Effect of resolvins on sensitisation of TRPV1 and visceral hypersensitivity in IBS

Peer-reviewed author version

Perna, Eluisa; Aguilera-Lizarraga, Javier; Florens, Morgane V; Jain, Piyush; Theofanous, Stavroula A; Hanning, Nikita; De Man, Joris G; Berg, Maya; De Winter, Benedicte; AGUIAR ALPIZAR, Yeranddy; Talavera, Karel; Vanden Berghe, Pieter; Wouters, Mira & Boeckxstaens, Guy (2021) Effect of resolvins on sensitisation of TRPV1 and visceral hypersensitivity in IBS. In: Gut, 70 (7), p. 1275 - 1286.

DOI: 10.1136/gutjnl-2020-321530
Handle: http://hdl.handle.net/1942/32447

EFFECT OF RESOLVINS ON SENSITIZATION OF TRPV1 AND VISCERAL HYPERSENSITIVITY IN IRRITABLE BOWEL SYNDROME

Word count: 4207

AUTHORS

Eluisa Perna[1†], Javier Aguilera-Lizarraga[1†], Morgane V. Florens[1], Piyush Jain[1], Stavroula Theofanous[1], Nikita Hanning[2], Joris G. De Man[2], Maya Berg[2], Benedicte Y. De Winter[2], Pieter Vanden Berghe[3], Yeranddy A. Alpizar[4], Karel Talavera[5], Mira M. Wouters[1], Guy E. Boeckxstaens[1].

AFFILIATIONS

[1] Laboratory for Intestinal Neuroimmune Interactions, Translational Research Center for Gastrointestinal Disorders, University of Leuven, Leuven, Belgium

[2] Laboratory of Experimental Medicine and Pediatrics (LEMP) and Infla-Med, research consortium of excellence, University of Antwerp, Antwerp, Belgium

[3] Laboratory for Enteric NeuroScience, Translational Research Center for Gastrointestinal Disorders, University of Leuven, Leuven, Belgium

[4] BIOMED, uHasselt, Hasselt, Belgium.

[5] Laboratory of Ion Channel Research, Dept. of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium; VIB Center for Brain & Disease Research, Leuven, Belgium

†Authors have contributed equally

PERSONAL CONTRIBUTIONS

EP, MVF, MMW and GEB planned and designed the experiments. BDW, NH, JDM and MB planned and designed the experiments in the post-TNBS colitis model. EP, JAL and PJ

performed and analyzed the Ca^{2+} experiments in DRG neurons and rectal biopsies experiments. MVF carried out the *in vivo* animal protocol and analyzed visceromotor responses and compliance. MVF and JAL performed and analyzed colonic permeability assessments. NH and JDM performed and analyzed the experiments in the post-TNBS-colitis model. ST and JAL performed the staining in retrograde labelled DRG neurons. PVB, YAA and KT provided technical expertise. EP, MVF, JAL and GEB wrote and revised the manuscript. All other authors corrected and approved the final version of the manuscript.

CORRESPONDING AUTHOR:

Prof. Dr. **Guy E. Boeckxstaens**

Department of Chronic Diseases, Metabolism and Ageing, Translational Research Center for Gastrointestinal Disorders (TARGID), University Hospital of Leuven, KU Leuven

Herestraat 49, 3000 Leuven, Belgium

Tel: secretary +32-16-330671 / +32-16-330237

E-mail address: guy.boeckxstaens@kuleuven.be

DISCLOSURE: The authors have nothing to disclose.

ABBREVIATIONS

DRG: Dorsal root ganglion; **GPCR**: G protein-coupled receptor; **GPR18**: G protein receptor 18; **IBS**: Irritable Bowel Syndrome; **OVA**: ovalbumin; **PI-IBS**:Post-infectious IBS; **HV**: healthy volunteer; **PTX:** pertussis toxin; **RvD1**: Resolvin D1; **RvD2**: Resolvin D2; **RvE1**: Resolvin E1; **SN**: supernatans**; TNBS**: 2,4,6-trinitrobenzene sulphonic acid; **TRPV1**: Transient receptor potential vanilloid 1; **VHS**: visceral hypersensitivity; **VMR**: visceromotor response;

ABSTRACT

Objective: Resolvins (RvD1, RvD2 and RvE1) are endogenous anti-inflammatory lipid mediators that display potent analgesic properties in somatic pain by modulating TRPV1 activation. To what extent these molecules could also have a beneficial effect on TRPV1 sensitization and visceral hypersensitivity (VHS), mechanisms involved in irritable bowel syndrome (IBS), remains unknown.

Design: The effect of RvD1, RvD2 and RvE1 on TRPV1 activation and sensitization by histamine or IBS supernatans was assessed on murine dorsal root ganglion (DRG) neurons using live Ca^{2+} imaging. Based on the results obtained *in vitro*, we further studied the effect of RvD2 *in vivo* using a murine model of post-infectious IBS (PI-IBS) and a rat model of post inflammatory VHS. Finally, we also tested the effect of RvD2 on submucosal neurons in rectal biopsies of IBS patients.

Results: RvD1, RvD2 and RvE1 prevented histamine-induced TRPV1 sensitization in DRG neurons at doses devoid of an analgesic effect. Of note, RvD2 also reversed TRPV1 sensitization by histamine and IBS supernatant. This effect was blocked by the GPR18 antagonist O-1918 (3-30 µM) and by pertussin toxin. In addition, RvD2 reduced the capsaicin-induced Ca^{2+} response of rectal submucosal neurons of IBS patients. Finally, treatment with RvD2 normalized pain responses to colorectal distention in both preclinical models of VHS.

Conclusions: Our data suggest that RvD2 and GPR18 agonists may represent interesting novel compounds to be further evaluated as treatment for IBS.

SUMMARY

What is already known about this subject?

- Abnormal activation and sensitization of TRP channels, i.e. TRPV1, is recognized to be an important mechanism of VHS, both in preclinical models of VHS and patients with IBS.
- Sensitization of TRPV1 is mediated by mast cells mediators such as histamine.
- Resolvins, a novel class of endogenous mediators with potent analgesic properties, have been demonstrated to reduce somatic pain via interaction with TRP channels.

What are the new findings?

- Our study shows that resolvins and especially RvD2 are able to normalize histamine- and IBS supernatant-induced sensitization of TRPV1 in DRG neurons. This effect is mediated by GPR18.
- RvD2 restores pain responses to colorectal distension in two preclinical models of VHS, i.e. a post-infectious mouse model and a post-inflammatory rat model.
- RvD2 normalizes the TRPV1 activation of submucosal neurons in rectal biopsies of IBS patients

How might it impact on clinical practice in the foreseeable future?

