

## **$\alpha$ -MELANOCYTE-STIMULATING HORMONE ANTI-INFLAMMATORY ACTION IN HUMAN DERMAL FIBROBLAST CELLS**

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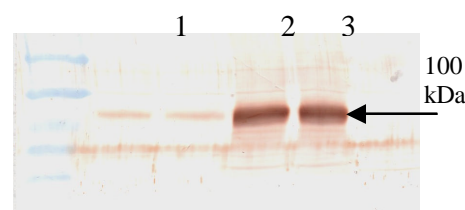
**INTRODUCTION:** Excessive inflammation resulting from a partial thickness or deep burn injury to skin tissue compromises autograft or allograft acceptance. The use of steroidal drugs as a means to alleviate inflammation is associated with a number of side effects including inhibition of wound healing. We have previously reported on the potency of several melanocyte stimulating hormone (MSH) peptides as anti-inflammatory molecules. The MSH peptides act on a number of different cell types via the melanocortin-1 (MC-1) receptor, generating a cyclic AMP signal. The anti inflammatory biology has previously been traced to the carboxyl terminal tripeptide (Lys-Pro-Val / Lys-Pro-D-Val)[1,2]. The aim of this investigation was to identify if human dermal fibroblasts express the MC-1 receptor and confirm if  $\alpha$ -MSH and Lys-Pro-D-Val peptides are effective at inhibiting cytokine stimulated NF- $\kappa$ B activity (a transcription factor that controls expression of many proinflammatory genes) and cytokine stimulated intercellular adhesion molecule-1 (ICAM-1) upregulation.

**METHODS:** MC-1 receptor expression was determined by immunofluorescent microscopy on fibroblast cultures and on isolated membranes via SDS-PAGE and Western blotting using an anti MC-1 receptor antibody (Santa Cruz Inc., [N-19]). Human dermal fibroblasts grown in culture and whole skin were stimulated with TNF- $\alpha$  (10, 50, 200 and 1000 units/ml; 5 minutes to 24 hours). Nuclear versus cytoplasmic immunofluorescent labeling of NF- $\kappa$ B/p65 was used to determine the relative activation of NF- $\kappa$ B using anti-NF- $\kappa$ B/p65 antibody (Santa Cruz Inc. [sc-302]) with FITC detection ( $\lambda_{ex}$ =495 nm,  $\lambda_{em}$ =515nm). MSH peptides ( $10^{-10}$  to  $10^{-6}$ M) were then incubated with TNF- $\alpha$  to investigate potential inhibition of NF- $\kappa$ B. Expression of ICAM-1 was determined in control and TNF- $\alpha$  (200 units/ml; 4days) stimulated cells by SDS-PAGE/Western blotting using an anti-ICAM-1 antibody (Santa Cruz Inc, [H-108]) with 3,3'-diaminobenzidine detection.

**RESULTS:** Human dermal fibroblasts were positive for the MC-1 receptor by immunolabelling and Western blotting, identifying a predicted molecular weight product of 37 kDa.

TNF- $\alpha$  was observed to induce nuclear translocation of NF- $\kappa$ B as soon as 10 minutes. However, nuclear

activation was maximal using a TNF- $\alpha$  dose of 200 units/ml for 60 minutes. In contrast, dermal fibroblasts contained within whole human skin tissue took 4 hours for maximum activation. The presence of  $\alpha$ -MSH ( $10^{-10}$  to  $10^{-6}$ M) + TNF- $\alpha$  or Lys-Pro-D-Val ( $10^{-10}$  to  $10^{-6}$ M) + TNF- $\alpha$  decreased the degree of NF- $\kappa$ B nuclear translocation caused by TNF- $\alpha$  alone by approximately 50% for fibroblasts in culture.



*Figure 1: Upregulation of ICAM-1 in dermal fibroblasts in response to TNF- $\alpha$  (lanes 3 and 4). Control unstimulated cells (lanes 1 and 2).*

TNF- $\alpha$  stimulation (200 units/ml) over 4 days caused upregulation of ICAM-1, identified as a 100kDa band by Western blotting.

**DISCUSSION & CONCLUSIONS:** MC-1 receptor presence was observed in human dermal fibroblasts by immunolabelling and Western blotting. This is fundamental for MSH peptide signaling in dermal fibroblast cells. TNF- $\alpha$  challenge lead to a rapid activation of NF- $\kappa$ B and considerable upregulation of ICAM-1. Use of MSH peptides was observed to inhibit TNF- $\alpha$  activation of NF- $\kappa$ B. The ability of MSH peptides to inhibit TNF- $\alpha$  stimulated ICAM-1 upregulation is currently underway. Preliminary data are consistent with MSH as an antiinflammatory peptide in fibroblast cells, supporting a potential use in augmenting therapy for dermal burns injury in grafting and engineered applications.

**REFERENCES:** <sup>1</sup>M. Moustafa, M. Szabo, G. Ghanem et al (2002) *J Invest Dermatol.* (In Press). <sup>2</sup>J.W. Haycock, S.J. Rowe, S. Cartledge et al (2000) *J Biol Chem* **275** (21), 15629-36

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