In fact, it is able to act directly as a radical scavenger and it also has an indirect activity, stimulating the production of antioxidant enzymes (i.e. glutathione peroxidase and glutathione reductase) and increasing electron transport chain efficiency at mitochondrial cristae level. Due to this property, it is present in moderate amounts in the cell nucleus, where it protects DNA from oxidative damage, potentially acting as a key factor in cancer prevention [3].

Our experiments relied on the antioxidant properties of melatonin and the aim of our work was to test melatonin effects on the cytotoxicity induced by various chemical triggers, in murine C2C12 skeletal muscle cells. Muscle apoptosis is attracting growing interest, due to its demonstrated involvement in several pathological conditions, such as sarcopenia and disuse muscle atrophy [4], myotonic dystrophy, Duchenne muscular dystrophy [5], collagen myopathies [6] and others. It has also been demonstrated that oxidative stress is involved, at least in part, in most of these conditions involving muscle apoptosis [7]. In literature we can find numerous evidences which indicate that melatonin treatment is able to normalize or prevent oxidative stress, leading to an improvement of certain pathological conditions [8]. Furthermore, in previous works carried out by our research group, it was shown that melatonin prevents apoptosis induced by UVB [9] and H_2O_2 [10], in human U937 hematopoietic cells.

For our purpose, C2C12 myoblasts were pre-treated with 100 µM melatonin for 24 hours and then treated for further 24 hours with various chemical triggers: 0.5 mM H_2O_2, 30 µM cisplatin, 0.25 µM staurosporine, 25 µM etoposide. These chemicals were chosen on the basis of their know pro-apoptotic effect and of their different mechanisms of action. Cellular growth and viability were monitored using inverted microscopy (IM), while the cellular response to treatments was investigated by means of transmission electron microscopy (TEM), TUNEL technique and supravital propidium iodide staining [11], analyzed by means of confocal laser scanning microscopy (CLSM), and DNA gel electrophoresis.

All treatments resulted in the alteration of cellular morphology with typical apoptotic features, such as detachment from the substrate, membrane blebbing, chromatin condensation and micronuclei formation, production of apoptotic bodies. Autophagic features were also observed. Our results indicate that melatonin pre-treatment prevents apoptosis induced by H_2O_2 and, to a lesser extent, by cisplatin and etoposide (Figure 1); conversely, no protective effect was observed in samples treated with staurosporine, and this may be due to its mechanism of action (Figure 2). Agarose gel electrophoresis revealed that DNA oligonucleosomal cleavage is absent in all experimental conditions. Intriguingly, melatonin increases the autophagic rate, probably as a further protective mechanism.

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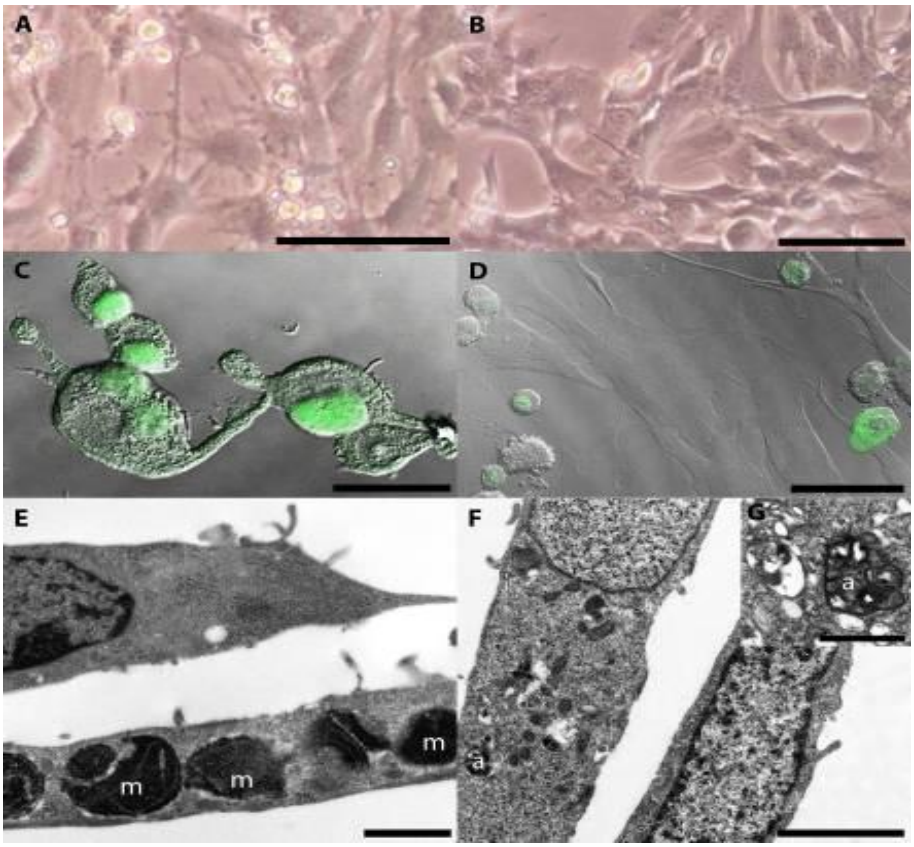

Figure 1. IM of cisplatin-treated (A) and melatonin pre-incubated cisplatin-treated (B) myoblasts; CLSM of TUNEL reaction after H_2O_2 exposure (C) and melatonin pre-treatment H_2O_2-exposure (D); TEM of etoposide-treated myoblasts (E) and melatonin pre-treated etoposide-exposed ones (F); detail of autophagic vacuoles (G). m = micronuclei, a = autophagic vacuole. Scale bar: A, B = 15 µm; C = 20 µm; D = 25 µm; E = 2 µm; F = 5 µm; G = 1 µm.

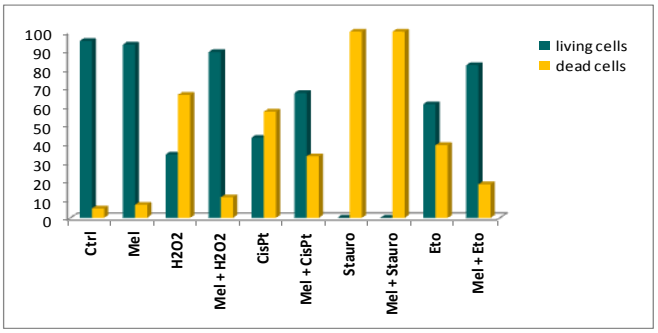

Figure 2. Histogram showing the cell viability, using the trypan blue exclusion assay, after all treatments. * = p<0,05