Sphingosine 1-Phosphate Mediates Hyperalgesia via a Neutrophil-Dependent Mechanism

Amanda Finley1, Zhoumou Chen1, Emanuela Esposito2, Salvatore Cuzzocrea2, Roger Sabbadini3, Daniela Salvemini1*

1 Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis, Missouri, United States of America. 2 Department of Clinical and Experimental Medicine and Pharmacology, University of Messina, Messina, Italy. 3 Lpath, Inc., and Department of Biology, San Diego State University, San Diego, California, United States of America

Abstract

Novel classes of pain-relieving molecules are needed to fill the void between non-steroidal anti-inflammatory agents and narcotics. We have recently shown that intraplantar administration of sphingosine 1-phosphate (S1P) in rats causes peripheral sensitization and hyperalgesia through the S1P1 receptor subtype (S1PR1): the mechanism(s) involved are largely unknown and were thus explored in the present study. Intraplantar injection of carrageenan in rats led to a time-dependent development of thermal hyperalgesia that was associated with pronounced edema and infiltration of neutrophils in paw tissues. Inhibition of 1) S1P formation with SK-I, a sphingosine kinase inhibitor, 2) S1P bioavailability with the S1P blocking antibody Sphingomab, LT1002 (but not its negative control, LTY017) or 3) S1P actions through S1PR1, with the selective S1PR1 antagonist, W146 (but not its inactive enantiomer, W140) blocked thermal hyperalgesia and infiltration of neutrophils. Taken together, these findings identify S1P as an important contributor to inflammatory pain acting through S1PR1, to elicit hyperalgesia in a neutrophil-dependant manner. In addition and in further support, we demonstrate that the development of thermal hyperalgesia following intraplantar injection of S1P or SEW2871 (an S1PR1 agonist) was also associated with neutrophilic infiltration in paw tissues as these events were attenuated by fucoidan, an inhibitor of neutrophilic infiltration. Importantly, FTY720, an FDA-approved S1P receptor modulator known to block S1P-S1PR1 signaling, attenuated carrageenan-induced thermal hyperalgesia and associated neutrophil infiltration. Targeting the S1P/S1PR1 axis opens a therapeutic strategy for the development of novel non-narcotic anti-hyperalgesic agents.

Introduction

One-quarter of Americans over the age of 20 suffer from some sort of persistent pain [1]. Current treatment options, such as non-steroidal anti-inflammatory agents and narcotics, result in deleterious side-effects making them unattractive options for persistent use [2]. Therefore, novel classes of pain-relievers are severely needed. In addition to their pro-inflammatory roles [3], sphingolipids including ceramide [4–10] and sphingosine 1-phosphate (S1P) [6,7,10–15] are emerging as important modulators of pain. S1P derived from the conversion of ceramide to sphingosine by ceramidase, and is a product of the phosphorylation of sphingosine by sphingosine kinase isoenzymes, plays an important role in peripheral and central sensitization. S1P resulting from ceramide bioconversion has been shown to contribute to NGF-induced excitation of rat sensory neurons [11] and is required for the development of ceramide-induced peripheral sensitization following intraplantar injection of ceramide in rats [7]. Furthermore, S1P has the ability to directly increase the excitability of rat sensory neurons in vitro [14] and cause thermal hyperalgesia following intraplantar injection in rats [12]. However, apart from S1P’s ability to directly increase nociceptor sensitivity in vitro and in vivo [13] and our previous reports that S1P exerts its actions at least in part via the upregulation of peroxynitrite [12], S1P’s mechanism of action remains largely uninvestigated.

