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KEY PAPER EVALUATION

Resolvin inflammation with pain


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Abstract

Background: Inflammation and pain coexist in conditions such as arthritis, inflammatory bowel disease, and lower back pain. The drugs currently used to treat the combination of inflammation and pain all have disadvantages. Thus, new drugs and new approaches are needed to treat inflammation with pain. The resolvins are considered to be part of the natural resolving mechanism for inflammation, and have been shown to prevent inflammation in animal models.

Objectives/methods: To evaluate a paper suggesting that the resolvins RvE1 and RvD1 attenuate inflammatory pain in animal models. Results: RvE1 has been shown to attenuate inflammation and, to a lesser extent, pain in animal models. Limited results are presented of the effectiveness of RvD1 against inflammatory pain. Conclusion: Drugs that mimic or potentiate the effects of the resolvins may be useful for the treatment of some inflammation with pain.

Key words animal models, inflammation, pain, resolvins, RvE1, RvD1
1. Introduction

Inflammation and pain coexist in conditions such as arthritis, inflammatory bowel disease, and lower back pain. Of these, mechanical low back pain is the second most common symptom-related reason for seeing a physician in the United States, and occurs at least once in 85% of adults below the age of 50. Nearly all of them will have at least one recurrence. About 1% of the world’s population is afflicted by rheumatoid arthritis with women being affected three times more often than men. Another 1% of the population is affected by osteoarthritis. Like low back pain, arthritis is a leading cause of disability. Inflammatory bowel disease is less common (0.2%). However, inflammatory bowel disease reduces quality of life greatly by causing pain, diarrhea and vomiting.

The drugs most commonly used to treat a combination of inflammation and pain are the cyclooxygenase (COX) inhibitors. These are only effective in mild-to-moderate inflammation and pain. When inflammation is severe, the glucocorticoids are often needed to decrease the inflammation, and when pain is severe, the opioids may be used to decrease pain. All of these drugs have disadvantages. Notably, non-selective COX inhibitors cause gastrointestinal bleeding, and the COX-2 selective inhibitors increase the risk of cardiovascular disease. The glucocorticoids are not suitable for the long term systemic use in chronic inflammation, as they cause a wide range of side effects on chronic use. The opioids cause drowsiness, constipation, nausea, sedation, and cognitive disturbances. Thus, there is a need for new drugs that are useful in the treatment of conditions associated with a combination of inflammation and pain.

Diets supplemented with omega-3 polyunsaturated fatty acids (ω-3 PUFAs) have been suggested to have antiinflammatory actions. These actions of ω-3 PUFAs may be useful in the treatment of inflammatory conditions such as arthritis, inflammatory bowel disease, and cardiovascular disease [1]. Notably in cardiovascular disease, the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico-Prevenzione trial enrolled subjects who had a recent myocardial infarction and showed that there was a reduction in mortality with ω-3 PUFAs [2].

Recently, studies have been undertaken to determine the cellular and molecular mechanisms of the antiinflammatory effects of ω-3 PUFAs. Serhan et al showed that the inflammatory exudates from mice treated with ω-3 PUFAs and aspirin generated a novel array of bioactive lipid signals [3], and these signals have been named resolvins. The resolvins are discussed more in the next section, and then there is an evaluation of a recent paper discussing the antiinflammatory and analgesic properties of resolvins in animal models of inflammatory pain, and the mechanism of action of the resolvins (Section 3, [4])
2. **Resolvins – the story so far**

The resolvins are derived from ω-3 PUFAs and are part of the natural resolving mechanism for inflammation. The levels of resolvins can be increased by increasing the levels of the ω-3 PUFAs. The major ω-3 PUFAs are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). When cyclooxygenase-2 (COX-2) is inhibited with aspirin, EPA is metabolised by a pathway involving lipoxygenase (LOX) to RvE1 (5S,12R,18R-trihydroxy-6Z,8E,14Z,16E-eicosapentaenoic acid) and RvE2 (5S, 18-dihydroxyeicosapentaenoic acid) [5]. The resolvin D series are formed from DHA and also use the LOX pathway [5]. RvE1 and RvE2 can also be formed from EPA by p450 pathway in human polymorphonuclear leukocytes [5]. RvE1 is present in human plasma, and the levels of RvE1 are increased by taking aspirin [5]. RvD1 is generated from DHA in human blood [6].

The effects of RvE1 are mediated by a G-protein-coupled receptor, GPCR ChemR23, and leads to the attenuation of nuclear factor (NF)-κB [5]. The effects of RvD1 are probably mediated by two receptors; ALX, a lipoxin A₄ receptor and another GPCR, GPR32 [7]. Activation of the receptors leads to a reduction in tumour necrosis factor (TNF)-α-stimulated NF-κB [7]. RvD1 had no effect at the RvE1 receptor ChemR23 [7].