- Our study provides evidence that RvD2 represents a novel potential approach to intervene with VHS and suggests that RvD2 and GPR18 agonists may represent interesting novel compounds to be further evaluated as treatment for IBS.

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by an altered defecation pattern in the absence of a known organic cause[1–4]. The main hallmark of IBS is abdominal pain or discomfort that is linked to abnormal perception of visceral stimuli such as intestinal distension or nutrient infusion, also referred to as visceral hypersensitivity (VHS)[5–9]. The mechanisms underlying VHS, however, remain largely unknown.

To date, evidence is accumulating that VHS can result from aberrant mast cell activation. Indeed, colonic and rectal biopsies from IBS patients release higher levels of tryptase[10–14] and histamine[11,13,15], compared to healthy controls. These mediators can activate and sensitize gut afferents via a variety of cell surface receptors and channels[16]. In particular, transient receptor potential (TRP) cation channels are thought to play a crucial role in visceral nociception as they can be directly or indirectly activated by pro-inflammatory mediators. These channels are involved in several cellular functions such as maintainance of cellular homeostasis, detection and transduction of chemical and physical stimuli from their environment or transduction of cell activation mediated by G protein-coupled receptors (GPCRs) or ion channels. TRP channels are present on peripheral nerve endings where their activation and signaling to the central nervous system results in nociception. Alterations in this process lead to VHS. Interestingly, upregulation and/or sensitization of TRP channels is now recognized to be an important mechanism of VHS both in preclinical models of VHS and patients with IBS[17–20]. TRPV1, a receptor responding to capsaicin, heat, acidosis and endovanilloids, is one of the most studied nociceptors involved in this process[21]. The expression of TRPV1 is indeed increased in preclinical models of VHS and in rectal biopsies of IBS patients[22,23], while we recently provided evidence of TRPV1 sensitization in IBS patients[20]. Rectal submucosal neurons of IBS patients responded more to the TRPV1 agonist capsaicin, a phenomenon that could be mimicked by incubation of neurons from healthy controls with histamine. Of interest, treatment of IBS patients with the histamine 1 receptor antagonist ebastine resulted in a significant improvement of abdominal pain in 46% of IBS patients compared to 13% in the

placebo group[20]. It should be emphasized though that only half of the patients improve on histamine 1 antagonism, stressing the need for an alternative therapeutic strategy.

Recently, a novel class of endogenous mediators with potent analgesic properties, *i.e.* resolvins, has been identified. Resolvins are generated from ω-3 poly-unsaturated fatty acid (PUFA) precursors docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and are classified into D- and E-series, respectively[24–28]. In preclinical models of acute and chronic somatic inflammatory pain, RvD1, RvD2 and RvE1 act as potent endogenous inhibitors of thermal and mechanical hypersensitivity upon modulation of TRP channels[29–31]. A similar effect has been shown in a murine model of reserpine-induced fibromyalgia, where RvD2 injections were able to decrease the nociceptive animal behaviour[32]. Resolvins exert their analgesic effect at different levels: 1) by reducing inflammation via the downregulation of pro-inflammatory signals, including nuclear factor-κB, several cytokines, and leukotrienes[29,33,34] and 2) by activating specific GPCRs that modulate the activity of specific TRP channels[24,29,30]. For example, through the activation of ChemR23 on DRG neurons, RvE1 inhibits the ERK-dependent activation of TRPV1 and TNF-α-mediated hyperalgesia, thereby reducing inflammatory pain[29].

To what extent these new mediators can also affect TRP channels sensitization involved in VHS remains however to be studied. In the present work, we therefore investigated the effect of D (RvD1, RvD2) and E (RvE1) resolvins on TRPV1 sensitization *in vitro*. Based on these data, RvD2 was further evaluated *in vivo* using two preclinical models of VHS, namely a mouse model of post-infectious VHS and a rat model of post-inflammatory VHS[35]. Finally, we studied the effect of RvD2 on the capsaicin-induced TRPV1 activation of submucosal neurons in rectal biopsies of healthy volunteers and IBS patients.

MATERIAL AND METHODS

Ca^{2+} imaging of murine DRG neurons and human submusal neurons:

The experiments were performed as previously described[20]. For details please see the Supplementary Methods.

In vivo experiments:

Mouse model of post-infectious VHS

Balb/c mice of at least 20 grams were implanted with Physiotel ETA-F10 telemetric transmitters (Data Sciences International St. Paul, Minnesota, USA) connected to electromyography (EMG) electrodes that were sutured into the abdominal wall musculature to record visceromotor responses (VMR)[36]. Mice remained on a heating pad for 12 h after the surgery and were then left to recover for 10 days[36]. VMRs were evoked at different time points (**Fig. 6A**) by colorectal distensions (increasing volumes from 20 µL to 80 µL) using a distension catheter (Fogarty catheter for 106 arterial embolectomy, 4F; Edwards Lifesciences, Irvine, California, USA) inserted into the colon (3 cm from the rectum). Data were calculated as percentage VMR relative to the maximum pain response before infection (*i.e.* 80 µL distention is set at 100%) and were presented as area under the curve (AUC) of the volume-response curve. Mice with an AUC superior to 4.8 %*mL were considered hypersensitive, based on the 95th percentile of measurements performed in naïve mice.

To induce VHS, eight- to nine-week old mice were infected by oral gavage of 1 x 10^{11} colony forming units (CFU) *C. rodentium* and first exposed to ovalbumin (OVA) by oral gavage (50 mg, dissolved in 0.9% NaCl) (Grade II, Sigma-Aldrich® St Louis, Missouri, USA) 3 h after infection[37]. From then onwards, OVA was dissolved in the drinking water (1% v/v) and renewed daily for 10 consecutive days. Five weeks after infection, mice were re-exposed to OVA (50 mg) by oral gavage every other day until sacrifice, resulting in VHS to colorectal distension[37]. To assess the effect of RvD2 on VHS, starting from the 9th OVA gavage at week 7 post-infection, hypersensitive mice were injected intraperitoneally (*i.p.*) with 300 ng RvD2 (solubilized in ethanol, Cayman Chemical, Ann Arbor, Michigan, USA) or vehicle (= ethanol 100%, VWR, Radnor, Pennsylvania, USA), diluted in 100 µL saline, 30 min after OVA gavage

every other day for one week (4 treatments in total). The dose of RvD2 was previously reported to be effective in a mouse model of fibromyalgia[32]. Mice were randomly assigned to the treatment or vehicle group. After the last assessment of visceral sensitivity to colorectal distention, mice were sacrificed by CO_2 intoxication and colonic tissue was collected for Ussing chamber experiments.

