PRELIMINAR STUDIES OF THE ANTIOXIDANT ACTIVITY OF MELATONIN FORMULATED WITH DIFFERENT SUNSCREENS FOR TOPICAL USE

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INTRODUCTION

Today it is evident that the use of sunscreen is necessary to prevent not only sunburn but also skin cancer. In recent years there has been an increased interest in the use of antioxidants, and their use in combination with sunscreens can provide advantages to sunscreen formulations. Melatonin is an antioxidant which scavenges and detoxifies Reactive Oxygen Species (ROS) (1) and able to suppress ultraviolet (UV)-induced damage to skin cells and shows strong antioxidant activity in UV exposed cells (2). Considering these relevant biological properties and its extremely low toxicity, melatonin could be candidate as a drug for several diseases and presently it is indicated for therapeutic applications.

The aim of this study was to evaluate if the presence of a solar filter in combination with melatonin in different formulations influences in permeation and retained amounts in skin of melatonin, and to evaluate the influence of this hormone in the antioxidant activity of these formulations.

MATERIALS AND METHODS

Materials

- Melatonin
- Excipients: Benzophenone-3 (3-Bph), Octyl salycilate (OS), Octyl methoxycinnamate (OMC), Perhidroescualen, Medium chain triglycerides and Montanov®68 .
- 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH).

Methods:

There were elaborated 3 different glucidic emulsions using perhidroescualen, medium chain triglycerides and Montanov^{68}, a 5% of a solar filter (Octyl salicylate, Octyl methoxycinnamate or Benzophenone-3) and a 1% of melatonin.

- In vitro permeation studies: Franz diffusion cells of 2,54 cm^2. Human abdominal skin (0,4 mm). Temperature: 32±0.5ºC. Samples obtained at 24 and 50h.
- Skin retention study: Temperature of 32+0.5ºC for 24h in different extraction medium by ultrasound technique.
- Antioxidant activity: DPPH radical scavenging assay: Samples measured at 515 nm on a UV/visible light spectrophotometer. Samples were kept in the dark for 30 min at room temperature. (3)
- Statistical analysis: All the results were compared by non-parametric analysis (Kruskall-Wallis) with a significance level of 0.05.

RESULTS AND DISCUSSION

Tables 1 and 2: (1) Quantities of Melatonin (µg) permeated at each time for the 3 formulations, expressed by mean and (2) Non parametric test of Kruskall-Wallis

	MLT + OS	MLT + OMC	MLT + 3-Bph
t (h)	Mean (µg)	Mean (µg)	Mean (µg)
4,00	5,183	3,089	2,696
18,08	84,199	69,843	74,268
21,10	122,376	98,612	126,633
24,03	161,388	127,284	136,948
27,03	204,602	159,714	175,108

Kruskal-Wallis test	
P value	0.8694
Exact or approximate P value?	Gaussian Approximation
P value summary	ns
Do the medians vary signif. (P < 0.05)	No
Number of groups	3
Kruskal-Wallis statistic	0.28

There were no differences in the quantity of melatonin permeated (p=0.8694), reaching values over 100 µg after 24 h of experience in all cases,

Table 3. Results of the 3 extractive solutions studied, with the average and the standard deviation for different solvents. ACN (acetonitrile).

	% of recovery	
	Average (%)	DE
ACN/water	23,2	23,2
Meth./water	37,2	17,6
Eth./water	86	26,8

According to the data, the means with the best recovery rates and therefore the one used in our study was ethanol / water, with a value of 86%.

Formulation	Q (*) (µg)	Skin weight (g)	Qext (µg/g/cm^2)	Qret 50h (µg/g/cm^2)
MLT	3,90E+01	1,41E-01	1,17E+02	1,36E+02
MLT + OS	4,59E+01	1,93E-01	1,25E+02	1,45E+02
MLT + OMC	5,49E+01	1,92E-01	1,07E+02	1,25E+02
MLT + BP3	4,62E+01	1,64E-01	1,07E+02	1,25E+02

Table 4. Extractive amount (mg), skin weight (g), extractive amount normalized by g and cm^2 on skin and retained amount in skin at 50h, for each formulation, expressed by median

The remaining amounts in skin at 50h for the 3 formulations were about 125 and 145 µg/g/cm^2. (*) P > 0,05 the results showed non significantly statistical differences between the values of retained amounts from the different formulations

Melatonin (**MLT**)	35.088
Octyl salicylate (**OS**)	0.000
Octyl metoxycinnamate (**OMC**)	10.526
3-benzophenone (**3Bph**)	1.096
Perhidroescualen (**PHE**)	Inactivo
Medium chain triglycerides (**TG**)	Inactivo
Mentanov 68 (**Mv**)	Inactivo
[PHE+TG+Mv]	Inactivo
MLT+[PHE+TG+Mv]	Inactivo
MLT+OS+[PHE+TG+Mv]	1.974
MLT+OMC+[PHE+TG+Mv]	0.439
MLT+3Bph+[PHE+TG+Mv]	2.851
OS+OMC+3Bph+[PHE+TG+Mv]	Inactivo
MLT+OS+OMC+3Bph+[PHE+TG+Mv]	Inactivo
OS+[PHE+TG+Mv]	Inactivo
OMC+[PHE+TG+Mv]	Inactivo

Table 5. % of reduction of radical DPPH at 1.000 ppm (w/v) of each compound or formulation.

There were compared the sunscreen formulation with and without melatonin. In absence of melatonin, none of the 3 formulations have antioxidant activity. In contrast, the addition of 1% of melatonin increased the antioxidant activity of the formulation containing 3-Bph and OMS, with a percentage of reduction of 64,52%±2,26 and 23,10%±1,15 respectively. In the case of the formulation with OS, the addition of melatonin to the formula didn't offer any improvement in antioxidant activity.

CONCLUSION

The results of the study shows that melatonin formulated at 1% with different solar filters as topical formulations applied to human skin, presented permeation and retention capacity. The different nature of the filters didn't influence in the quantity of melatonin permeated and retained in the skin. The use of melatonin in combination with some solar filters enhances the antioxidant activity of sunscreens.

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