In animal models of inflammation (periodontal disease, colitis, retinal neovascularisation), RvE1 has been shown to protect against inflammation [5]. RvE1 has also been shown to dampen airway inflammation and hyperresponsiveness in a mouse model of asthma [8]. Recently, RvE1 has been shown to protect the rat heart against reperfusion injury [9]. In these experiments, RvE1 was administered after the ischemia but prior to the reperfusion and was shown to reduce infarct size [9].

Cerebral ischemia produced by cerebral artery in mice is used as a model of ischaemic stroke, and in this model RvD1 is produced, and the administration of RvD1 into the ventricles reduces leukocyte polymorphonuclear infiltration into the brain [10]. Similarly, in peritonitis, RvD1 reduces polymorphonuclear infiltration [11]. RvD1 is produced by mouse kidneys in response to ischemia/reperfusion injury [12]. Administration of RvD1 before the ischemia or 10 minutes after the reperfusion reduced the damage to the kidney [12].

3. **Resolvins – latest findings**

3.1 Methods and results
The methods and results of the paper showing that resolvins may be useful in the treatment of inflammatory pain [4] are summarised in this section. As an acute model of inflammation, injection of formalin into the feet of mice (intraplantar) produces immediate and secondary pain, and the secondary pain is due to central mechanisms. When RvE1 at 0.3 and 1 ng was injected into the mouse spine intrathecally, it had no effect on the immediate pain, but inhibited the secondary pain, and in doing so, was equieffective to morphine at 100 ng, and to NS-398 (a COX-2) inhibitor at 10 μg. This shows that RvE1 is more potent than morphine or NS-398 at inhibiting the secondary pain in this model. This also suggests that RvE1 inhibits pain by a central mechanism.

The G protein subunit Gαi is probably associated with the ChemR23 receptor for RvE1, and inhibition of this subunit with pertussis toxin, reduced the analgesic response to RvE1 in the formalin model. This result shows the involvement of the Gαi subunit in the response to RvE1. Chemerin, which is a peptide agonist at ChemR23, reduced the secondary pain response in the formalin model, which shows that the analgesic response can be mediated via ChemR23. The opioid antagonist naloxone reduced the analgesic response to morphine, but not to RvE1, and this shows that the analgesic response to morphine, but not to RvE1, is mediated by opioid receptors. Knockout of ChemR23 with siRNA abolished the analgesic response to RvE1, which is a good demonstration that the analgesic effect of RvE1 is mediated by ChemR23.

Localisation studies showed that ChemR23 mRNA was found in the dorsal route ganglion and neurons, and in the spinal cord and the primary afferent terminals of this. Thus, the receptors are located in regions, which support central mechanisms of action for RvE1.

As a model of persistent inflammation, complete Freund’s adjuvant was injected into the feet of mice. In this model the pain and inflammation lasts for weeks, and the intrathecal administration of resolvins reduced the hyperalgesia response to heat. For instance, 10 ng of RvE1 inhibited the hyperalgesia by about 75%. RvE1 was also more potent that the COX-2 inhibitor NS-398 in this model. A stable analog of RvE1 that is resistant to metabolic breakdown, 19-(p-fluorophenoxy)-RvE1, caused a prolonged (6 hour) reduction in hyperalgesia. Much higher concentrations of the precursors of RvE1 and RvF1, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively, than the resolvins, were needed to inhibit the hyperalgesia.

As a model of chronic inflammation, carrageenan was injected into the feet of mice, and this caused pain and inflammation. Intraplantar pretreatment with RvD1 or RvE1 reduced the heat hyperalgesia response in these mice. RvE1 reduced the carrageenan-induced oedema and
neutrophils infiltration. RvE1 also reduced the expression of the cytokines; tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6, and the expression of the chemokines; monocyte chemotactic protein-1 and macrophage inflammatory protein-1α. As intraplantar administration of resolvins was effective in this model, it suggests that peripheral mechanisms are involved in the analgesic response. In support of this, intraplantar administration of RvE1 also reduced formalin-induced acute pain in the mouse.

TNF-α is major contributor to both pain and inflammation, and it does this by both central and peripheral mechanisms. Transient receptor potential vanilloid subtype-1 (TRPV1) is predominantly found on sensory neurones where it has an important role in pain transmission. The formalin-elicited and complete Feund’s adjuvant models of pain and inflammation are much reduced in TNF-α knockout mice. Intrathecal TNF-α induces heat hyperplasia in mice, which is reduced by spinal administration of RvE1. The response to TNF-α is not present in mice lacking the gene for TRPV1, which implicated TRPV1 in the response to TNF-α. The pain and inflammatory response to formalin is observed in TRPV1 knockout mice, as this response is inhibited by RvE1, and this suggests that TRPV1-independent mechanisms are also involved in the antiinflammatory effects of RvE1.

