Therapeutic Promise of Proteinase-Activated Receptor-2 Antagonism in Joint Inflammation


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ABSTRACT

Biological therapies such as tumor necrosis factor-α inhibitors have advanced the treatment of rheumatoid arthritis, but one-third of patients do not respond to such therapy. Furthermore, these inhibitors are now usually administered in combination with conventional disease-modifying antirheumatic drugs, suggesting they have not achieved their early promise. This study investigates a novel therapeutic target, proteinase-activated receptor (PAR)-2, in joint inflammation. Intra-articular carrageenan/kaolin (C/K) injection in mice resulted in joint swelling suggesting they have not achieved their early promise. This study investigates a novel therapeutic target, proteinase-activated receptor (PAR)-2, in joint inflammation. Intra-articular carrageenan/kaolin (C/K) injection in mice resulted in joint swelling that was associated with synovial PAR2 up-regulation using small interfering RNA technology, as confirmed by immunoblotting, substantially reduced the inflammatory response in the joint. Serine proteinase-inhibited joint swelling was mediated primarily via PAR2 activation, since the response to exogenous application of trypsin and trypstatin was absent in PAR2 knockout mice. Furthermore, serine proteinase inhibitors were effective anti-inflammatory agents in this model. Disrupting proteolytic activation of PAR2 using antiserum (B5) directed to the receptor cleavage/activation site also attenuated C/K-induced inflammation, as did the similarly targeted PAR2 monoclonal antibody SAM-11. Finally, we report the activity of a novel small molecule PAR2 antagonist, N\(^-\)3-methylbutyryl-N\(^-\)6-aminohexanoyl-piperazine (ENMD-1068), that dose dependently attenuated joint inflammation. Our findings represent a major advance in collectively identifying PAR2 as a novel target for the future treatment of arthritis.

Proteinase-activated receptor (PAR)-2 is one of a unique subfamily of G protein-coupled receptors originally cloned from murine and human sources (Nystedt et al., 1994, 1995; Bohm et al., 1996). It is activated by a novel mechanism involving the proteolysis of the receptor N terminus to expose a “tethered ligand” sequence. This proteolytically revealed N-terminal tethered ligand then binds to and activates the receptor, resulting in signaling and internalization of the receptor complex (for reviews, see Macfarlane et al., 2001; Hollenberg and Compton, 2002; Ossovskaya and Bunnett, 2004). Unlike other members of the PAR family, where the primary activating proteinase is thrombin, PAR2 is preferentially activated by trypsin and related serine proteinases, including mast cell tryptase (Nystedt et al., 1994; Corvera et al., 1997; Molino et al., 1997). In addition to this cleavage mechanism, PAR2 can also be activated selectively by the application of synthetic peptides modeled on the sequence of the tethered ligand such as the human sequence SLIGKV-NH\(_2\), and the more potent murine variant, SLIGRL-NH\(_2\) (Blackhart et al., 1996; Hollenberg et al., 1997; Al-Ani et al., 1999; Ferrell et al., 2003).

PAR2 has been implicated in inflammatory responses, and its actions include increased vascular permeability (Kawabata et al., 1998), leukocyte infiltration (Vergnolle

ABBREVIATIONS: PAR, proteinase-activated receptor; C/K, carrageenan/kaolin; ENMD-1068, N\(^-\)3-methylbutyryl-N\(^-\)6-aminohexanoyl-piperazine; hPAR, human proteinase-activated receptor; siRNA, small interfering RNA; APPA, 4-amidinophenylpyruvic acid; ANOVA, analysis of variance; SBTI, soybean trypsin inhibitor; LLC, Lewis lung carcinoma.
et al., 1999), and smooth muscle relaxation (Al-Ani et al., 1995). There has also been increasing interest in PAR2 as a mediator of nociception (Vergnolle et al., 2001a) and neurogenic inflammation (Steinhoff et al., 2000), with the latter study demonstrating coexpression of PAR2 on sensory nerves along with substance P and calcitonin gene-related peptide. Because the edema formation induced by SLIGRL-NH₂ was blocked by neuropeptide antagonists, or by depleting sensory nerve endings with capsaicin, PAR₂ was concluded to mediate neurogenic inflammation via release of sensory neuropeptides. Inflammation in the joint has also previously been reported to involve a neurogenic component mediated by sensory neuropeptide release (Lam and Ferrell, 1991), although it is not known whether this process involves PAR₂. We recently demonstrated that administration of specific PAR₂ agonists induced joint swelling and hyperemia, both cardinal signs of inflammation (Ferrell et al., 2003). Moreover, chronic joint inflammation is attenuated in PAR₂ knockout mice (Ferrell et al., 2003), arguing for a critical proinflammatory role for this receptor in the joint. From a clinical perspective, the key question to be addressed in this study is whether PAR₂ presents a new therapeutic target for the treatment of arthritis. An acute model of arthritis was used to establish proof of concept, assessing various approaches of preventing PAR₂ activation from genomic intervention to use of a novel PAR₂ antagonist (Fig. 1).

