OP2.031

The influence of anti-asthma drugs on the transcriptional regulation of chemokine receptor 3 (CCR3)

G.M. Grech[1], A.G. Fenech[1], G. Grech[2], R. Ellul-Micallef[1]

[1]Department of Clinical Pharmacology and Theraputics, University of Malta, Msida, [2]Departmet of Pathology, University of Malta, Msida

Introduction: Chemokine receptor 3 (CCR3), the major chemokine receptor expressed on eosinophils, binds promiscuously to several ligands, mainly the eotaxin family of chemokines which are up-regulated in inflammatory response. CCR3 expression in airway epithelial cells, has been reported to be upregulated in asthma, and has been proposed to play an important role in airway inflammation by amplifying the expression of chemokine transcripts. The promoter region of CCR3 gene has recently been characterized in the literature and contains promoter elements which include a TATA box and motifs for transcription factors such as NF-κB, AP-1 and GATA-1.

Aim: The aim of this study was to investigate the effects of transcription modifier anti-asthma drugs on the transcriptional regulation of the CCR3 promoter.

Methodology: pGL3E luciferase-based reporter deletion constructs were generated for the 1.6kb CCR3 promoter region, using standard cloning approaches in DH5α E.Coli cells. Each promoter construct was transfected to A549 cells in microwell plate format and stimulated with dexamethasone, cortisol, and theophylline, in a dose dependent manner.

Results: A CCR3 promoter tri-phasic response (i.e. activation at low concentration 10^{-8}M, repression at medium concentration 10^{-7}M, and activation at high concentration 10^{-6}M) to dexamethasone was observed, indicating a complex transcriptional regulatory mechanism. Unlike dexamethasone, cortisol did not activate CCR3 promoter activity at any of the concentrations investigated, but rather showed significant transcriptional repression at concentrations of 10^{-6}M and 10^{-7}M. Theophylline showed significant transcriptional repression at all three concentrations investigated (10^{-4}M, 10^{-5}M and 10^{-6}M).

Conclusions: Dexamethasone-induced transcriptional regulation of the CCR3 promoter in A549 cells appears to occur in a complex dose-dependent manner, potentially involving additional mechanisms besides the established NF-κB and AP-1 transcriptional pathways. Changes in CCR3 promoter activity in response to cortisol were different from those observed for dexamethasone, and can be explained by dose-related increases in transcriptional repression. Our results have also shown that theophylline significantly represses CCR3 promoter activity in the absence of glucocorticoids, suggesting that this may be another mechanism by which theophylline exerts its pharmacological effects.