To date, five subtypes of G-protein coupled S1P receptors (S1PRs) have been identified: S1PR1-5 [16]. These receptors are differentially expressed on all cell types and can bind to multiple different heterotrimeric G-proteins [16,17], thereby having varying effects, depending on the signaling cascade they activate. In order to examine the signaling pathways and mechanisms involved in S1P-mediated hyperalgesia it is important to identify the receptor subtype(s) involved. We have focused our studies on S1PR1, as we have shown this receptor subtype to be of particular importance in S1P-mediated peripheral hyperalgesia [12]. In addition, enhanced excitability in peripheral sensory neurons in response to S1P been shown to occur, at least in part, through the activation of S1PR1 [18] and S1P hypersensitivity is significantly reduced in mice with a conditional nociceptor-specific deletion of S1PR1 [13] or those with local knockdown of S1PR1 in the DRG [19].

* E-mail: salvemini@slu.edu
Another important action of S1P is its ability to enhance immune cell migration [20]. Specifically, S1P via activation of S1PR1 upregulates the expression of the adhesion molecules ICAM-1 and E-selectin on the surface of endothelial cells [21–24] thereby initiating neutrophilic infiltration [25,26]. Many models of inflammation-induced hyperalgesia rely on neutrophilic recruitment [27–30] and this neutrophil-dependent hyperalgesia underlies pain of several etiologies.

Taken together, we hypothesize and demonstrate herein that neutrophils contribute to the development of S1P-induced hyperalgesia acting through the S1PR1 subtype. Targeting the S1P-to-S1PR1 pathway may offer a novel approach in the management of pain.

Materials and Methods

Materials

S1P (sphingosine 1-phosphate), W146 (3- amino- 4- (3- hexylphenylamino)- 4- oxobutyl phosphonic acid), W140 (3- amino- 4- (3- hexylphenylamino)- 4- oxobutyl phosphonic acid), JTE-013 (N- (2, 6- dichloro- 4- pyridinyl)- 2- [1, 3- dimethyl- 4- (1- methylethyl)- 1H- pyrazolo[3, 4- b]pyridin- 6- yl]- hydrazinecarboxamide), CAY10444 (2- undecyl- thiazolidine- 4- carboxylic acid) and FTY720 (2- amino- 2- [2- (4- octylphenyl)ethyl]- 1, 3- propanediol, hydrochloride) were all purchased from Cayman Chemical (Ann Arbor, MI). Carrageenan, fucoidan and o- dianisidine (3,3'-Dimethoxybenzidine dihydrochloride) were purchased from Sigma-Aldrich (St. Louis, MO). The SK inhibitor, SK-I [2-(p-hydroxyanilino)-4-(p-chlorophenyl) thiazole] was purchased from Calbiochem (La Jolla, CA) and the bichinchoninic acid (BCA) assay was from Thermo-Fisher (Rockford IL). The murine monoclonal anti-S1P antibody, Sphingomab/LT1002 and its isotype mAb control, LT1017, was generated as described previously [31].

Animals

Male Sprague Dawley rats (200–220 g) were purchased from Harlan (USA) and housed 3–4 per cage and maintained in a controlled environment (12 h light/dark cycle) with food and water available ad libitum. All experiments were performed in accordance with the International Association for the Study of Pain and the National Institutes of Health guidelines on laboratory animal welfare and the recommendations by Saint Louis University Institutional Animal Care and Use Committee.
Drug Administration

Male Sprague Dawley rats were lightly anesthetized [CO$_2$ (80%)/O$_2$ (20%)] and given a subplantar injection of S1P (0.3 mg; using a Hamilton gauge needle 3 K’; 5 mL) or of 1% carrageenan (100 mL) into the left hindpaw. All drugs or their vehicle (6% EtOH in saline for S1P; saline for carrageenan) were given by intraplantar injection 30 minutes prior to intraplantar S1P or carrageenan injection unless otherwise stated. LT1002 and LT1017 were given in a volume of 40 mL while SK-I, W146, W140, JTE-013 and CAY10444 were given in a volume of 5 mL. Fucoidan was given i.p. in 200 mL saline, 30 minutes prior to S1P injection. FTY720 was given p.o. in 10% DMSO in saline, 30 min prior to carrageenan injection.