Materials and Methods

Animals. Wild-type (PAR₂⁺/⁺) C57BL/6J and PAR₂-deficient (PAR₂⁻/⁻) mice (Ferrell et al., 2003), body weight 25 to 30 g, were used. All procedures were performed in accordance with United Kingdom regulations.

Joint Inflammation. To establish proof of concept by multiple therapeutic interventions, an acute model of inflammation was used. Approaches used in the present study to inhibit PAR₂ activation from genomic intervention to use of a novel PAR₂ antagonist (Fig. 1).

Western Blotting. Protein extracts derived from 100 ng of tissue were prepared from inflamed (C/K) and control synovium using standard procedures, separated by SDS-polyacrylamide gel electrophoresis, and transferred onto nitrocellulose membrane (GE Healthcare, Little Chalfont, Buckinghamshire, UK) in a buffer containing 20 mM Tris, 20 mM glycine, and 20% methanol at a constant voltage of 100 V for 25 min. Residual binding sites were blocked, and the membrane was incubated with a PAR₂ monoclonal primary antibody (SAM-11, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at a 1:1000 dilution. This antibody is directed to amino acids 37 to 50 (SLIGKVDTSHVTG) of hPAR₂. After a further washing period the membrane was incubated with goat anti-mouse IgG conjugated to horseradish peroxidase (BD Biosciences, Oxford, UK) at a 1:2000 dilution for 1 h. The membrane was developed using ECL Plus, and antibody detection was conducted with Hyperfilm (GE Healthcare). B5 Antiserum. A polyclonal antiserum, B5 (Kong et al., 1997; Al-Ani et al., 1999), raised to a peptide corresponding to the rat PAR₂ sequence (GPNSGKR) rSLIGRLDT46P-yggc, coupled to keyhole limpet hemocyanin (1, trypsin cleavage site; yygc added for conjugation), was sequenced and purified (B5 kits from LASERGEM, Minneapolis, MN). The peptide sequence SKGRSLIGRL of the immunogen corresponds to the sequence of murine PAR₂ incorporating the tethered ligand. B5 detects both the preactivated and activated forms of PAR₂ (i.e., total PAR₂ immunoreactivity) in various species, including rat and mouse (Wang et al., 2003). The antiserum (1:1000 dilution) was injected (20 μl) into the knee joint 5 min before administration of C/K. As a control, nonimmune rabbit serum, in the same total protein concentration, was administered in separate animals.

Immunohistochemistry. To confirm PAR₂ selectivity, cryopreserved brains from PAR₂⁺/⁺ and PAR₂⁻/⁻ mice were analyzed using B5 since all four PARs have been detected in the central nervous system (Striggow et al., 2001). Sections (6 μm) were acetone fixed, treated to quench endogenous peroxidase, and then incubated with blocking buffer. Sections were incubated with B5 at 1:1000 (12 h; 4°C). Endogenous biotin was blocked using an Avidin/Biotin blocking kit (Vector Laboratories, Peterborough, UK). Biotinylated secondary antibody (Santa Cruz Biotechnology, Inc.) was applied for 30 min and then incubated with peroxidase-conjugated streptavidin for 30 min. Antigen-antibody complexes were visualized using 3,3'-diaminobenzidine. Slides were counterstained with Mayer’s hematoxylin and mounted in di-n-butyl phthalate polystyrene xylene.

