Final Report Summary - INFLAMMATIONPAIN (Role of spinal anti-inflammatory lipid mediators in inflammation and arthritis-induced pain)

Summary

Introduction

More than 100 million Europeans are affected by arthritis and other rheumatic diseases that cause stiffness, inflammation and swelling in the joints. Notably, pain is one of the most bothersome symptoms reported by this group of people. Pain not only impairs the ability to function and reduces the quality of life, but also forms the basis for an increasing economical burden. Hence, it is important that we increase our understanding of mechanisms regulating persistent pain in order to be able to identify new targets for development of pain relieving therapeutics.

Lipoxins and resolvins represent novel classes of lipid mediators that function as ‘braking signals’ in inflammation. My work prior to this project started showed that while systemic injection of lipoxins reduces both pain and edema, spinal delivery reduces inflammatory hyperalgesia without altering the peripheral inflammatory state. The lipoxin receptor, ALXR, is found expressed on spinal astrocytes, indicating that not only neurons, but also spinal non-neuronal cells participate in the regulation of pain signaling. These findings served as the platform for the current proposal, which had the aim to examine the role of another class of anti-inflammatory lipid mediators, the resolvins, in order to determine if the antinociceptive effect of lipoxins are unique to this class of lipid metabolites, or if also other anti-inflammatory lipid metabolites has this property.

Further, the first studies had been undertaken in a model of transient (48 h long) model of inflammation, and one goal with the proposed work was to expand this to also include studies of the antinociceptive effect in a model of longer lasting inflammation, and for this purpose a model of rheumatoid arthritis was chosen. Lastly, the aim was to dissect the molecular mechanisms that give lipoxins and resolvins their anti-nociceptive properties.

Aim one. Characterise the roles of spinal lipoxins and resolvins in arthritis-induced pain.

We have investigated the antinociceptive effect of i.t. injection of RvD1 and RvE1 and found both to have potent dose-dependent anti-allodynic effects in the carrageenan model, without affecting the peripheral inflammation. This data is included in manuscript (1), which has been submitted for publication. Carrageenan induces a local inflammation that lasts for 36-48 hours. In order to explore the ability of RvD1 and RvE1 to reverse inflammatory hypersensitivity in models of more long-lasting pain we characterised pain behavior in two well-established models of rheumatoid arthritis (K/BxN serum transfer arthritis and collagen-antibody-induced arthritis, (CAIA)). Prior to work these models had not been used for pain research. The work characterizing pain behavior in the K/BxN model was published in 'pain' 2010 (2, 3) and the work describing pain behavior in the CAIA model in 'arthritis and rheumatism' in 2012 (4,5). These models are based on injection of antibodies (serum containing glucose-6-phosphate isomerase and collagen type II antibodies, respectively), which generates a transient episode of joint inflammation (lasting approximately three weeks). One striking feature of these two models is that while pain behavior concurs with the onset of inflammation, it outlasts the inflammation by weeks. We refer to this as pain behavior during the...
'inflammatory' and 'post-inflammatory' phases. We have injected RvE1 and RvD1 i.t. in the CAIA model in both phases and found that RvD1 and RvE1 reverse already established hypersensitivity during the inflammatory phase, but are without any antihyperalgesic effect in the post-inflammatory phase. These data indicate that resolvins are functioning as pain antagonists during ongoing inflammation and that the mechanisms that drive pain behavior in the sequel of joint inflammation in the arthritis models are different, and not sensitive to resolvins. This work is has been submitted for publication (6). Aim two. Determine if lipoxins and/or resolvins are formed in the spinal cord during spontaneous resolution of inflammatory pain.

We have characterised spinal gene and protein expression of the enzymes, the lipoxygenases (LO), which generates resolvins and lipoxins from polyunsaturated fatty acids. Both 5-LO,12-LO and 15-LO are constitutively expressed in the spinal cord and elevated subsequent to induction of peripheral inflammation. We have searched for RvD1 and RvE1 and associated precursors in spinal cord and CSF using LC-MS/MS and a lipidomics approach at different time points subsequent to carrageenan-induced paw inflammation, but not been able to detect these or associated lipid metabolites (7). Hence, though we have not yet been able to detect resolvins and lipoxins in spinal cord or CSF after carrageenan-induced inflammation, the machinery for their synthesis is present in the spinal cord.

Aim three. Examine how lipoxins and resolvins, through activation of their respective receptors, attenuate spinal sensitisation induced by peripheral inflammation.

Note-worthy, we have found that the receptors for RvD1 and lipoxins (lipoxigenase A receptor, ALXR and G-protein coupled receptor 32, GPR32) are exclusively expressed on spinal astrocytes, while the receptor for RvE1 (ChemR23) is only expressed on neurons. We have assessed this in mice, rats and human tissues and cells, and found this to be the case in all three species. This indicates that RvD1 and lipoxins attenuate pain transmission through mechanisms that are distinctly different from the mechanisms through which RvE1 operates. The data described above is included in manuscript 1.

During work related to Aim 3 we noticed that our rat spinal astrocyte cultures grew faster and had a slightly different morphology in Sweden as compared to when we cultured these cells in the United States. We have changed two factors upon the move, source of fetal bovine serum (FBS) and Sprague-Dawley substrains. Hence we determined to investigate if the characteristics and purity of the astrocytes differ when established from rats from two different substrains (adult Harlan and Charles River Sprague-Dawley rats) or subjected to different sera (fetal bovine serum from Sigma, Gibco, Hyclone or defined growth supplement from AM), and also to compare rat primary astrocytes to human primary astrocytes. Taken together, this study showed that rat substrain and growth medium composition affect purity, expression profile and response to starvation of primary astrocytes suggesting that cultures of Harlan rats in AM media have optimal astrocyte characteristics, purity, and similarity to human astrocytes. This work is now published (8). Lastly we have found that RvD1, but not RvE1, blocks TNF-induced activation of ERK in primary astrocyte cultures. No change in TNF-induced p38 or JNK MAPK activity was observed in the presence of RvD1 or RvE1 in astrocyte and neuronal cultures. Though outside the scope we found that RvD1 and LXA4, but not RvE1, attenuates LPS-induced TNF release in primary rat and human astrocyte cell cultures and carrageenan-induced spinal TNF and interferon-gamma release. This work is included in manuscript 1. Taken together, our studies point to a multifaceted role of resolvins and lipoxins in the resolution of pain transmission in conditions of peripheral inflammation.


(5) Sandor K, Nandakumar KS, Holmdahl R, Svensson CI. Collagen antibody-induced arthritis model, a disease-relevant model...
Web site: www.ki.se/research/camillasvensson

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Reported by

KAROLINSKA INSTITUTET
2, Nanna Svartz väg
17177 STOCKHOLM
Sweden
See on map

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