

Antagonizing the 5-HT₂ receptors represents a novel mechanism for suppressing inflammation in arthritis

C. Wenglén¹, H. Arozenius¹, M. Siller¹, N. Palmqvist¹, A-C. Ryde¹, A. Sjödin¹, C. Klint¹, C. Flood¹, A. Pramhed¹, E. Seifert², A. Boman², P. Lek², T. Lundstedt², G. Ekström¹
¹AnaMar AB, R&D, Lund, Sweden, ²Acure Pharma AB, Uppsala, Sweden.

Background: In the inflammatory arthritic joint thrombocytes, the major source of blood serotonin (5-HT), accumulate and become activated. 5-HT is released into the synovial fluid where it boosts the inflammatory response by binding to 5-HT receptors on inflammatory cells. 5-HT has previously been shown to synergistically increase the Lipopolysaccharide (LPS)-induced release of pro-inflammatory mediators. We have developed several compounds antagonizing the 5-HT₂ receptors that have been shown to reduce IL-6 and TNF- α release *in vitro* and to effectively diminish arthritis activity in various animal models.

Objectives: The objective of the present study was to (1) evaluate the impact of 5-HT and the 5-HT₂ receptors on the release of pro-inflammatory cytokines, (2) investigate whether the synergistic effect of 5-HT and LPS on cytokine release is due to LPS-induced up-regulation of 5-HT₂ receptors, (3) evaluate various 5-HT₂ receptor antagonists in LPS-induced inflammation (LII) in the mouse.

Methods: *In vitro*, the 5-HT₂ receptor expression pattern on inflammatory cells present in the arthritic joint was investigated by RT-PCR. To investigate the importance of 5-HT and the 5-HT₂ receptors on LPS-induced IL-6 release, commercial 5-HT₂ receptor antagonists or our compounds were added to rat synoviocytes together with 5-HT or commercial 5-HT₂ receptor agonists. The level of 5-HT₂ receptor mRNA in rat synoviocytes after LPS and 5-HT induction was investigated using RT-qPCR. *In vivo*, LII in the mouse was used to study the modulation of cytokines after treatment with 5-HT₂ receptor antagonists.

Results: *In vitro* target validation experiments showed that 5-HT₂ receptors are expressed on cells present in the arthritic joint and that 5-HT as well as selective 5-HT₂ receptor agonists in synergy with LPS induce an inflammatory response that could be reversed by selective 5-HT₂ receptor antagonists. Our compounds targeting the 5-HT₂ receptors were shown to reduce IL-6 release in rat synoviocytes as efficiently as commercially available antagonists. LPS treatment did not modify the level of 5-HT₂ receptor mRNA in rat synoviocytes. *In vivo*, 5-HT₂ receptor antagonists were shown to effectively suppress LPS-induced cytokine release.

Conclusions: The present study shows the importance of 5-HT in inflammation and convincing anti-inflammatory effects are established both *in vivo* and *in vitro* by antagonizing the 5-HT₂ receptors. The synergistic pro-inflammatory effects achieved by LPS and 5-HT stimulation need to be further investigated. In conclusion, the study supports the clinical development of compounds targeting the peripheral 5-HT₂ receptors for the treatment of rheumatoid arthritis.