Rat model of post-inflammatory VHS

Colitis was induced in male Sprague-Dawley rats by 2,4,6-trinitrobenzene sulphonic acid (TNBS) as previously described[35,38]. Three days later, the presence of colitis was confirmed and scored colonoscopically. From day 10 onwards, colonoscopy was repeated every 4 days until the colonic mucosa did not show any remaining signs of inflammation. After full mucosal healing, EMG electrodes were implanted in the abdominal musculature and the animals received an *i.p.* injection with either RvD2 (1500 ng) or its vehicle (250 µL of a 2.4% ethanol solution). The RvD2 dose chosen is based on that used in the post-infectious mouse model but adjusted for rats as previously described[39]. Treatment was repeated every day after mucosal healing until the VMR measurements 3 days later, resulting in the total administration of 4 dosages. Thirty min after the last treatment, VMR was assessed followed by the colonic compliance.

Finally, animals were sacrificed (exsanguination under 100 mg/kg pentobarbital anaesthesia) and the inflammatory parameters (colonoscopy, macroscopy, microscopy and myeloperoxidase (MPO) activity) were scored. Unequal group sizes are due to loosening of the EMG electrodes in one animal, part of the control group treated with RvD2.

Human studies

Patients that fulfilled the Rome III criteria for IBS were recruited at the outpatient clinic of the University Hospital Leuven. Healthy volunteers free of symptoms, without history of gastrointestinal disease or previous gastrointestinal surgery and not taking any gastrointestinal

medication were recruited by public advertisement. All participants gave informed consent and Leuven University Hospital Human Ethics Committee approved the protocol (ethical approval number ML9438 and S55484).

Eleven healthy volunteers (7 F and 4 M, median age of 31 years IQR [28-50]) and 15 IBS patients (14 F and 1 M, median age of 34 years IQR [27-58]; 3 IBS-C, 9 IBS-D, 3 IBS-U) were invited to undergo a proctoscopy to collect rectal biopsies. Eight biopsies were incubated for 24 h in Roswell Park Memorial Institute medium (RPMI) (Lonza, Verviers, Belgium) supplemented with fetal calf serum (FCS) (10%) (Pan Biotech, Aidenbach, Germany), Penicillin/Streptomycin (1%) (Lonza, Verviers, Belgium), Amphotericin B/gentamycin (0.2%) (Invitrogen, Gent, Belgium) at 37°C, 5% CO_2 to generate supernatants (SN). After overnight incubation, SN were collected and stored at -80 °C until further experiments[40]. In addition, freshly isolated biopsies were processed for Ca^{2+} imaging of submucosal neurons, as previously described [20,41]. For more details, please see Supplementary Methods.

Statistical analyses

All statistical analyses were performed using GraphPad Prism 8 (La Jolla,USA) or SPSS 24.0 (IBM, New York, USA). The results are presented as the mean ± standard error of the mean (mean ± SEM) or median ± interquartile range (median ± IQR), as indicated in the figure legends. Statistical analyses of the peak F340/380 ratio for the Ca^{2+} imaging experiments were performed after correction for the individual baseline. The effect of resolvins on TRPV1 activation on DRG neurons was analyzed by mixed effects model (RELM) followed by Dunnett's multiple comparisons. The effect of resolvins on TRPV1 sensitization and on the effect on the VMR in the murine model was analyzed by Kruskal Wallis test followed by Dunn's multiple comparison test. The effect of RvD2 on TRPV1 sensitization in submucosal neurons from human biopsies was analyzed by Friedman test followed by Dunn's multiple comparison test. The effect of resolvins on the visceromotor response and in colonic permeability in our murine model was analyzed by two-tailed Mann-Whitney test. The effect of resolvins on the visceromotor response in our rat model was analyzed by generalized estimating equations

(GEE) followed by least significant difference post hoc test. Inflammatory parameters (assessed by endoscopy) in rats were analyzed by two-way ANOVA, followed by Student-Newman-Keuls (S-N-K) *post hoc* test when appropriate. A p-value less than 0.05 was considered statistically significant.

RESULTS

Effect of RvD1, RvD2 and RvE1 on TRPV1 in murine DRG neurons

To evaluate if RvD1, RvD2 or RvE1 interfered with the direct activation of TRPV1, DRG neurons were pre-treated for 30 min with different doses of RvD1, RvD2 or RvE1 or vehicle prior to administration of increasing concentrations (10 nM-100 nM-1 µM) of the TRPV1-agonist capsaicin. As shown in **Figure 1**, administration of capsaicin resulted in a concentration-dependent increase in the Ca^{2+} response of vehicle-treated DRG neurons. Incubation of DRG neurons with RvD1 (10 nM-100 nM-1 µM), did not inhibit the Ca^{2+} response to capsaicin compared to vehicle (**Fig. 1A).** Incubation with a low dose of RvD2 or RvE1 (10 nM) did not decrease TRPV1 activation while higher concentrations (100 nM-1 µM) led to a decrease of the capsaicin-induced TRPV1 response (**Fig. 1B-C**).

Taken together, these results demonstrate that higher concentrations of RvD2 and RvE1, but not of RvD1, inhibit TRPV1 activation, thereby confirming their analgesic properties at higher concentrations.

Effect of RvD1, RvD2 and RvE1 on histamine-induced sensitization of TRPV1

We previously demonstrated that TRPV1 is sensitized by histamine and by biopsy supernatant from IBS patients[20]. First, we examined whether sensitization of TRPV1 by histamine could be prevented by pre-treatment of DRG neurons with RvD1, RvD2 or RvE1 (**Fig. 2A**). To avoid an effect of the tested dosages on TRPV1 activation, we selected concentrations that did not affect the TRPV1 concentration-response curve. As shown in **Figure 2**, histamine (10 µM)

induced an increased response of TRPV1 to capsaicin (10 nM) compared to vehicle, an effect that was prevented by the 3 resolvins tested (**Fig. 2B-D**).

Next, we evaluated if RvD1, RvD2 or RvE1 could revert histamine-induced TRPV1 sensitization (**Fig. 2E**). To this end, DRG neurons were first sensitized with histamine and subsequently treated with RvD1 (100-300 nM) (**Fig. 2F**), RvD2 (10 nM) (**Fig. 2G**), RvE1 (30 nM) (**Fig. 2H**) or vehicle. The highest dose of RvD1 (300 nM) and RvD2 (10 nM), but not 100 nM of RvD1 or RvE1 (30 nM), were able to reverse established histamine-mediated TRPV1 sensitization compared to vehicle treated cells (**Fig. 2**).

Taken together, these results indicate that resolvins, in particular RvD2, can prevent and reverse TRPV1 sensitization at concentrations that do not affect TRPV1 activation.