Behavioral Analysis

Behavioral testing was done with experimenter blinded to treatment conditions. Hyperalgesic responses to heat were determined by the Hargreaves’ Method using a Basile Plantar Test [32] with a cut-off latency of 20 s employed to prevent tissue damage. Rats were individually confined to plexiglass chambers and allowed to habituate. A mobile unit consisting of a high intensity projector bulb was positioned to deliver a thermal stimulus directly to an individual hindpaw from beneath the chamber. The withdrawal latency period of injected paws was determined with an electronic clock circuit and thermocouple. Results are expressed as paw-withdrawal latency(s).

Carrageenan-Induced Paw Edema

Changes in paw volume were measured as previously described [33]. Briefly, paw volume was measured with a plethysmometer (Ugo Basile, Comerio, Varese, Italy) immediately prior to the injection of carrageenan and thereafter at hourly intervals for 6 h. Edema was expressed as the increase in paw volume (mL) after carrageenan injection relative to the pre-injection value for each animal. Results are expressed as paw volume change (mL).

Histological Examination

For histopathological examination, biopsies of paws were taken 2 hours following the intraplantar injection of carrageenan, tissue from the pads of the rats hindpaw was removed with a scalpel. The

Figure 2. Carrageenan-induced thermal hyperalgesia is blocked by SK-I. Intraplantar injection of carrageenan (1%) led to a time-dependent development of thermal hyperalgesia that was attenuated in a dose-dependent manner by intraplanlar injection of SK-I (250 ng, 500 ng, or 1000 ng; n = 6). Results are expressed as mean ± SEM and analyzed by two-way repeated measures ANOVA with Bonferroni post hoc test where *P<0.01, **P<0.001 vs. carrageenan. doi:10.1371/journal.pone.0055255.g002

Figure 3. Inhibition of S1P attenuates carrageenan-induced thermal hyperalgesia and the recruitment of neutrophils. A) Intraplantar injection of LT1002 (484 mg, n = 6) but not of LT1017 (572 mg; isotype control, n = 6) attenuated carrageenan-induced thermal hyperalgesia. B) Intraplantar injection of carrageenan led to an increase in neutrophilic recruitment as evidenced by increased levels of MPO activity in paw tissues and this was blocked by LT1002 but not LT1017. Results are expressed as mean ± SEM and analyzed by two-way repeated measures ANOVA with Bonferroni post hoc test for behavior and one-way ANOVA with Dunnett’s post hoc test for MPO, where *P<0.01, **P<0.001 vs. carrageenan; † P<0.05, ‡ P<0.001 vs. vehicle. doi:10.1371/journal.pone.0055255.g003
tissue slices were fixed in Dietrich solution (14.25% ethanol, 1.85% formaldehyde, 1% acetic acid) for 1 week at room-temperature, dehydrated by graded ethanol and embedded in Paraplast (Sherwood Medical). Section (thickness 7 \( \mu \)m) were deparaffinized with xylene, stained with hematoxylin and eosin and observed in Dialux 22 Leitz microscope.

**Myeloperoxidase Assay**

Myeloperoxidase (MPO; a peroxidase enzyme released by neutrophils and a marker of neutrophilic infiltration [34,35]) activity was assessed by taking tissue at 2 h (time of peak inhibition). Flash-frozen plantar soft tissue was pulverized in liquid nitrogen-chilled mortar and pestle, and then homogenized in 1 mL 0.05% HTAB in 50 mM potassium phosphate buffer and kept on ice. Homogenates were sonicated with an ultrasonicator.