Small Interfering RNA. Small interfering RNA (siRNA) sequences were designed as described previously (Elbashir et al., 2001). A sequence 5’-AA(N19)UU-3’ was selected from the open reading frame of the cDNA sequence of the PAR₂ gene ~100 nucleotides downstream of the start codon. This sequence, siRNAPAR₂ (AAU GGC AUG GCC CUG UGG AUC), and a scrambled sequence derived from it, siRNACON (AAG UCG UGA CGC UGG GCU CAU), were synthesized (Dharmacon Research, Inc., Lafayette, CO). Both sequences were checked (www.ncbi.nlm.nih.gov/BLAST) to ensure that only the PAR₂ gene was targeted by the siRNAPAR₂ and that the siRNACON sequence was nonpecific. Either siRNAPAR₂ or siRNACON was injected i.p. at a dose of 2 nmol/day for 3 days before C/K administration.
In Vitro Studies. NCTC2544 cells, expressing hPAR2 (clone G) were preincubated with 10 μM LiCl for 30 min and stimulated for a further 40 min with trypsin (50 nM) or the PAR2 agonist SLIGKV-NH₂ (100 μM). Reactions were terminated and [3H]inositol phosphates extracted using 1 ml of methanol/0.5 ml of chloroform. Water-soluble inositol phosphates were separated from phosphorylids by addition of 0.5 ml of chloroform and 0.8 ml of H₂O followed by centrifugation. Total inositol phosphates were then assayed by ion exchange chromatography on Dowex-1 columns (Plevin et al., 1990) and measured by liquid scintillation counting.

Drugs. Trypsin (catalog no. T1426), soybean trypsin inhibitor (catalog no. T9003), thrombin (catalog no. T6634), and nonimmune rabbit serum were obtained from Sigma Chemical. β-Tryptase was expressed in Pichia pastoris (Niles et al., 1998) and purified to apparent homogeneity (specific activity >90% of the theoretical value). 4-Aminophenylpyruvic acid (APPa) was a gift from J. Stürzebecher (Germany). SLIGKV-NH₂ was obtained from NeoSystem (Strasbourg, France).

Statistics. Data are expressed as mean ± S.E.M., and comparisons were performed using two-tailed t test or one- or two-way repeated measures ANOVA, with Bonferroni post hoc testing (SigmaStat, SPSS Inc., Chicago, IL). Swelling was evaluated as change in joint diameter as percentage of preinjection values.

Results

PAR2 Inhibition at the Genomic Level. Intra-articular injection of C/K resulted in progressive knee joint swelling in PAR2+/+ mice, a response significantly (p = 0.014; two-way ANOVA; n = 4–6) attenuated in PAR2−/− mice (Fig. 2A). The involvement of PAR2 in acute joint inflammation was further highlighted by immunoblot analysis of PAR2+/+ mouse synovium, which showed a marked up-regulation of PAR2 protein expression in the inflamed joint in a time-dependent manner (Fig. 2A, inset). Immunoblot analysis also revealed an up-regulation of several glycosylated PAR2 species (in the 50- to 100-kDa range) known to be synthesized by cells or tissues, in agreement with the findings of Compton et al. (2002).

Using another genomic approach, siRNA_Par2 was administered i.p. to PAR2+/+ mice 3 days before induction of acute joint inflammation. This down-regulated PAR2 as confirmed by immunoblot analysis (Fig. 2B, inset), with a corresponding significant (p < 0.0001; two-way ANOVA; n = 8–11) reduction of joint swelling (Fig. 2B). siRNA_CON had minimal effect on PAR2 expression relative to siRNA_Par2 treatment (Fig. 2B, inset). In contrast to siRNA_Par2 treatment, siRNA_CON did not reduce joint swelling (p = 0.09), with the two siRNA treatments differing significantly (p < 0.0001; Bonferroni; n = 8–11).

