Topical melatonin: evaluation of the expression of antioxidant genes *versus* topical control

Campos C.[1], Mullor J.[2], Sánchez Sánchez A.[2], Trullàs C.[3]
1. ISDIN, Medical Affairs Area, Barcelona, Spain, 2. Bionos Biotech, Technical Department, Valencia, Spain, 3. ISDIN, Innovation Area, Barcelona, Spain

INTRODUCTION AND OBJECTIVES

Free radicals are key factors in skin aging process. To provide protection against oxidative stress, the skin uses endogenous antioxidants compounds and enzymes.

Nuclear factor erythroid-2 like-2 (known as Nrf2) is a transcription factor that regulates the basal and stress-inducible expression of a battery of genes encoding key components of the antioxidant systems. It initiates transcription and translation of antioxidant genes into proteins such us Superoxide Dismutase 1 (SOD1), Catalase (CAT) and Glutathione Peroxidase 1 (GPx1) (Figure 1). The topical application of a molecule able to stimulate the expression of these genes will be a good strategy to increase the skin's own capacity for fight against oxidative stress.

A product containing Melatonin was tested on an *in vitro* model using medaka eleutheroembryos (ME) to demonstrate the indirect antioxidant activity. At the molecular level, signal pathways and mechanisms are well conserved between fish and humans. This method effectively simulates the topical application on the skin and allows employing the finish formulation without dilution. This way it facilitates the extrapolation of the data to *in vivo*.

This study measures the effects of a product containing Melatonin on the expression of antioxidant SOD1, CAT, Nrf2 and GPx1 genes *versus* control.

MATERIAL AND METHODS

Same formulation with and without Melatonin were evaluated undiluted and topically applied on ME.

Assessments were performed after 2 hours (response time) and after 3 days (cumulative response). Total RNA was purified, quantified and used to synthesize complementary DNA (cDNA). The cDNA from treated or untreated ME (control) was used to determine the relative gene expression of SOD1, CAT, Nrf2 and GPx1 through real-time quantitative PCR (Polymerase Chain Reaction). Actin was used as reference gene.

RESULTS

Topical application of Melatonin serum increased expression of all genes at all time points significantly (Figure 2 and 3).

Since the Nrf2 gene codes for a potent transcription factor, it was moderately activated, whereas the rest of the genes had a higher response (Table 1).

CONCLUSIONS

Efficient ROS detoxification is particularly important in the skin, which is challenged by UV light, mechanical insults or exposure to various irritants, allergens, pollutants and pathogens.

Melatonin, after topical application, behaves as an indirect antioxidant by up regulating the gene expression of the antioxidant enzymes, from the start of the metabolic chain (by increasing the Nrf2) to the expression of genes responsible of antioxidant enzymes.

Therefore Melatonin has demonstrated to be effective to reinforce the skin against oxidative stress and can be a new compound of the anti-aging armamentarium.

REFERENCES

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Figure 1 Schematic presentation of (Nrf2)-antioxidant response element (ARE) pathway activation by reactive oxygen species (ROS). [Chen J Diabetes Metab J. 2014]

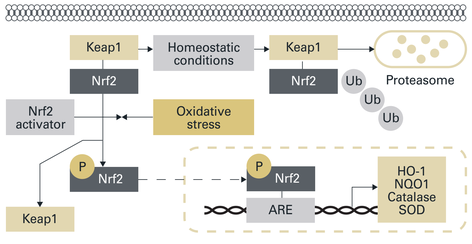


Table 1 Expression changes vs. control of SOD1, CATt, Nrf2 and GPx1 genes after topical treatment on medaka eleutheroembryos using RT-qPCR

		SOD 1	CAT	Nrf2	GPx1
2 hours	Vehicle serum	22,5%	19,1%	17,6%	38,8%
	Melatonin serum	42,8%	39,5%	11,8%	107,6%
3 days	Vehicle serum	4,4%	15,03%	10,88%	-1,65%
	Melatonin serum	52,3%	31,06%	28,68%	56,64%

Figure 2 Expression of antioxidant SOD1, CAT, Nrf2 and GPx1 genes after treating medaka eleutheroembryos during 2 hours

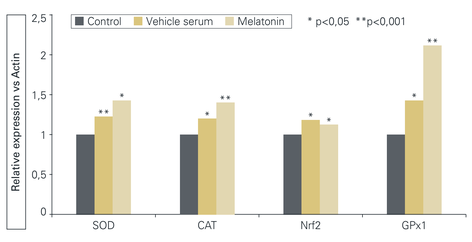
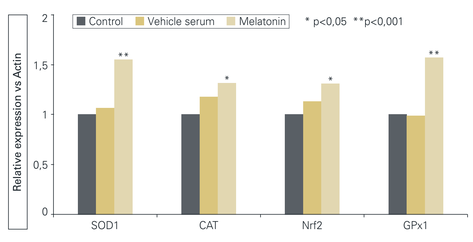


Figure 3 Expression of antioxidant SOD1, CAT, Nrf2 and GPx1 genes after treating medaka eleutheroembryos during 3 days



14th EADV Spring Symposium. Brussels, Belgium. 25-28 May 2017