**Figure 4. Blockade of S1PR1 inhibits carrageenan-induced thermal hyperalgesia and the recruitment of neutrophils.** A) Intraplantar injection of carrageenan led to a time-dependent development of thermal hyperalgesia that was blocked in a dose-dependent manner by the selective S1PR1 antagonist, W146 (0.3–1.2 \( \mu \)g, \( n = 6 \)) but not by its inactive S-enantiomer, W140 (1.2 \( \mu \)g, \( n = 6 \)). B) W146 (1.2 \( \mu \)g, \( n = 6 \)) but not W140 (1.2 \( \mu \)g, \( n = 6 \)) attenuated neutrophilic recruitment. Results are expressed as mean ± SEM and analyzed by two-way repeated measures ANOVA with Bonferroni post hoc test for behavior and one-way ANOVA with Dunnett’s post hoc test for MPO, where *\( P < 0.05 \), **\( P < 0.001 \) vs. carrageenan; \( \dagger P < 0.01 \), \( \ddagger P < 0.001 \) vs. vehicle. doi:10.1371/journal.pone.0055255.g004

**Figure 5. Fucoidan blocks S1P-induced thermal hyperalgesia.** Intraplantar injection of A) S1P (0.3 \( \mu \)g) or B) the S1PR1 agonist, SEW2871 (0.3 \( \mu \)g), led to a time-dependent development of thermal hyperalgesia that was attenuated by pretreatment with fucoidan (40 mg/kg, i.p.). Results are expressed as mean ± SEM for 6 rats and analyzed by two-way repeated measures ANOVA with Bonferroni post hoc test where *\( P < 0.05 \), **\( P < 0.001 \) vs. carrageenan; \( \dagger P < 0.01 \), \( \ddagger P < 0.001 \) vs. vehicle. doi:10.1371/journal.pone.0055255.g005
for 5×10 s, centrifuged 40,000 g @ 4°C for 15 min, then
supernatants were pulled off and stored at 4°C. For the assay,
7 μL of sample was added to 193 μL of 0.167 mg/mL o-
dianisidine in 50 mM potassium phosphate buffer with or without
0.0005% H₂O₂. Absorbance of each sample was read immediately
and at 1 min intervals for 3 min at 460 nm. To calculate MPO activity,
we plotted absorbance over time to obtain slope and used
slope to calculate units of activity per mg (U/mg) using the
equation U/mg = (ΔA₄₆₀/min)/(11.3 × mg enzyme/ml reaction
mixture).

Statistical Analysis
Differences in thermal hyperalgesia were assessed using two-way
analysis of variance (ANOVA) with Bonferroni post hoc compar-
sions to S1P or carrageenan-treated animals. Differences in MPO
activity levels were assessed by one-way ANOVA followed by
Dunnett’s post hoc comparisons to S1P or carrageenan-treated
animals. Differences in paw volume were analyzed by using
student’s unpaired t test. Significant statistical difference was
defined when P-value <0.05.

Results
Carrageenan-induced thermal hyperalgesia is associated
with an increase in neutrophilic recruitment which is
blocked by fucoidan
The carrageenan model is a well-characterized model of
inflammation-induced thermal hyperalgesia which has been
suggested to rely on neutrophilic infiltration [28]. The develop-
ment of edema and thermal hyperalgesia in response to
intraplantar injection of carrageenan (1%, n = 6) seen at peak
(6 h) was associated with increased infiltration of neutrophils as
shown by an increase in myeloperoxidase activity (MPO; a
peroxidase enzyme released by neutrophils and a marker of
neutrophilic infiltration [34,35]) and by histological examination
of paw tissues (Figure 1). Administration of fucoidan (40 mg/kg,
n = 6), a well-characterized P- and L-selectin blocker, that is well
established in the literature as a potent inhibitor of neutrophil
adhesion, rolling and infiltration at inflammatory sites [28,36,37],
prevented the edema associated with carrageenan injection
(Figure 1A), blocked the thermal hyperalgesia (Figure 1B) and
significantly reduced myeloperoxidase activity (Figure 1C). Upon
histological examination, the paws revealed pathologic changes
that correlated closely with the increases in MPO activity. Paw
biopsies showed that after carrageenan administration, marked
inflammatory changes were observed including pronounced
neutrophilic infiltration (Figure 1D, see arrows). Treatment with
fucoidan significantly reduced overall pathological changes and
neutrophil infiltration in the paw tissues (Figure 1D).