PAR2 Inhibition by Proteinase Suppression. Intra-articular injection of trypsin (210 μmol) in PAR2+/+ mice caused a rapid increase in knee joint swelling, peaking at approximately 4 h and slowly subsiding thereafter (Fig. 3A). This effect is specific for PAR2 as the same procedure repeated in PAR2−/− mice was ineffective. Likewise, intra-articular injection of human β-tryptase (5 μg) also resulted in knee joint swelling over a comparable time course (Fig. 3B). Again, this effect is specific for PAR2 because β-tryptase had no effect in PAR2−/− mice. In both cases, the responses in PAR2+/+ mice were dose-dependent (data not shown). To investigate the ability of trypsin to activate PAR2, further experiments were performed in NCTC2544 cells expressing human PAR2. PAR2 activation by trypsin (50 nM) in these cells, mimicked by the PAR2-activating peptide SLIGKV-NH₂, resulted in an increase in the intracellular levels of inositol-1,4,5-trisphosphate, an effect that was significantly (p < 0.0001; Bonferroni; n = 3) and dose dependently inhibited by soybean trypsin inhibitor (SBTI; Fig. 3C). The effect of SBTI did not involve receptor blockade, since administration of the human PAR2-activating peptide SLIGKV-NH₂.
resulted in PAR2 activation and an elevation of inositol-1,4,5-trisphosphate irrespective of the SBTI dose. The proinflammatory effects of trypsin and β-trypsin suggested that such serine proteinases might participate in C/K-induced joint inflammation. This possibility was therefore investigated using serine proteinase inhibitors. Preadministration of soybean trypsin inhibitor (5 mg), which can block a number of trypsin family members, significantly (p < 0.0001; Bonferroni; n = 4–5) inhibited C/K-induced joint swelling. Likewise, APPA (5 μg), a tryptase inhibitor (Stürzebecher et al., 1992; Sommerhoff et al., 2000) that can also inhibit other serine proteinases, was even more effective (Fig. 3D) at attenuating the inflammatory response triggered by C/K (p < 0.0001; Bonferroni; n = 4–5).

PAR2 Inhibition by Ocluding the Tethered Ligand.

To first check the specificity of B5 for PAR2, we examined the effect of this antiserum on PAR1 activation. Thrombin is known to activate PAR1, -3, and -4, but not PAR2, which is preferentially activated by other serine proteinases, such as trypsin. We therefore examined the effect of intra-articular injection of thrombin (20 units) on joint swelling. Thrombin induced modest swelling (7.5 ± 2%), but this was not significantly (p = 0.57; two-way ANOVA; n = 4) inhibited by prior administration of B5 (Fig. 4A), demonstrating pharmacologically the specificity of B5 for PAR2. This result was confirmed further by immunohistochemical analysis of PAR2 expression using B5 in brain as a control tissue known to express all four PARs (Striggow et al., 2001). Immunohistochemical analysis revealed PAR2 expression in neurons of the PAR2+/− mouse, but no staining was evident in the PAR2−/− mouse (Fig. 4B), despite the presence of PAR1, PAR3, and PAR4 in the latter.

Having established the specificity of B5 antiserum for PAR2, experiments were then performed to determine whether prevention of receptor cleavage and activation by the revealed tethered ligand using B5 would attenuate the proinflammatory role of PAR2 in this acute model of joint inflammation. Prior intra-articular injection of the B5 antiserum was also significantly different (p < 0.002; Bonferroni; n = 4–6). The attenuated response in the presence of the B5 antiserum did not significantly attenuate the inflammatory response (p = 0.3; Bonferroni; n = 4–6). The attenuated response in the presence of the B5 antiserum was also significantly different (p < 0.0001; Bonferroni; n = 6) from inflammation observed in the presence of nonimmune serum (Fig. 4C). SAM-11, a monoclonal antibody to human PAR2 that targets the residues SLIGXXD in common with B5, also significantly (p < 0.0001; two-way ANOVA; n = 4) and dose dependently attenuated joint swelling (Fig. 4D), with both doses of antibody differing from vehicle treatment (p < 0.0001; Bonferroni; n = 4) and from each other (p < 0.002; Bonferroni; n = 4). Together with the B5 studies, these results suggest that proteinase activation of PAR2 via its revealed tethered ligand is necessary for its proinflammatory role.

PAR2 Inhibition by Receptor Antagonism. ENMD-1068 is a novel selective PAR2 antagonist, based on a disubstituted piperazine (Fig. 5A), whose selectivity was confirmed both in vitro and in vivo. Figure 5B shows a
representative inhibition study using murine Lewis lung carcinoma (LLC) cells stimulated with 200 μM SLIGKV-NH₂. ENMD-1068 dose dependently inhibited calcium signaling, this being complete at 5 mM. Similar inhibition was observed on both human and murine cells stimulated with either human or murine agonist peptide (data not shown). Proteolytically activated PAR₂ studies were performed using trypsin, and Fig. 5C shows complete inhibition of trypsin signaling in murine LLC cells at 5 mM. ENMD-1068 had no effect on the proteolytic activity of trypsin (data not shown). ENMD-1068 specificity was demonstrated by inhibition of calcium signaling, also observed in human cell lines (data not shown). ENMD-1068 specificity was confirmed by pretreatment with the PAR₁ agonist peptide TFLLRN (Fig. 5D) and ATP (data not shown) in the presence of increasing concentrations of ENMD-1068. In all cases, no inhibition of calcium signaling was observed with ENMD-1068. Finally, ENMD-1068 did not inhibit murine platelet aggregating stimulated by the addition of thrombin, demonstrating that up to 5 mM ENMD-1068 has no inhibitory activity against thrombin-mediated PAR₂ and PAR₄ signaling (data not shown).

