Local Delivery of Anti-inflammatory Drugs for Epithelial Cells

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INTRODUCTION: Acute peripheral inflammation creates an extremely hostile environment in the wound bed, which in addition to its remedial functions can harm healthy cells. This can result in increased healing times, hypertrophic scarring and graft rejection. Anti-inflammatory drugs can modulate inflammation to reduce the above problems, associated with acute tissue damage. The control of inflammation via the melanocortin receptors (MCRs) has been a topic of recent interest following observations that α-MSH has anti-inflammatory activity in a number of cell types. This has led to further investigation of the properties of the α-MSH peptide, its analogues and novel compounds designed to specifically target the MCRs. It is known that α-MSH acts by binding to the MC1R, causing an intracellular increase in cyclic AMP and calcium that interrupt proinflammatory pathways. There are five MCRs cloned to date[1,2] and an increasing amount of literature suggests that MC3R and MC4R may have roles in inflammatory control (as well as other effects such as control over satiety and sexual behaviour). In order to delineate these signalling pathways and resultant functions we are investigating MC1R, MC3R and MC4R individually, using CHO cell lines stably transfected with each receptor. Elevation of cyclic AMP and Ca^{2+} was investigated in response to stimulation with α-MSH, Nle^4-$_D$-Phe^7-α-MSH (a potent analogue of α-MSH) and BMS-470539, a novel MC1R agonist developed by Bristol-Myers-Squibb[3].

METHODS: Relative NF-κB activity was assessed by measuring light output from HBL melanoma cells transfected with an NF-κB-luciferase reporter construct, stimulated with TNF-α. The inhibitory effect of BMS-470539 was investigated by pre-incubating cells with the compound prior to TNF-α addition. Stably transfected CHO clones were developed by co-incubating cells with transfection reagent and a DNA prep coding the receptor of interest. Clones were selected using G418 sulphate. For cyclic AMP assays, cells were labelled using ^{3}H-adenine. Cyclic AMP production was measured by stimulating cells with either α-MSH, Nle^4-$_D$-Phe^7-α-MSH or BMS-470539 (10^{-12}M to 10^{-6}M), to form labelled cAMP. This was separated from other nucleotides by neutral alumina chromatography and the radioactivity of resultant samples measured using a beta counter. For intracellular calcium measurement, cells were grown on glass cover slips and labelled with Fura-2AM. Ca^{2+} elevation in response to drug addition was measured in real time using cell-averaged fluorimetry.

RESULTS: BMS-470539 was found to significantly reduce NF-κB activation stimulated by TNF-α in a dose dependent manner. Results indicate a potential for elevating cAMP by stimulation of MC1R, MC3R and MC4R with α-MSH and Nle^4-$_D$-Phe^7-αMSH. Interestingly, no cyclic AMP elevation was observed in response to BMS-470539, contrary to findings by Bristol-Myers-Squibb[3]. All three agonists were found to elevate intracellular calcium levels through MC1R. Work is ongoing to investigate intracellular Ca^{2+} signalling via MC3R and MC4R.

DISCUSSION & CONCLUSIONS: In conclusion, our results show that novel MC1R agonists cause intracellular elevations that may inhibit cellular responses linked to inflammation, suggesting that these compounds may have therapeutic potential in controlling the inflammatory response. These novel compounds are ideal to deliver locally for preventing inflammation as they are straightforward to manufacture. Future work is underway to link intracellular events measured from each receptor with functional control over p65/NF-κB, and in turn in situ delivery to establish suitability for local delivery.

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