RvD2 inhibits histamine-mediated sensitization of TRPV1 through GPR18 and Gαi signaling pathway

Next, we further investigated the receptor and intracellular signaling pathway mediating the effect of RvD2 on TRPV1 sensitization. GPR18 and FPR2/ALX were recently identified as the receptor for RvD2 and RvD1, respectively[42]. To investigate if the modulating effect of RvD2 on TRPV1 sensitization is mediated by GPR18, DRG neurons were incubated with increasing concentrations of the GPR18 antagonist O-1918 (3-30 μM)[42,43] (**Fig. 3A**). As shown in **Figure 3B**, O-1918 dose-dependently inhibited the effect of RvD2 on TRPV1 sensitization. In contrast, the FPR2/ALX receptor antagonist Boc2 (10 μM)[44] failed to affect TRPV1 response, suggesting that RvD2 acts on GPR18 but not FPR2/ALX to block histamine-induced TRPV1 sensitization.

As GPCRs can regulate TRPV1 via Gα-mediated modulation of intracellular phospholipases or kinases[21,45], and knowing that GPR18 acts through the inhibitory Gαi GPCRs signaling pathway[46], we hypothesized that RvD2 inhibits TRPV1 sensitization via Gαi proteins. Hence, we incubated DRG neurons with pertussis toxin (PTX), known to prevent Gαi from binding GPCRs[47]. As shown in **Figure 3C-D**, PTX incubation blocked the effect of RvD2 on histamine-induced TRPV1 sensitization.

Next, to investigate if GPR18 could be linked to visceral nociception, we evaluated the expression of this receptor in DRG neurons projecting to the colon. To this end, thoracolumbar (TL; splanchnic) and lumbosacral (LS; pelvic) DRG were collected from healthy mice one week after injection of retrograde fluorescent tracer Fast Blue in the colon. We observed that GPR18 was widely present in DRG neurons projecting to both splanchnic and pelvic nerves (**Fig. 3E**).

Taken together, these results suggest that RvD2 blocks histamine-induced TRPV1 sensitization via GPR18 coupled to Gαi proteins and expressd by colon-innervating DRG neurons.

RvD2 interferes with TRPV1 sensitization mediated by IBS supernatants

Knowing that RvD2 can prevent and reverse histamine-induced TRPV1 sensitization, we next evaluated if similar results could be obtained using DRG neurons sensitized by IBS supernatants. In a first series of experiments, DRG neurons were incubated with RvD2 (10 nM) in the presence or absence of O-1918 (10 μM) and subsequently exposed to biopsy supernatants from HV or IBS patients (**Fig. 4A**). As previously reported[20], DRG neurons incubated with histamine (10 μM) or IBS supernatant showed an increased Ca^{2+} response to capsaicin compared to neurons treated with RPMI medium (vehicle) or with supernatants from healthy subjects. Notably, RvD2 was able to prevent TRPV1 sensitization by IBS supernatant, an effect that was blocked by the GPR18 antagonist O-1918. No effect of RvD2 or O-1918 on HV supernatant was detected (**Fig. 4B**).

Next, we tested if RvD2 could revert TRPV1 sensitization induced by IBS supernatant. To this end, DRG neurons were first sensitized by overnight incubation with supernatants from IBS patients or with histamine. RPMI (vehicle) and supernatant from HV were used as negative controls. After an overnight incubation, DRG neurons were treated for 30 min with GPR18 antagonist O-1918 or vehicle, followed by a 30-min treatment with RvD2 10 nM prior to capsaicin administration (**Fig. 4C**). RvD2 incubation reverted TRPV1 sensitization mediated

by IBS supernatant and histamine. This effect was abolished in the presence of O-1918 (**Fig. 4D**).

Altogether, these results show that RvD2, through GPR18 signaling, blocks TRPV1 sensitization by IBS supernantant, suggesting potential therapeutic properties for RVD2 and GPR18 agonists to treat VHS.

RvD2 reverses TRPV1 sensitization in neurons of IBS patients

As we previously demonstrated TRPV1 sensitization of submucosal neurons in IBS patients compared to HV[19,20] , we next evaluated the effect of RvD2 on the Ca^{2+} response to capsaicin (10 nM) in rectal biopsies of IBS patients (n=7). As shown in **Figure 5**, 1 nM of capsaicin activated between 40 and 100% of submucosal neurons in rectal biopsies of IBS patients. The % of responding neurons nor the amplitude of this response was affected by 30 min of perfusion with vehicle, indicating that the response of submucosal neurons to 1 nM capsaicin is reproducible. In contrast, after 30 min perfusion with RvD2 (10 nM), the amplitude of the Ca^{2+} response to 1 nM capsaicin was significantly decreased compared to vehicle. The number of responding submucosal neurons, however, remained unaffected. These data indicate that RvD2 is also able to revert TRPV1 sensitization in human submucosal ganglia (**Fig. 5**).

RvD2 reverses VHS in two preclinical models of IBS

Based on the results obtained *in vitro*, we next studied the effect of RvD2 *in vivo* in two preclinical models of VHS.

1. Mouse model of post-infectious VHS: We previously reported that BALB/C mice infected with *C. rodentium* in the presence of ovalbumin (OVA) develop an aberrant local immune response to OVA leading to the production of OVA-specific IgE antibodies in the colon. Serum levels of these antibodies and an ear prick test to OVA are however negative confirming the local nature of the immune response[48]. Re-exposure to OVA by oral gavage leads to mast cell activation and histamine-mediated VHS in the absence of intestinal inflammation[37]. In the present study, re-exposure to OVA in mice exposed to OVA during *C. rodentium* infection

resulted in VHS (VMR response: AUC > 4.8 %*mL) compared to baseline in 13 out of 20 mice. As shown in **Figure 6B**, all hypersensitive mice treated with vehicle (n=6) remained hypersensitive. In contrast, RvD2 significantly reduced the VMR to colorectal distension (**Fig. 6C-D**) resulting in normalization of visceral sensitivity to colorectal distention in 6 out of 7 mice. RvD2 had no effect on colonic compliance (**Supplementary Fig.1**).

In addition to visceral pain, we also assessed colonic permeability using Ussing chambers. Here, we observed no significant difference in the passage of Na^+-fluorescein or transepithelial electrical resistance (TEER) between mice treated with RvD2 versus vehicle, while both showed increased colon permeability compared to uninfected control mice (**Fig. 6E-F**).

2. Rat model of post-inflammation VHS

As shown in **Figure 7B**, post-inflammation animals treated with vehicle displayed higher VMR responses compared to control animals treated with vehicle, indicating the development of VHS. In control (normosensitive) rats, RvD2 did not affect visceral pain responses to colorectal distension, confirming the lack of an analgesic effect of the dose used. In contrast, RvD2 significantly reduced the VMR in post-inflammation rats restoring pain responses to the same level as that of control animals. RvD2 had no effect on colonic compliance (**Supplementary Fig. 1**) or post mortem inflammatory parameters (**Supplementary table 1-4**).

