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Prevention of UVB radiation-induced skin damage: “in vitro” studies.

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The ultraviolet component of sun light consists of UVA, UVB and UVC rays. They differentially penetrate the skin barrier, thus prevalently affecting epidermal (UVB) or dermal (UVA) cells and causing various pathologies.

UVB represent an environmental hazard because of their role in skin aging, cancer and infection exacerbation.

UVA and UVB rays stimulate indeed the production of reactive oxygen species (ROS) in epidermal cells, resulting in skin lesions, accelerating aging and eliciting malignancies.

At least 50% of UVB-induced damage is attributable to the formation of reactive ROS [1]. Although ROS also appear in normal physiologic processes, including aerobic metabolism, they may cause cellular damage if antioxidant defense mechanisms are down-regulated [2]. Skin cells are also equipped with an elaborate system of antioxidant substances and enzymes, which maintains the balance between oxidative stress and anti-oxidant defense and keeps the cells away from oxidative stress damage. Thus, exogenous supplementation of antioxidants may be an effective strategy to reduce or prevent skin damage.

In the last years, we demonstrated the antioxidant effects of melatonin (Mel) in hemopoietic human cells [3]. On the other hand, data concerning its antioxidant effect on keratinocytes have been also reported [4]. Recently, our research group demonstrated an anti-oxidant and anti-apoptotic action of hydroxytyrosol (HyT), and its derivatives in muscle and hemopoietic cells exposed to H2O2. These data suggest interesting properties of HyT, and its potential application in biology and clinic [5].

Therefore, in this project we propose to evaluate the antioxidant and anti-apoptotic effect of Mel and HyT in HaCaT human keratinocytes exposed to UVB rays, well known cell death inducers.

Keratinocytes in the non-irradiated condition are morphologically similar in Mel and HyT-treated and untreated group. Cells appear flattened and closely confluent, with fusiform or, sometimes, polygonal shape (Fig. 1 A, B, C). In HyT-treated ones a significant confluence increase can be revealed (Fig. 1 D, E, F), cells appear polygonal, slightly swollen and closely in contact each other. TUNEL reaction appears negative in both conditions (Fig 1 C,F).

UV-B radiations induce a significant decrease in cell confluence with the occurrence of cell detachment and the appearance of consequent empty spaces (Fig 1I). Detached cells appear rounding with surface blebbing (Fig. G, H, I) .

TUNEL reaction, observed at confocal microscopy, evidences several nuclei with DNA fragmentation in UV-B treated keratinocytes. In addition, cell viability evaluated by means of propidio iodide (PI) supravital staining [6] evidences a diffuse positivity.

Pre-treatment with HyT or Mel reduce cell death. Cells maintain unchanged the shape, appear fusiform or polygonal and monolayer confluence can be again revealed.

We can conclude that HyT and Mel evidentiate an intringuing capability to prevent cell death in keratinocytes too. They could so represent a potential tool in skin protection from UVB radiations.

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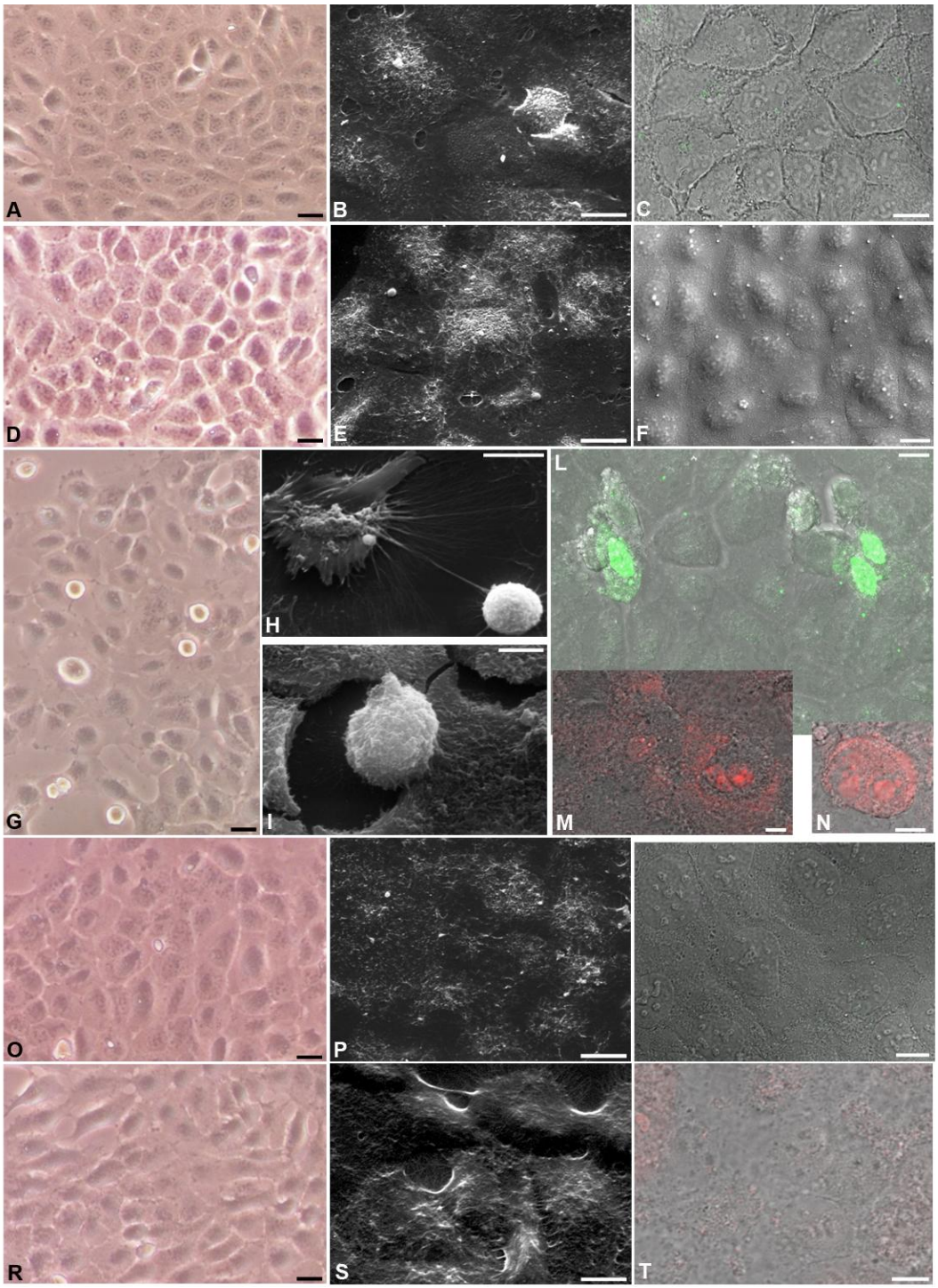

Figure 1. Inverted Microscope: A,D,H,N,Q; Scanning Electron Microscopy: B,E,H,I,P,S; TUNEL: C,F,L,T, PI staining: M,N,Q. Keratinocytes in different conditions: A,B,C control cells; D,E,F, HyT treated cells; G,H,I,L,M,N UVB-treated cells; O,P,Q; HyT pre-treated cells; R,S,T Mel pre-treated cells.
A,D Bar = 25µm; C,F,G,H,L,Q,P,S, , Bar = 12µm; B,E,O,R Bar = 9µm; I,M,N Bar = 6µm.