Having demonstrated the in vitro selectivity of ENMD-1068, we next confirmed its selectivity in vivo. The observation that C/K-induced swelling was not completely abolished in PAR₂−/− mice (Fig. 2A), presented an opportunity to assess specificity by administering the antagonist before induction of inflammation in these mice. ENMD-1068 (4 mg i.p.) in PAR₂−/− mice had no significant effect across 24 h (p = 0.544; two-way ANOVA; n = 3–6) compared with vehicle-treated PAR₂−/− mice (Fig. 5E), confirming ENMD-1068 is not influencing inflammatory pathways other than those mediated via PAR₂. The specificity of this compound was further confirmed (Fig. 5E) by the observation that ENMD-1068 (4 mg i.p.) had no effect on thrombin-mediated knee joint swelling over 48 h (p = 0.99; two-way ANOVA; n = 4–5).

The culmination of this study is the key finding that joint inflammation was dose dependently attenuated by prior i.p. administration of ENMD-1068 (Fig. 6), this effect being highly significant (p < 0.0001; two-way ANOVA; n = 5–6). Compared with i.p. injection of vehicle, joint swelling was significantly reduced by ENMD-1068 at both the 1- and 4-mg doses (p < 0.0001 in both cases; Bonferroni; n = 5–6), which differed from each other (p = 0.0065; Bonferroni; n = 5–6).

**Discussion**

Investigation of the pathophysiological roles for PAR₂ has been limited to date by the lack of a selective receptor antagonist. The current study presents the first evidence demonstrating the inhibition of acute joint inflammation by multiple strategies targeting PAR₂ (Fig. 1), including the use of a novel PAR₂ antagonist. PAR₂ has previously been reported to mediate acute inflammatory responses by neurogenic (Steinhoff et al., 2000) and non-neurogenic mechanisms (Damiano et al., 1999). Several lines of evidence are presented in the current murine study to demonstrate a role for PAR₂ in acute knee joint inflammation. First, the inflammatory response in the acute model of arthritis was substantially attenuated in PAR₂−/− deficient mice, supporting a key role for this receptor in inflammation. Second, the expression of PAR₂ protein in
murine articular tissue was substantially up-regulated during acute joint inflammation. Preventing such up-regulation by post-transcriptional gene silencing of PAR2, using siRNA designed against the receptor sequence, resulted in inhibition of joint inflammation, supporting the findings of the PAR2 \( / \) murine studies. The significant reduction of joint swelling in the PAR2 \( / \) animals suggested that preventing PAR2 activation would have significant anti-inflammatory potential. Indeed, inhibition of joint inflammation by preventing PAR2 activation using 1) proteinase inhibitors, 2) antibodies targeted against PAR2, and 3) an antagonist for the ligand binding domain on PAR2 together provide proof of principle that PAR2 plays a key role in joint inflammation via receptor up-regulation, proteinase cleavage, and endogenous activation by the revealed tethered ligand.

Post-transcriptional gene silencing using siRNA technology proved an effective means of reducing synovial expression of PAR2, thereby inhibiting joint inflammation (Fig. 2B).

Although siRNA sequences have been administered in vivo with success previously (Soutschek et al., 2004), this study is the first to show both that intraperitoneal administration of siRNA can be effective in reducing levels of targeted proteins within joint tissues and that such technology can be applied to down-regulate PAR2 expression specifically, with potential therapeutic value. Further work is required to delineate which cells express PAR2 in the inflamed joint and whether the siRNA-induced reduction in expression was in the resident articular and/or infiltrating inflammatory cells.