DISCUSSION

In the present study, we showed that RvD1 and, in particular, RvD2, prevent and reverse TRPV1 sensitization mediated by histamine or IBS supernatants at concentrations that do not affect normal TRPV1 activation. The effect of RvD2 was mediated by GPR18 and blocked by PTX, suggesting the involvement of Gαi signaling. Notably, in two preclinical models of VHS treatment with RvD2 reversed VHS. Of interest, in rectal biopsies from IBS patients, treatment with RvD2 also reversed TRPV1 sensitization of submucosal neurons. Taken together, our

results suggest that RvD2 or agonists of GPR18 could be an interesting novel approach to treat VHS in IBS patients.

Resolvins are endogenous lipid mediators mainly studied for their anti-inflammatory properties and their role in the recovery phase of an inflammatory process[24]. In inflammatory models, resolvins exert their analgesic effect not only indirectly by dampening the inflammatory process, but also through binding to their GPCRs on afferent nerve fibers. Acute spontaneous pain evoked by intraplantar injection of TRPV1, TRPV4 and TRPA1 agonists is indeed potently inhibited by RvD1, RvD2 and RvE1, even at concentrations in the nanomolar range[30,31,49]. In line with these findings, these resolvins inhibited TRPA1, TRPV1 and TRPV4 activation in DRG neurons and transiently transfected HEK cells[30,31]. In a first set of *in vitro* experiments using DRG neurons, we confirmed the inhibitory properties of RvD2 and RvE1, while RvD1 did not alter TRPV1 activation at any of the doses used, in keeping with a previous study by Bang *et al*[30]. As previously shown[20], incubation of DRG neurons with histamine induced an increased Ca^{2+} response to capsaicin, indicative of TRPV1 sensitization. RvD2 and, to a lesser extent, RvD1 and RvE1, prevented and reversed this sensitizing effect of histamine. The effect of RvD2 was inhibited by O-1918, a blocker of the recently identified receptor for RvD2, *i.e.* GPR18[42], expressed by DRG neurons projecting to the colon. Along the same line, TRPV1 sensitization evoked by incubation with IBS supernatant was prevented and reversed by RvD2. This is of particular interest as we previously demonstrated TRPV1 sensitization as an important mechanism underlying abnormal pain perception in IBS[20]. Of note, we here show that this response in IBS biopsies can also be reduced by RvD2, indicating that our findings can be translated to the human intestine. Similar results showing resolvin-mediated inhibition of TRPV1 sensitization were previously reported by Jo *et al*[50]. In this study, TRPV1 potentiation in murine DRG neurons was induced by substance P and was blocked by RvE1 acting on its receptor ChemR23.

To further explore its therapeutic potential, the effect of RvD2 was evaluated in two preclinical models of VHS. In a first model, mice were infected with *Citrobacter rodentium* while exposed

to OVA. These mice developed pronounced increased pain responses to colorectal distention and altered mucosal permeability[37] (*i.e.* 2 characteristic symptoms of IBS) when re-exposed to OVA in the post-infectious phase. This process results from an aberrant local immune response to OVA (but not a systemic immune response as in food allergy) with IgE-mediated sensitization of colonic mast cells and mast cell activation upon re-exposure to OVA leading to histamine release and sensitization of TRPV1[51]. Of note, visceral hypersensitivity in this post-infectious model is not associated with upregulation of pro-inflammatory genes, influx of inflammatory cells or changes in morphology[37], illustrating that intestinal inflammation is not involved. Interestingly, the VMR to colorectal distension was normalized in hypersensitive mice treated with RvD2. These results were further supported in a rat model of post-inflammatory VHS. RvD2 normalized the aberrant VMR to colorectal distension in post-TNBS colitis rats while it had no effect on colonic compliance or inflammatory parameters. In control rats, RvD2 did not affect pain responses suggesting that pain transmission under normal conditions remains unaffected by the dosis tested. The VHS present in this post-inflammatory model was however potently prevented by treatment with RvD2.

Similar pain modulating properties of resolvins were previously reported in somatic pain models. Local application of RvD1, RvD2 or RvE1 efficiently reduced mechanical and thermal somatic allodynia evoked by carrageenan, formalin and complete Freund's adjuvant[32,49,52,53]. Together with the *in vitro* data, our findings suggest that RvD2 and GPR18 agonists are interesting compounds to reverse VHS, most likely by normalizing TRPV1 sensitization.

We recently reported that histamine potentiates TRPV1 via the activation of histamine 1 receptor[19,20] leading to phospholipase (PL) C activation and accumulation of cyclic AMP by adenylyl cyclase[20]. cAMP-dependent activation of protein kinase (PK) A subsequently triggers the direct phosphorylation of TRPV1 on Ser116, resulting in sensitization of TRPV1 on primary afferents[54]. The mechanism by which resolvins inhibit TRP channel activation and sensitization is not entirely resolved. Of interest, however, morphine and μ-opioid agonists block TRPV1 sensitization via activation of inhibitory GPCR signaling (Gαi), resulting in the inhibition of

adenylyl cyclase-dependent cAMP production[55]. Along the same line, we showed that, in the presence of the Gαi inhibitor pertussis toxin, RvD2 was unable to normalize the reponse of DRG neurons to histamine, suggesting that the effect of RvD2 on TRPV1 sensitization is mediated via inhibitory GPCR signaling.

Besides altered bowel habits, the hallmark of IBS is abnormal pain perception or VHS, reported by ~60% of patients[5–9]. Our group recently showed that TRPV1, TRPV4 and TRPA1 are sensitized in IBS patients[19,20], identifying these channels as promising drug targets to treat VHS. Interfering with TRP channel activation is however not an attractive approach. TRPV1, TRPV4 and TRPA1 are indeed widely expressed and are involved in crucial physiological functions such as heat sensation, and can thus lead to severe side effects (*e.g.* hyperthermia, impaired noxious heat sensation). On the contrary, the use of RvD2 in the treatment for VHS in IBS appears particularly appealing since this compound did not interfere, in our experiments, with TRPV1 activation by itself. Of interest, a recent study demonstrated lower serum concentrations of RvD1 in IBS-C patients compared to healthy controls, correlating with abdominal pain severity[56].

To conclude, based on the current study, we propose an alternative approach, *i.e.* to interfere with the process underlying TRP sensitization using resolvins, in particular RvD2 or GPR18 agonists. To what extent sensitization of TRPV4 or TRPA1 and sensitization of TRP channels by other mast cell mediators, such as proteases[40,57] are similarly affected by RvD2 remains yet to be studied. Moreover, resolvins are rather unstable and difficult to synthesize, while safety issues remain to be evaluated[58,59]. Nevertheless, a clinical trial on infantile eczema reported the first successful treatment with a pro-resolving mediator (15(R/S)-methyl-LXA(4), a topical stable LXA4 analogue) in humans[60]. In addition, the synthetic resolvin RX-10045 (a derivative of RvE1) has progressed to phaseI/II clinical evaluation in patients with ocular inflammation and pain in cataract surgery (NCT02329743). Although further research is definitely required, based on our data, we propose that RvD2 and GPR18 agonists should be further explored as novel compounds to treat VHS in IBS.

