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Evaluation of protective effect of Thymol on UVB-induced damage in an ex-vivo human skin tissue model: morphological analysis and genotoxic evaluations

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Skin, the most superficial tissue of our body, is the first target of environmental insult, among which is the most important solar ultraviolet (UV) radiation (Bernerd et al., 2001). For this reason, the use of human skin tissue obtained from plastic aesthetic surgery represents a simple but efficient experimental approach to reproduce a physiological condition to test the early effects of an exogenous stimulus as UV radiation and the possibility of preventing or reducing the early epidermal effects. Normal human skin explants were obtained from healthy young non smoking women 20-40 years old (n=5) after informed consent and cultured epidermal side up at the air-liquid interface overnight in a Transwell system before treatment (Donetti et al., 2005; Bedoni et al., 2007). They were further divided in two groups: the first was exposed to UVB doses ranging from 0.24 J/cm^2 to 0.72 J/cm^2 and the other one pretreated for 1 h with Thymol (natural monoterpene phenol, 6.6 μM), before the UVB irradiation. In each experiment a cultured sample was not UVB exposed and represented the internal control. Samples were harvested 24 hours after of UVB exposure. Lactate Dehydrogenase (LDH) assay and alkaline comet and micronucleus tests were used to assess cytotoxicity and genotoxicity, respectively. Bioptic fragments were processed both for transmission electron (TEM) and light (LM) microscopy. Epidermal proliferation was investigated by indirect immunofluorescence after incorporation of 5-Bromo-2'-deoxyuridine (BrdU).

UVB induced evident ultrastructural alterations in nucleus and cytoskeleton, while the pretreatment with Thymol showed a reduction of damage in UVB exposed samples both from the morphological point of view that genotoxic aspects. Cell proliferation was strongly inhibited by UVB exposure, while in Thylom pretreated samples was comparable to control.

Furthermore these results strongly support the use of ex vivo human skin as a relevant method for safety evaluation of UV skin exposure.

References

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Ultraviolet radiation, Thymol, organotipic cultures, keratinocyte proliferation, transmission electron microscopy, cytotoxicity, genotoxicity.