Further experiments supported the involvement of both TNF-α and TRPV1 in the antiinflammatory actions of RvE1. In patch-clamp studies in spinal cord slices from mice, there were spontaneous excitatory postsynaptic currents in the lamina II neurones, and the frequency, but not the amplitude, of these was increased by TNF-α. This response to TNF-α was abolished by the TRPV1 antagonist capsazepine, and by RvE1. RvE1 also reduced the increased frequency due to the administration of the TRPV1 agonist capsaicin. Chemerin also abolished the response to capsaicin, which suggests the involvement of ChemR23. Intrathecal capsaicin induced acting pain in the mouse, and this was prevented by intrathecal RvE1. Intraplantar capsaicin also induced pain, and this was reduced by peripheral RvE1.

In the complete Freund’s adjuvant model and after intrathecal TNF-α in the mouse, mechanical allodynia (a reduction in paw withdrawal threshold) is also observed, and RvE1 (administered 3 days after the adjuvant) reduced this response. Increased activity of the glutamate NMDA receptors on dorsal horn neurones is associated with mechanical allodynia. In preparations of these neurones, TNF-α increased the NMDA currents, and RvE1 reversed this.

In a mouse model of nerve injury/spinal nerve ligation-induced neuropathic pain, post-treatment with RvE1 caused a small reduction in the paw withdrawal latency. RvD1 was tested in a mouse
model of chronic pain (incision-induced postoperative pain) and intraplantar pretreatment was shown to be effective.

### 3.1 Discussion
The authors concluded that resolvins may represent a new family of analgesic useful in treating inflammation-associated pain states such as arthritic and post-operative pain [4].

### 4 Expert opinion

#### 4.1 Are resolvins underactive in excessive inflammation?
As the resolvins have only recently been discovered, we are at the early stages of characterising their physiological and pathophysiological roles. Much more needs to be done in this area, and one of the interesting questions is whether the resolvin system is underactive in reducing inflammation in some pathophysiological conditions associated with excessive inflammation. If this was the case, bolstering the levels of resolvins by administering ω-3 PUFAs would be a logical approach to treating excessive inflammation. Drugs that increase the levels or mimic the effects of resolvins may be a new approach to the treatment of conditions associated with excessive inflammation (e.g. rheumatoid arthritis, inflammatory bowel disease).

#### 4.2 Can we extrapolate from RvE1 to RvD1?
The authors of the paper on RvE1 and RvD1 seem to be extrapolating the results with one of the resolvins to the other. From reading the published manuscript and supplementary information, the authors have shown that RvE1 is effective against the formalin model of acute inflammation, the complete Freund’s adjuvant model of persistent inflammation, the carrageenan model of chronic inflammation, TNF-α-induced heat hyperplasia, and nerve injury/spinal nerve ligation-induced neuropathic pain. RvD1 was shown to be effective in the mouse carrageenan model and the model of chronic pain (incision-induced postoperative pain), but data was not shown or claimed for other models. However, from this data, the authors seem to me to be suggesting that both RvE1 and RvD1 have similar abilities to attenuate inflammatory pain when they have only presented data for RvD1 in chronic inflammation (not acute or persistent inflammation), and have not presented data for RvE1 in their model of postoperative pain. Until the results for both resolvins in all the models are presented, this seems to me, to be an extravagant claim.

#### 4.3 Persistent and chronic inflammation
To be useful in relieving persistent and chronic inflammation, drugs need to be effective when administered after the chronic inflammation has developed. In the model of persistent
inflammation, Complete Freund’s adjuvant in mice, resolvins were effective when administered 3 days after the adjuvant. The ongoing effects of resolvins in adjuvant inflammation need to be assessed. In chronic inflammation, the resolvins, RvE1 and RvD1, are only reported to inhibit the heat hyperalgesia response in mice, when they are administered before the carrageenan. The effects of resolvins, after the development of carrageenan-induced pain and inflammation, needs to be studied and reported. The resolvins will only be useful in the treatment of persistent or chronic inflammation, if they are effective on an ongoing basis after the establishment of the inflammation.

4.4 Potency as analgesics
As pointed out by Sommer & Birklein ([13]), the effect of the resolvins on TNF-induced pain is moderate, and this casts doubt as whether drugs that mimic resolvins will be useful analgesics [13]. To support this assertion, they cited that the drug lacosamide, which had stronger effects than the resolvins in an animal model of pain induced by TNF [14], had failed in clinical trial by giving similar responses to placebo in painful diabetic neuropathy [15]. In the study of lacosamide in an animal model of pain induced by TNF, lacosamide was more potent than gabapentin and pregabalin [14]. Gabapentin and pregabalin have been shown to be useful in painful diabetic neuropathy [16]. This suggests that there is no direct correlation between TNF-induced pain animal models and effects in painful diabetic neuropathy. Clearly, further studies of resolvins in other animal models of pain are needed as we continue to assess whether they will be powerful and/or useful analgesics in a variety of painful conditions.

4.5 Conclusions
We are at the early stages of characterising the physiological and pathophysiological roles of resolvins. There is good evidence that RvE1 can reverse inflammation whereas there is less testing with RvD1. Diets or drugs that increase the levels of RvE1, and drugs that mimic the effects of RvE1 or prevent the breakdown of RvE1 may be useful in the treatment of inflammation. More evidence is required to support an analgesic effect of the resolvins.

References