ACKNOWLEDGEMENTS

We would like to thank the Cell and Tissue Imaging Cluster for the 470 use of the NIKON inverted TillVision 471 microscope, epifluorescence microscope BX 41 Olympus and Zeiss LSM880 – Airyscan 472 confocal microscope. Cell and Tissue Imaging Cluster, CIC, Supported by Hercules 473 AKUL/15/37_GOH1816N and FWO G.0929.15 to Prof. Pieter Vanden Berghe, KU Leuven.

This work was supported by the Research Foundation - Flanders (FWO-SBO grant nr. S001017). MVF, MMW, PJ, JAL, and YAA were supported by an FWO doctoral or postdoctoral fellowship (1110017N/1110019N, 1248513N, 12R5219N, 12X9820N and 12H8217N). EP was supported by an Early Stage Researcher Marie Curie fellowship (NeuroGUT).

LEGENDS

Figure 1. Effect of resolvins on TRPV1 activation by capsaicin. Dose-response curves after 30 min incubation with (**A**) RvD1**,** (**B**) RvD2 and (**C**) RvE1 or vehicle on the Ca^{2+} response evoked by increasing concentrations of capsaicin (10 nM, 100 nM and 1 μM) in murine DRG neurons. The number of neurons evaluated is depicted in the legend. *, $P < .05$; **, $P < .01$; ****, $P < .0001$. Mixed effects model (RELM) analysis was performed followed by Dunnett's multiple comparisons test compared to vehicle. Data are shown as mean ± SEM.

Figure 2. Effect of RvD1, RvD2 and RvE1 on histamine-induced TRPV1 sensitization. (**A**) Outline of experimental design evaluating the ability of resolvins to prevent histamine-induced TRPV1 sensitization on DRG neurons. Vehicle or resolvins (RvD1, RvD2 and RvE1) were administered before histamine perfusion and activation of TRPV1 by capsaicin. Effect of pre-treatment with (**B**) RvD1, (**C**) RvD2, or (**D**) RvE1 on the Ca^{2+} response to capsaicin of DRG neurons sensitized by histamine. (**E**) Outline of experimental design testing the ability of resolvins to reverse histamine-induced TRPV1 sensitization on DRG neurons. Treatments with

vehicle or RvD1, RvD2 and RvE1 were administered after 10 min of histamine perfusion. Effect of (**F**) RvD1, (**G)** RvD2, (**H**) RvE1 or vehicle on the Ca^{2+} response to capsaicin of DRG neurons sensitized by histamine. The number of neurons evaluated is depicted above each column. *, *P* < .05; **, *P* <.01; ***, *P* < .001; ****, *P* < .0001. Kruskal Wallis test was performed followed by Dunn's multiple comparison test. Data are shown as mean ± SEM.

Figure 3. RvD2 activates GPR18 and triggers Gαi signaling. (**A**) Outline of experimental design evaluating the effect of O-1918 or Boc2 on the ability of RvD2 to prevent histamine-induced TRPV1 senstization. (**B**) Effect of the GPR18 antagonist O-1918 (3-30 μM) and the FPR2/ALX antagonist Boc2 (10 μM) on the Ca^{2+} response to capsaicin of RvD2-treated DRG neurons sensitized by histamine. (**C**) Outline of experimental design evaluating the effect of pertussis toxin (PTX) on the ability of RvD2 to prevent histamine-induced TRPV1 senstization. (**D**) Effect of PTX on the Ca^{2+} response to capsaicin of RvD2-treated DRG neurons sensitized by histamine. The number of neurons evaluated is depicted above each column. (**E**) Representative images (n = 3 mice) showing GPR18 immunoreactivity (red) in DRG neurons retrogadely labelled from the colon (Fast Blue positive; green). White arrowheads indicate GPR18 protein expression in colon-innervating DRG neurons and yellow arteriks indicate DRG neurons projecting to the colon without GPR18 expression. *P < .05, **P < .01, ***P < .001, ****P < .0001. Kruskal Wallis test was performed followed by Dunn's multiple comparison test. Two-tailed Mann-Whitney test was performed in **D** to compare the Ca^{2+} response after incubation with histamine + RvD2 with PTX vs without PTX (§, *P* < .05). Data are shown as mean ± SEM.

Figure 4. RvD2 inhibits and reverses IBS SN-induced TRPV1 sensitization. (**A**) Outline of the experimental design testing the ability of RvD2 to inhibit IBS SN-induced TRPV1 sensitization on DRG neurons. (**B**) Effect of pre-treatment with RvD2 in the absence or presence of the GPR18 antagonist O-1918 on the Ca^{2+} response to capsaicin of DRG neurons incubated with histamine, SN from HV (n = 8) or SN from IBS patients (n = 8). (**C**) Outline of

experimental design testing the ability of RvD2 to reverse IBS SN-induced TRPV1 sensitization on DRG neurons. (**D**) Effect of RvD2 in the absence or presence of the GPR18 antagonist O-1918 on the Ca^{2+} response to capsaicin of DRG neurons pre-incubated with histamine, SN from HV (n = 8) or SN from IBS patients (n = 8). The number of neurons evaluated is depicted above each column. *P < .05, **P < .01, ***P < .001, ****P < .0001. Kruskal Wallis test was performed followed by Dunn's multiple comparison test. Data are shown as mean ± SEM.

Figure 5. Effect of RvD2 on the Ca^{2+} response to capsaicin of submucosal neurons from rectal biopsies of IBS patients. (**A**) Representative tracing and (**B**) amplitude of the Ca^{2+} response of submucosal neurons in biopsies of IBS patients in response to capsaicin (1 nM) before and after 30-min treatment with RvD2 (or vehicle). (**C**) Percentage of TRPV1 responding submucosal in response to capsaicin (1 nM) before and after 30-min treatment with RvD2 (or vehicle). *, P < .05. Friedman test was performed followed by Dunn's multiple comparison test. Data are shown as box-and-whiskers showing the median ± 1.5 IQR.