Trypsin is well recognized as a proteinase activator of PAR2, as corroborated in the present study on murine synovium. Significantly, trypsin as well as \( / \)-tryptase were able to mimic C/K-induced joint swelling, an effect that was abrogated by inhibitors of these proteolytic enzymes. It was also significant that neither trypsin nor \( / \)-tryptase caused joint swelling in PAR2 null animals, a result that is entirely in keeping with a lack of effect of these proteinases in other
inflammatory models using PAR²-deficient animals (Vergnolle et al., 2001b). An endogenous source of trypsin-like enzymes in the murine joint has not yet been established, although it has been shown in the rat lung that PAR² colocalizes immunohistochemically with an unidentified trypsin family member in epithelial and endothelial cells (Cocks and Moffatt, 2000). Extrapancreatic trypsin can also be found in a number of sites in humans, such as in human dermal endothelial cells, wherein trypsinogen mRNA has been detected (Shpacovitch et al., 2002). Furthermore, members of the trypsin family are now known to be up-regulated in the setting of a murine model of infectious colitis, which accounts for the inflammatory response mediated by PAR² (Hansen et al., 2005). Mast cells are found in large numbers in the inflamed human rheumatoid synovium (Woolley and Tetlow, 2000) and can be implicated in PAR²-mediated inflammation in humans since these cells release serine proteinases such as tryptase. Tryptase, an activator of PAR² (Corvera et al., 1997; Molino et al., 1997), is known to be present in human mast cells and is thus an endogenous candidate for PAR² cleavage/activation within the joint in humans. Murine connective tissue mast cells express mouse mast cell proteinase-6 and -7 (MMCP-6 and -7), which have approximately 75% amino acid sequence identity with human mast cell tryptases (Reynolds et al., 1991), but it cannot be excluded that other serine proteinases may have contributed to the inflammation generated by the C/K stimulus. Remarkably, both soybean trypsin inhibitor, which can potentially block a number of the murine trypsins, and APPA, another serine proteinase inhibitor that can target various serine proteinases, including human tryptase, were effective in attenuating C/K-induced joint inflammation. Thus, proteinase inhibitors themselves may represent an effective therapeutic modality for arthritis. Interestingly, the joint swelling induced by intra-articular application of either trypsin or tryptase was effectively abolished in PAR²−/− mice (Fig. 3, A and B), arguing that the inflammatory actions of both potent proteinases seem to be mediated primarily via PAR² activation. In this context, both proteinases provide potentially powerful anti-inflammatory agents for treatment of arthritis.

Although the B5 antiserum was originally raised against rat PAR² (Kong et al., 1997), the sequence of the immunizing rat peptide is the same in much of its sequence as the comparable murine sequence. The specificity of the B5 antiserum for murine PAR² was confirmed immunohistochemically by the selective positive staining in the brain of PAR²+/+ mice, but not PAR²−/− mice, which express PAR₁, PAR₃, and PAR₄, but clearly not PAR² in this tissue. The selectivity of B5 for PAR² inhibition was confirmed further by the observation that the antiserum did not significantly inhibit thrombin-induced joint swelling. The effectiveness of B5 in detecting murine PAR² protein is because of the close homology of PAR² between rat and mouse (97% sequence identity on the DNA level; BLAST). The selectivity of SAM-11 for human PAR₂ has previously been established (Molino et al., 1998), and for the present study it was also confirmed immunohistochemically in murine tissues (data not shown).

Importantly, this study provides the first demonstration that antibodies raised against the cleavage site on the tethered ligand, thereby inhibiting receptor activation, have significant anti-inflammatory activity (Fig. 4, C and D). However, the therapeutic potential of this approach is limited by the need for local intra-articular administration to ensure efficacy. The small molecule antagonist for PAR², ENMD-1068, allowed a more traditional pharmacological approach to blocking PAR² activation and offers considerably greater potential for future anti-inflammatory therapy because this compound, when administered systemically, powerfully and dose dependently reduced knee joint swelling (Fig. 6). ENMD-1068 inhibits PAR² selectively in vitro and in vivo (Fig. 5), although requiring relatively high concentrations, possibly reflecting low receptor affinity. This is reminiscent of peptides described previously that inhibited PAR activation by trypsin but again with low potency (Al-Ani et al., 2002). Even so, ENMD-1068 and analogs provide good “lead” compounds for future development of therapeutically valuable agents.

Using multiple strategies to prevent PAR² activation (Fig. 1), including the first use of a receptor antagonist, our findings support the unifying conclusion that PAR² is a therapeutic target in the treatment of inflammatory joint disease. This is the first study to establish such proof of concept and identifies antagonists and/or antibodies targeting PAR² as potentially powerful anti-inflammatory agents for treatment of arthritis.

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References


