

## Identification of a phytochemical stimulant for the proliferation of mouse melanocytes in culture

A. RAMAN\*, Z. LIN\* \*\* AND J. R. S. HOULT†

\*Pharmacognosy Research Laboratories, Department of Pharmacy, and †Pharmacology Group, King's College London, Manresa Road, London SW3 6LX, and \*\*Institute of Chinese Medicine, 44-46 Chandos Place, London WC2N 4HS

Vitiligo is a common skin pigment disorder characterised by the development of patchy depigmented macules due to loss of functional epidermal melanocytes. A prospective successful treatment should be able to repopulate the lesions with melanocytes (Ortonne et al, 1993). During a herbal screening programme, we found that an aqueous extract of the fruit of *Piper nigrum* L. possessed melanocyte proliferant activity. In the present study, the effect of piperine, the main alkaloid derived from *Piper nigrum* on the proliferation of melan-a cell, a non-tumorigenic pigmented mouse melanocyte line (Bennett et al, 1987) was investigated.

Subconfluent melan-a cells were subcultured and  $6 \times 10^3$  cells/well were inoculated into 96-well plates containing piperine dissolved in MeOH/H<sub>2</sub>O. The final concentrations of piperine were 0.1 and 1  $\mu$ M, with 20 nM 12-*o*-tetradecanoyl-phorbol-13-acetate (TPA), a phorbol ester capable of stimulating melanocyte proliferation as positive control. The plates were incubated for 2, 4 and 6 days before they were subjected to SRB assay for cell number (Skehan et al, 1990). The growth pattern of melan-a cells in the presence or absence of piperine and TPA was monitored (Fig.). One way ANOVA and Dunnett's comparison were used to determine the significance of any difference.

TPA and piperine at both dose levels was found to stimulate the proliferation of melanocytes ( $P < 0.01$  when compared to cells only on days 4 and 6). The results were repeatable in several replicate experiments. The proliferant effect of piperine on

melan-a cells was effectively blocked by RO-31-8220, a selective inhibitor for protein kinase C (data not shown), indicating that the mechanism of action may involve activation of a PKC cell signalling pathway.

In the light of the above findings, we regard piperine as a prospective candidate for the stimulation of hair follicular outer root sheath melanocytes in vitiligo.

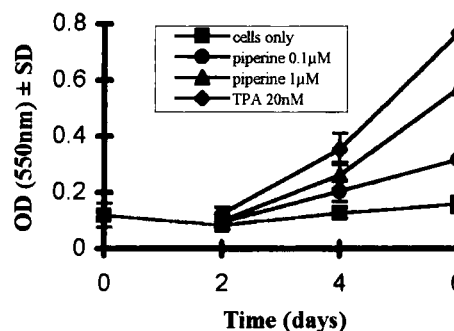


Fig. Effects of piperine and TPA on the growth of melan-a cell line (n = 6)

Bennett, D.C. et al (1987) *Internatl. J. Cancer*. 39:414-418.  
Ortonne, J. et al (1993) *Pigment Cell Research*. 6:61-72.  
Skehan, P. et al (1990) *J. Natl. Cancer Insti.* 82:1107-1112.

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