Figure 6. Effect of RvD2 on the VMR to colorectal distention in a mouse model of post-infectious visceral hypersensitivity. (**A**) Schematic representation of the experimental design of the post-infectious murine model of visceral hypersensitivity**.** (**B, C**) Visceromotor response to colorectal ditention in mice treated with vehicle (n = 6) or RvD2 (300 ng; n = 7). Panel **C** depicts the visceromotor response after treatment with RvD2 vs. vehicle. (**D**) Colonic permeability expressed as passage of fluorescein sodium (left) and transepithelial electrical resistance (right) in mice treated with vehicle (n = 6) or RvD2 (300 ng; n = 7) and uninfected control mice (n = 6). *, P < .05; ***, P < .001. Kruskal Wallis test was performed followed by Dunn's multiple comparison test for **B** and **D**. Two-tailed Mann-Whitney test was performed for **C**. Data are shown as box-and-whiskers showing the median ± 1.5 IQR in **B** and as median ± IQR for **C** and **D**.

Figure 7. Effect of RvD2 on the VMR in a post TNBS colitis rat model of visceral hypersensitivity. (**A**) Schematic representation of the experimental design of the post-TNBS colitis rat model of visceral hypersensitivity**.** (**B**) Visceromotor response to colorectal ditention

in rats treated with vehicle or RvD2. Generalized Estimating Equations (GEE) was performed followed by least significant difference *post hoc* test (n = 5-6). **, $P < .01$; ***, $P < .001$; significantly different from control + vehicle. §§, $P < .01$; §§§, $P < .001$; significantly different from post-colitis + vehicle. Data are presented as median ± IQR.

References

1. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. Clin Epidemiol 2014;6:71-80.

2. Lovell RM, Ford AC. Effect of Gender on Prevalence of Irritable Bowel Syndrome in the Community: Systematic Review and Meta-Analysis. Am J Gastroenterol 2012;107:991-1000.

3. Sperber AD, Dumitrascu D, Fukudo S, et al. The global prevalence of IBS in adults remains elusive due to the heterogeneity of studies : a Rome Foundation working team literature review. 2016;0:1-8.

4. Simrén M, Tack J. New treatments and therapeutic targets for IBS and other functional

bowel disorders. Nat Rev Gastroenterol Hepatol 2018;10:589-605.

5. Kuiken SD, Lindeboom R, Tytgat GN, et al. Relationship between symptoms and hypersensitivity to rectal distension in patients with irritable bowel syndrome. Aliment Pharmacol Ther 2005;22:157-164.

6. Van der Veek PPJ, Van Rood YR, Masclee AAM. Symptom Severity but Not Psychopathology Predicts Visceral Hypersensitivity in Irritable Bowel Syndrome. Clin Gastroenterol Hepatol 2008;6:321-328.

7. Kanazawa M, Palsson OS, Thiwan SIM, et al. Contributions of pain sensitivity and colonic motility to IBS symptom severity and predominant bowel habits. Am J Gastroenterol 2008;103:2550-2561.

8. Farzaei MH, Bahramsoltani R, Abdollahi M, et al. The role of visceral hypersensitivity in irritable bowel syndrome: pharmacological targets and novel treatments. J Neurogastroenterol Motil 2016;22:558-574.

9. Mertz H, Naliboff B, Munakata J, et al. Altered rectal perception is a biological marker of patients with irritable bowel syndrome. Gastroenterology 1995;109:40-52.

10. Cenac N, Andrews CN, Holzhausen M, et al. Role for protease activity in visceral pain in irritable bowel syndrome. J Clin Invest 2007;117:636-647.

11. Barbara G, Wang B, Stanghellini V, et al. Mast Cell-Dependent Excitation of Visceral-Nociceptive Sensory Neurons in Irritable Bowel Syndrome. Gastroenterology 2007;132:26-37.

12. Vicario M, Guilarte M, Alonso C, et al. Chronological assessment of mast cell-mediated gut dysfunction and mucosal inflammation in a rat model of chronic psychosocial stress. Brain Behav Immun 2010;24:1166-1175.

13. Buhner S, Li Q, Vignali S, et al. Activation of Human Enteric Neurons by Supernatants of Colonic Biopsy Specimens From Patients With Irritable Bowel Syndrome.

Gastroenterology 2009;137:1425-1434.

14. Barbara G, Cremon C, Carini G, et al. The immune system in irritable bowel syndrome. J Neurogastroenterol Motil 2011;17:349-359.

15. Klooker TK, Braak B, Koopman KE, et al. The mast cell stabiliser ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome. Gut 2010;9:1213-1221.

16. Brierley SM. Autonomic Neuroscience : Basic and Clinical Molecular basis of mechanosensitivity. Auton Neurosci Basic Clin 2010;153:58-68.

17. Hughes PA, Harrington AM, Castro J, et al. Sensory neuro-immune interactions differ between Irritable Bowel Syndrome subtypes. Gut 2013;62:1456-1465.

18. Anavi-goffer S, Coutts AA. Cellular distribution of vanilloid VR1 receptor immunoreactivity in the guinea-pig myenteric plexus. Eur J Pharmacol 2003;458:61-71.

19. Balemans D, Mondelaers SU, Cibert-Goton V, et al. Evidence for long-term sensitization of the bowel in patients with post-infectious-IBS. Sci Rep 2017;7:1-11.

20. Wouters MM, Balemans D, Van Wanrooy S, et al. Histamine Receptor H1-Mediated Sensitization of TRPV1 Mediates Visceral Hypersensitivity and Symptoms in Patients with Irritable Bowel Syndrome. Gastroenterology 2016;150:875-887.

21. Julius D. TRP Channels and Pain. Annu Rev Cell Dev Biol 2013;29:355–84.

22. Hughes PA, Harrington AM, Castro J, et al. Sensory neuro-immune interactions differ between Irritable Bowel Syndrome subtypes. Gut 2012;0:1-10.

23. Cenac N, Bautzova T, Le Faouder P, et al. Quantification and potential functions of endogenous agonists of transient receptor potential channels in patients with irritable bowel syndrome. Gastroenterology 2015;149:433-444.

24. Serhan CN, Chiang N, Dalli J, et al. Lipid Mediators in the Resolution of Inflammation. Cold Spring Harb Perspect Biol 2015;7:1-21.

25. Stewart AG. Mediators and receptors in the resolution of inflammation: Drug targeting opportunities. Br J Pharmacol 2009;158:933-935.

26. Spite M, Norling L V., Summers L, et al. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. Nature 2009;461:1287-1291.

27. Uddin M, Levy BD. Resolvins: Natural Agonists for Resolution of Pulmonary Inflammation. Prog Lipid Res 2011;50(1):75-88.

28. Recchiuti A. Resolvin D1 and its GPCRs in resolution circuits of inflammation. Prostaglandins Other Lipid Mediat 2013;107:64-76.

29. Xu ZZ, Zhang L, Liu T, et al. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med 2010;16:592-597.

30. Bang S, Yoo S, Yang TJ, et al. Resolvin D1 attenuates activation of sensory transient receptor potential channels leading to multiple anti-nociception. Br J Pharmacol 2010;161:707-720.