Blocking S1P inhibits carrageenan-induced thermal
hyperalgesia
Intraplantar injection of carrageenan led to a time-dependent
development of thermal hyperalgesia that peaked at 3 h and was
sustained through 5 h (Figure 2). Intraplantar injection of SK-I, a
well-characterized, competitive and reversible inhibitor of sphin-
gosine kinase, and thus S1P [38], given 30 minutes before
carrageenan, inhibited the development of carrageenan-induced
thermal hyperalgesia in a dose-dependent manner (250–1000 ng,
n = 6, Figure 2). Doses were chosen from previous studies [38–41].
Similarly, treatment with LT1002 (484 mg, n = 6), a monoclonal
antibody directed against S1P [31], was able to significantly
attenuate the carrageenan-induced hyperalgesic response, while its
IgG1 isotype control, LT1017 (572 mg, n = 6), had no effect
(Figure 3A). These results suggest that S1P contributes to the
development of carrageenan-induced thermal hyperalgesia.
Inhibition of S1P blocks the increased neutrophilic recruitment associated with carrageenan-induced thermal hyperalgesia

To determine whether S1P mediates the recruitment of neutrophils in carrageenan-induced thermal hyperalgesia, plantar tissues were taken from animals at 2 h (time of peak inhibition, data not shown) and assayed for MPO activity. As can be seen in Figure 3B, carrageenan injection led to a significant increase in MPO activity that was completely abrogated by pretreatment with LT1002 (484 μg, n = 6), but not by its negative control, LT1017 (572 μg, n = 6).

Blocking the S1P-S1PR1 axis attenuates carrageenan-induced thermal hyperalgesia and neutrophilic recruitment

In order to determine whether S1P recruits neutrophils through the S1PR1 receptor, we used the well-characterized, selective S1PR1 agonist, W146 [42]. As can be seen in Figure 3, intraplantar injection of W146 (0.3–1.2 μg, n = 6) [43], but not its inactive S-enantiomer, W140 (1.2 μg, n = 6) [43], dose-dependently abrogated the carrageenan-induced hyperalgesic response (Figure 4A).

When tested at the highest dose, W146 but not W140 (1.2 μg, n = 6) attenuated neutrophilic recruitment in response to carrageenan (Figure 4B). Doses of W146 and W140 were chosen from previous studies [42].

S1P and SEW2871-mediated thermal hyperalgesia is attenuated by fucoidan

To further strengthen the relationship between S1PR1 and neutrophil infiltration, we investigated whether the development of thermal hyperalgesia in response to exogenous intraplantar injection of S1P or the S1PR1 agonist, SEW2871 [43,44], was driven by neutrophils. As previously reported by our group [7,12], intraplantar injection of S1P (0.3 μg, n = 6) or SEW2871 (0.3 μg, n = 6) led to a time-dependent development of thermal hyperalgesia (Figure 5) which was blocked by i.p. injection of fucoidan (40 mg/kg, n = 6, Figure 5) given 30 min prior to S1P or SEW2871. S1P and SEW2871 were used at doses previously shown by our group to provide maximal hyperalgesia [7,12] and were chosen from previous studies [45]. We attempted to measure increased formation of MPO in paw tissues following intraplantar injection of S1P but our results yielded inadequate signal to detect changes in MPO formation between the groups. This may be due to insufficient sensitivity of the assay in these tissues or may have resulted from a highly localized infiltration of neutrophils at sites of damage that is capable of participating in the development of hyperalgesia, but whose signal is undetectable in a total paw preparation. Nevertheless, pharmacological targeting with a well-characterized anti-neutrophil agent [28,36,37] clearly supports the contribution of neutrophils in S1P-mediated thermal hyperalgesia.

