A183 An investigation into the role of protein kinase C in the melanocyte stimulatory effects of piperine and analogues

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Vitiligo is a skin disorder characterised by loss of pigmentation and active melanocytes from lesional areas. Numerous theories exist regarding its aetiology but as yet there is no concensus. The hair follicle is believed to harbour a melanocyte reservoir; stimulation of this reservoir to replicate can result in repopulation of the depigmented patches.

A potential future therapy under investigation by our group is the use of piperine (PIP) from black pepper (*Piper nigrum* L.). PIP has the capacity to stimulate melanocyte proliferation and alter cell morphology *in vitro*. This effect is blocked by the protein kinase C (PKC) inhibitor RO-31-8220 (1). PKC is one of the major signal transduction pathways regulating growth and cellular metabolism and has been implicated in a number of melanocyte functions.

Two analogues of PIP, tetrahydropiperine (THP) and the cyclohexylamide analogue (CHP), were synthesised and tested *in vitro* using a pigmented mouse melanocyte cell line to determine if they also possessed melanocyte stimulatory activity similar to the parent compound. To confirm the preliminary findings on mechanism of action, three inhibitors of PKC (RO-31-8220, staurosporine and calphostin C) were tested alone and in combination with PIP, THP or CHP to determine if cell stimulation by these compounds is affected and to outline a possible role for PKC.

Cells were seeded in 96-well plates ($6x10^3$ cells per well) and allowed to settle for 24 hours prior to addition of compound alone (PIP/THP/CHP) at 1 μM final concentration or compound (1 μM) plus inhibitor (10^{-4} to 10^{-9}M). Cells were fixed on experimental day five and assayed using sulphorhodamine B as described by Lin *et* al. (2).

Results showed that PIP and its analogues are able to stimulate melanocyte proliferation and inhibitors of PKC affect the activity of all three compounds. Therefore, the ability of these compounds to increase melanocyte cell numbers is likely to involve activation of PKC pathways.

References: 1. Lin et al., (1999) Planta Med. 65: 600-603. **2.** Lin et al., (1999) J. Ethnopharmacol. 66: 141-50.

A184 Antiproliferative effect of *Vernonia anthelmintica* (L.) Willd. seed extracts

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Vernonia anthelmintica seeds (VA) have been employed in traditional Ayurvedic medicine for a range of medical conditions including the common, chronic skin disorder, psoriasis (1, 2). The SVK-14 cell line was chosen as an *in vitro* model to investigate the effectiveness of various extracts of VA seeds, as it is a rapidly dividing immortalised human keratinocyte cell line. It was therefore capable of mimicking the hyper-proliferation of the epidermis characteristic of psoriatic plaques. Different VA extracts were prepared utilising sequential soxhlet extraction with light petroleum (LP), chloroform and methanol (MeOH) to afford extracts A3 (LP) and A7 (MeOH). A direct MeOH extraction gave MET and extraction of LP-defatted seeds with dichloromethane, resulted in extract DCM. Investigation of the effect of these extracts on SVK-14 cells revealed DCM to exhibit the highest level of activity (1.7 μg/mL), followed by MET (21.6 μg/mL). Extract A7 (73 μg/mL) gave a higher IC_{50} value than MET indicating that the potential active constituents in VA may not be completely polar. The oil extract, A3 displayed the least activity (170 μg/mL). Further *in vitro* experiments were undertaken to establish the mode of action of MET and DCM namely, lactate dehydrogenase (LDH) release and cellular adenosine triphosphate (ATP), indicators of membrane integrity and metabolic competence, respectively. DCM was found to cause LDH release and ATP depletion at concentrations above 1.56 μg/mL and 0.195 μg/mL respectively, unlike MET which showed no marked effect on ATP or LDH at concentrations up to 200 μg/mL, implying a difference in the mode of action of the two extracts.

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References: 1. Dymock, W. (1891). Vernonia anthelmintica Willd. Pharmacographia Indica - a history of the principle drugs of vegetable origin met with in British India, Hamdard. II: 224. **2.** Nadkarni, K.M. (1954). Dr K M Nadkarni's Indian Materia Medica. Bombay, Popular Prakashan.