31. Park CK, Xu ZZ, Liu T, et al. Resolvin D2 is a potent endogenous inhibitor for transient receptor potential subtype V1/A1, inflammatory pain, and spinal cord synaptic plasticity in mice: Distinct roles of Resolvin D1, D2, and E1. J Neurosci 2013;6:18433-18438.

32. Klein CP, Sperotto NDM, Maciel IS, et al. Effects of D-series resolvins on behavioral and neurochemical changes in a fibromyalgia-like model in mice. Neuropharmacology 2014;86:57-66.

33. Bento AF, Claudino RF, Dutra RC, et al. Omega-3 Fatty Acid-Derived Mediators 17(R)-Hydroxy Docosahexaenoic Acid, Aspirin-Triggered Resolvin D1 and Resolvin D2 Prevent Experimental Colitis in Mice. J Immunol 2011;187:1957-1969.

34. Arnardottir H, Orr SK, Dalli J, et al. Human milk proresolving mediators stimulate resolution of acute inflammation. Mucosal Immunol 2016;9:757-766.

35. Ceuleers H, Hanning N, Heirbaut J, et al. Newly developed serine protease inhibitors decrease visceral hypersensitivity in a post- in flammatory rat model for irritable bowel syndrome. Br J Pharmacol 2018;175:3516–3533.

36. Mondelaers SU, Theofanous SA, Florens MV et al. Effect of genetic background and postinfectious stress on visceral sensitivity in Citrobacter rodentium -infected mice. Neurogastroenterol Motil 2016;28:647-658.

37. Aguilera-lizarraga J, Mondelaers S, Florens M, et al. Evidence for a New Mechanism Underlying Persistent Visceral Hypersensitivity and Increased Permeability in a Model of Post-Infectious IBS. Gastroenterol AGA Abstr 2015;148:S-55.

38. Deiteren A, Vermeulen W, Moreels TG, et al. The effect of chemically induced colitis , psychological stress and their combination on visceral pain in female Wistar rats. Stress 2014;17:431-444.

39. Nair AB JSA. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 2016;2:27-31.

40. Valdez-Morales EE, Overington J, Guerrero-Alba R, et al. Sensitization of peripheral sensory nerves by mediators from colonic biopsies of diarrhea-predominant irritable bowel syndrome patients: A role for PAR2. Am J Gastroenterol 2013;108:1634-1643.

41. Cirillo C, Tack J, Vanden Berghe P. Nerve activity recordings in routine human intestinal biopsies. Gut 2013;62:227-235.

42. Chiang N, Dalli J, Colas RA, et al. Identification of resolvin D2 receptor mediating resolution of infections and organ protection. J Exp Med 2015;212:1203-1217.

43. Alsuleimani YM, Hiley CR. Mechanisms of vasorelaxation induced by oleoylethanolamide in the rat small mesenteric artery. Eur J Pharmacol 2013;702:1-

11.

44. Bylund J, Stenfeldt A, Karlsson J, et al. Cyclosporin H , Boc-MLF and Boc-FLFLF are Antagonists that Preferentially Inhibit Activity Triggered Through the Formyl Peptide Receptor. Inflammation 2007;30:224-229.

45. Balemans D, Boeckxstaens GE, Talavera K, et al. Transient receptor potential ion channel function in sensory transduction and cellular signaling cascades underlying visceral hypersensitivity. Am J Physiol - Gastrointest Liver Physiol 2017;312:635-648.

46. Yekkirala AS. Two to tango: GPCR oligomers and GPCR-TRP channel interactions in nociception. Life Sci 2013;92:438-445.

47. Mangmool S, Kurose H. Gi/o Protein-Dependent and -Independent Actions of Pertussis Toxin (PTX). 2011;3:884-899.

48. Florens M, Aguilera-Lizarraga J, Theofanous S, et al. Food Antigen-Specific Antibodies and Mast Cell activation in Post-Infectious Visceral Hypersensitivity. AGA Abstr 2017;152:S721.

49. Xu ZZ, Zhang L, Liu T, et al. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med 2010;16:592-597.

50. Jo YY, Lee JY, Park C. Resolvin E1 Inhibits Substance P-Induced Potentiation of TRPV1 in Primary Sensory Neurons. Mediators Inflamm 2016;0:1-9.

51. Javier Aguilera-Lizarraga, Dafne Balemans CDL et al. Food Antigen-Specific Sensitization of Nociceptive Nerves as an Underlying Mechanism of Visceral Pain in Ibs. Gastroenterol AGA Abstr 2018;154:S-214-S-215.

52. Weylandt KH, Chiu CY, Gomolka B, et al. Omega-3 fatty acids and their lipid mediators: Towards an understanding of resolvin and protectin formation. Omega-3 fatty acids and their resolvin/protectin mediators. Prostaglandins Other Lipid Mediat 2012;97:73-82.

53. Lima-garcia JF, Dutra RC, Silva K, et al. The precursor of resolvin D series and aspirin-triggered resolvin D1 display anti-hyperalgesic properties in adjuvant-induced arthritis in rats. Br J Pharmacol 2011;164:278-293.

54. Bhave G, Zhu W, Wang H, et al. cAMP-Dependent Protein Kinase Regulates Desensitization of the Capsaicin Receptor (VR1) by Direct Phosphorylation. Neuron 2002;35:721-731.

55. Taiwo YO, Bjerknes LK GE et al. Mediation of primary afferent peripheral hyperalgesia by the CAMP second messenger system. Neuroscience 1989;32:577-580.

56. Karatay E, Gul Utku O. Serum resolvin D1 levels as a marker of inflammation in constipation dominant irritable bowel syndrome. Turkish Soc Gastroenterol 2020:113-119.

57. Veldhuis NA, Poole DP, Grace M, et al. The G Protein-Coupled Receptor-Transient Receptor Potential Channel Axis: Molecular Insights for Targeting Disorders of Sensation and Inflammation. Pharmacol Rev 2014;67:36-73.

58. Fukuda H, Muromoto R, Takakura Y, et al. Design and Synthesis of Cyclopropane Congeners of Resolvin E2, an Endogenous Proresolving Lipid Mediator, as Its Stable Equivalents. Org Lett 2016;18:6224-6262.

59. Cholkar K, Gilger BC, Mitra AK, et al. Topical delivery of aqueous micellar Resolvin E1 analog (RX-10045). Int J Pharm 2016;498:(1-2): 326–334.

60. Wu D, Zheng S, Li W, et al. Novel Biphasic Role of Resolvin D1 on Expression of Cyclooxygenase-2 in Lipopolysaccharide-Stimulated Lung Fibroblasts Is Partly through PI3K / AKT and ERK2 Pathways. Mediators Inflamm 2013;0:1-11.