FTY720 inhibits carrageenan-induced thermal hyperalgesia and neutrophilic recruitment

To assess the therapeutic potential of targeting S1P-S1PR1 signaling in the inflammatory pain setting, we examined the ability of the orally active S1PR modulator, FTY720 (fingolimod), to block carrageenan-induced thermal hyperalgesia and neutrophilic recruitment. FTY720 has been recently FDA-approved for the treatment of multiple sclerosis and is postulated to exert its actions, at least in part, through the binding, internalization, and subsequent blockade of S1PR1 signaling [46,47]. Inhibition of S1PR signaling using FTY720 (0.1 mg/kg –1.0 mg/kg, n = 7), with doses chosen from previous studies [46], attenuated the carrageenan-induced hyperalgesia and associated neutrophilic infiltration (Figure 6).

Discussion

In the present study we demonstrate that S1P acting through the S1P1 receptor subtype plays an important role in the development of thermal hyperalgesia associated with inflammation. In addition, we present evidence that S1PR1-triggered neutrophil infiltration is a central component in this setting. Inhibition of sphingosine kinases 1 and 2 with SK-I, which prevents the phosphorylation of sphingosine to form S1P [38], inhibits the development of thermal hyperalgesia in the carrageenan model, a well-characterized model of inflammation-induced hyperalgesia. Similarly, neutralizing S1P with the anti-S1P blocking antibody, LT1002, prevents the development of the carrageenan hyperalgesic response.

Our present work focuses on the role of S1PR1 as it is emerging as an important subtype in the mediation of peripheral sensitization and hyperalgesia. As we have previously reported, blockade of S1PR1 with W146 attenuates S1P-induced thermal hyperalgesia [12] and the enhanced excitability in peripheral sensory neurons...
in response to S1P has been shown to occur at least in part through the activation of S1PR1 [10]. It has also been demonstrated that a S1PR1 agonist injected intracutaneously induces heat hypersensitivity in vivo and that mice lacking S1PR1 in Na+,l,β expressing nociceptors or in the DRG exhibit reduced S1P-induced hyperalgesia, suggesting that nociceptor sensitization by S1P predominantly occurs through activation of S1PR1 [13,19]. Our results support these previous findings and extend them to also implicate the role for this receptor subtype in inflammatory pain. Indeed, the selective S1PR1 antagonist, W146 [43], blocked carrageenan-induced thermal hyperalgesia.

Given that S1P plays a prominent role in the inflammatory process through its ability to recruit neutrophils, which are also implicated in pain [27–30], we hypothesized that S1P-induced peripheral sensitization and hyperalgesia may be triggered by neutrophils. In support, we show that carrageenan-induced neutrophil infiltration is dependent upon S1P and subsequent activation of S1PR1 as both neutralization of S1P with the anti-S1P mAb, LT1002, and blockade of S1PR1 activation with W146 was able to inhibit carrageenan-induced neutrophil infiltration. This evidence, taken with the ability of fucoidan to abrogate the development of thermal hyperalgesia in response to S1P alone, supports our hypothesis that S1P-mediated peripheral sensitization and hyperalgesia occurs via a neutrophil-dependent mechanism.

How neutrophils are recruited at sites of inflammation following activation of the S1P-to-S1PR1 pathway remains to be investigated and was not the focus of the present study. However, scientific literature allows us to speculate as to how this might occur. S1PR1 activation has been shown to increase the production of the adhesion molecules ICAM-1 and E-selectin in response to inflammatory stimuli, making this a promising candidate for a potential mechanism in our neutrophil-dependent induction of hyperalgesia [22,48]. Several studies have implicated S1PR1, in the activation of the inflammatory transcription factor NFκB and p38 MAP kinase as well [23,49]. Activation of both NFκB and p38 leads to the increased production of many pro-inflammatory cytokines and chemokines such as TNF-α, IL-1β, IL-6 and CINC-1, the rat homolog of human IL-8 [50–52]. These cytokines are known to enhance the migration of neutrophils through their ability to upregulate the expression of adhesion molecules such as ICAM-1 and E-selectin on resident endothelial cells [53] while the chemokine CINC-1 is a potent neutrophil attractant through a mechanism independent of adhesion molecule expression [54]. Interestingly, the potent proinflammatory and pronociceptive nitrooxidative species, peroxynitrite [55–57], has been shown to have comparable therapeutic efficacy to FTY720 in models of MS [65]. As we show in this study, FTY720, like the S1PR1 antagonist W146, blocked carrageenan-induced thermal hyperalgesia and neutrophilic recruitment. In addition, FTY720 has been shown to be efficacious in the treatment of rheumatoid arthritis [66] and similar effects are observed with the S1PR1 antagonist, TASP0277308 [67]. Our work suggests that FTY720’s clinical efficacy may extend into the chronic inflammatory pain setting, as in for the treatment of arthritis-induced pain.

