EFFECT OF NUTRIENTS ON REACTIVE OXYGEN SPECIES, ATP AND MELANOGENESIS STATUS IN CULTURED MELANOCYTES

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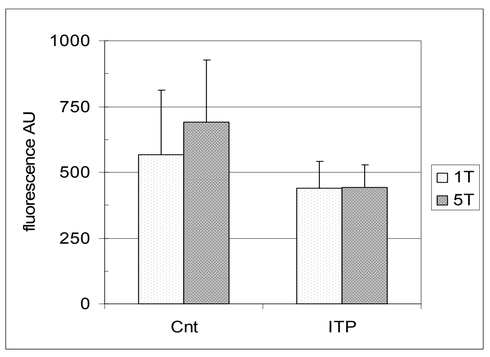
AIM

Normal human skin melanocytes greatly respond to both intrinsic and extrinsic factors.

In this study we tested effects of nutritional ingredients mixture Imedeen Time Perfection ™ (ITP) that includes antioxidants on parameters related to skin ageing: reactive oxygen species (ROS), ATP levels, and melanin production in cultures of human melanocyte monolayers.

RESULTS

The ITP nutrients caused a strong reduction of ROS levels. Reduced fluorescence of dihydrorhodamine 123 (DHR123) probe was demonstrated in a multiwell plate reader:

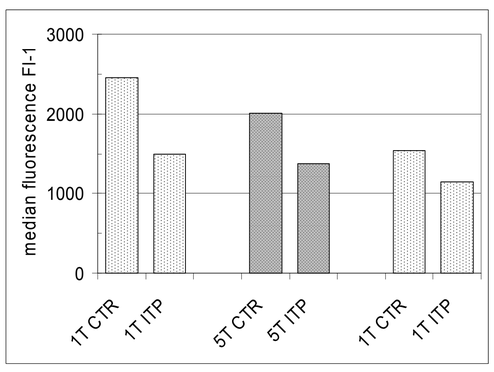


%	Cnt	ITP
1T	100	77
5T	100	64

Figure: Average DHR fluorescence for M9602p21 melanocytes in 12 wells incubated with DHR123.

Light hatch: 1T= low tyrosine cultures. Dark hatch: 5T = high tyrosine cultures. $p < 0.05$ for ITP in 5T cultures.

Reduction of ROS was confirmed by FACS measurements.



%	Cnt	ITP
1T left	100	60
5T	100	67
1T right	100	74

Figure: Mean DHR fluorescence for 10.000 gated cells measured by FACS.
Light hatch: 1T= low tyrosine cultures. Dark hatch: 5T = high tyrosine cultures.

The ATP levels remained relatively constant during the cell growth and only non-significant effects were found in the nutrient treated cultures.

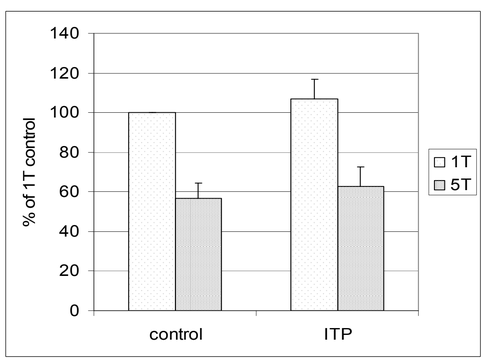


Figure: Summary of the results of ATP measurement. ATP was measured in 96 well plates on 4 different data in 2x diluted melanocyte cultures. Results of ITP treated cells were compared to the 1T control cultures (100%). On the average, 7% (1T) and 6 % (5T) higher ATP was found for the cells treated with ITP for the 1T and 5T cultures, respectively.

The nutrients caused reduced pigmentation in the melanocytes with tyrosine induced pigmentation (5T).

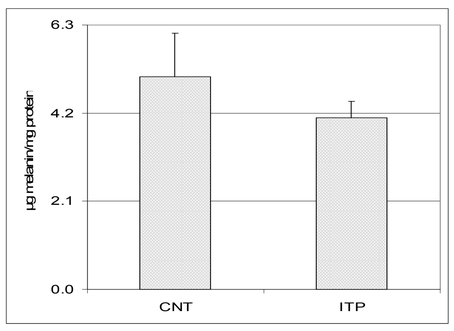


Figure: Average melanin content. A significant reduction (19%) of melanin content for the high tyrosine cultures (5T) in passages 15 to 17 ($p = 0.02$, $n = 6$).

The cell growth was not influenced by the ITP nutrients.

METHOD

The experiments were done in melanocytes from a skin type I donor according to a standard culture method [1]. These cells showed limited pigmentation in basic medium containing standard tyrosine concentration (1T) and a more pronounced pigmentation with increased tyrosine concentration (5-10T).

The Imedeen (ITP) nutrient mixture contained extracts of tomato, grape seed and acerola (antioxidant component) and Imedeen Marine Complex™ (nutritional component).

The ROS levels were measured fluorimetrically by using dihydrorhodamine 123 (DHR123) as a ROS sensitive probe. The ATP was measured using a bioluminescence assay. Melanin was measured spectrophotometrically. Cell growth was monitored by microscopic examination and by protein measurements.

CONCLUSIONS

The tested nutrients from Imedeen Time Perfection ™ (ITP) exhibited a strong antioxidant effect demonstrated by a reduction in ROS levels and reduced melanin production.

The effects of ITP shown in this study can be used to combat the oxidative stress and hyperpigmentation in vivo.

REFERENCES

[1] Smit et al. The combined effects of extracts containing carotenoids and vitamins E and C on growth and pigmentation of cultured human melanocytes. *Skin Pharmacol Physiol. 2004 ;17 (5):238-45.*

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