While current and emerging therapeutics like NSAIDS and TRPV1 antagonists have been shown to have potent antinociceptive actions in the inflammatory pain setting, in part through their ability to block neutrophilic recruitment, adverse side effect profiles limit their viability as a long-term solution to chronic pain. Novel classes of drugs, such as those targeting S1P, whether used in combination with current analgesics or as a stand-alone treatment, may represent a novel approach in effectively treating chronic pain while avoiding unattractive side effects.

In summary our findings show that S1P, through the activation of S1PR1, and the subsequent recruitment of neutrophils, plays a key role in inflammatory pain (summarized in Figure 7). Elucidating the mechanisms behind S1P’s involvement in inflammatory pain can serve to identify targets for new therapeutic agents that may fill the void between NSAIDs and narcotics in the management of pain.

Author Contributions

Conceived and designed the experiments: AF DS. Performed the experiments: AF ZC EE SC. Analyzed the data: AF EE SC. Contributed reagents/materials/analysis tools: RS. Wrote the paper: AF DS.

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S1P Mediates Pain via Neutrophil Infiltration

Whereas this study has clearly demonstrated the role of the S1PR1, we are not excluding the potential contribution of other receptor subtypes in S1P’s roles; however, this was not the focus of our work. Noteworthy, the tools that are available to examine other receptor subtypes are limited by off-target effects and selectivity issues. For example, the selective S1PR2 antagonist, JTE-013, has been shown to actually sensitize sensory neurons independently of S1PR2 activation [61]. The S1PR3 antagonist, CAY10444 has only been shown to be selective in vivo at very low dosages which may not be enough to sufficiently block due to low affinity of the compound for the receptor [62]. Also, CAY10444 has been shown to inhibit [Ca2+]i increases via purinergic P2 receptor or ß1A-adrenoceptor stimulation and ß1A-adrenoceptor-mediated contraction, while not affecting the S1P3-mediated decrease of forskolin-induced cAMP accumulation [63]. Inhibitors are not presently available for S1PR4 and S1PR5.

In the present study, FTY720 serves to demonstrate the potential clinical significance of targeting S1PR1 receptor activation in the inflammatory pain setting. It has been reported that blockade of the S1P-to-S1PR1 signaling pathway accounts for the observed beneficial effect of FTY720 in MS [64]. In support of this, recently developed S1PR3 antagonists, such as NIBR-0213, have been shown to have comparable therapeutic efficacy to FTY720 in models of MS [65]. As we show in this study, FTY720, like the S1PR1 antagonist W146, blocked carrageenan-induced thermal hyperalgesia and neutrophilic recruitment. In addition, FTY720 has been shown to be efficacious in the treatment of rheumatoid arthritis [66] and similar effects are observed with the S1PR1 antagonist, TASP0277308 [67]. Our work suggests that FTY720’s clinical efficacy may extend into the chronic inflammatory pain setting, as in for the treatment of arthritis-induced pain.

Author Contributions

Conceived and designed the experiments: AF DS. Performed the experiments: AF ZC EE SC. Analyzed the data: AF EE SC. Contributed reagents/materials/analysis tools: RS. Wrote the paper: AF DS.

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