Targeting the P2X$_7$ receptor in rheumatoid arthritis: biological rationale for P2X$_7$ antagonism

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Abstract

**Objective**

This paper aims to explore the functional significance of the P2X$_7$ receptor in preclinical models of rheumatoid arthritis.

**Methods**

Preclinical studies in vivo were performed using the rat streptococcal cell wall (SCW) arthritis model. Ex vivo cultures of lipopolysaccharide (LPS)/benzoylbenzoyl adenosine triphosphate (BzATP)-stimulated human monocytes were generated to test the activities of a novel, highly specific inhibitor of human P2X$_7$, AZD9056, on interleukin (IL)-1 and IL-18 release.

**Results**

P2X$_7$ receptor expression was detected in inflamed synovial tissue after onset of SCW-induced arthritis in rats. Inhibition of P2X$_7$ therein led to reduced articular inflammation and erosive progression. No effect was noted on acute-phase responses. Ex vivo, AZD9056 inhibited IL-1 and IL-18 release to BzATP in LPS-primed human monocytes.

**Conclusion**

P2X$_7$ receptor inhibition could represent a novel approach to the treatment of inflammatory arthritis. However, confirmatory clinical studies are warranted to further explore this possibility.

**Key words**

rheumatoid arthritis, inflammation, ion channels, P2X$_7$, antagonism, AZD9056
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Introduction

Rheumatoid arthritis (RA) is an inflammatory disorder associated with chronic synovitis and progressive articular damage, significant co-morbidity and accelerated mortality (1-4). Pathogenesis-based therapeutic interventions have recently substantially altered the management of RA, primarily based on the advent of cytokine-blocking therapeutics targeting tumour necrosis factor-α and, subsequently, the introduction of B-cell-depleting therapy and co-stimulatory blockade. However, a significant proportion of patients exhibit partial or non-response to such biological agents, few patients achieve sustained remission, and protein-based biological agents remain expensive and must be delivered by parenteral routes (5, 6). Thus, substantial unmet clinical need remains. There is intense interest, therefore, in identifying novel pathological pathways in RA, particularly those that might be tractable to inhibitory small-molecule entities (SMEs) that are orally bioavailable. In particular, it would be desirable to generate SMEs that can inhibit cytokines known to be up-regulated and pro-inflammatory in RA synovitis.

Ion channels are increasingly implicated in multiple facets of host immune function in rodent models and in vitro systems (7-11). The P2X<sub>7</sub> receptor is a member of the P2 family of ligand-gated ion channels that has been implicated in numerous biological processes involving immune regulation (reviewed in 12) and in bone biology (reviewed in 13, 14), making it a potentially interesting target for therapeutic intervention for the treatment of rheumatic disease. Recent studies further suggest a role for P2X<sub>7</sub>, signalling in the pathogenesis of primary Sjögren’s syndrome (15, 16), thus adding to a growing repertoire of biological pathways in RA, particularly those up-regulated in RA synovial membrane wherein they exhibit potent inflammatory properties (4, 21-23). We have explored the hypothesis that synovial P2X<sub>7</sub>, receptor expression in RA synovial membrane provides a novel therapeutic target. We present in vitro and rodent in vivo model evidence for P2X<sub>7</sub>, receptor expression and effector function.

Methods

In vitro culture methodology, streptococcal cell wall (SCW) rat arthritis model procedures and histology analyses are described online (see Supplementary Methods online).

Results and discussion

Validating P2X<sub>7</sub>, as an inflammatory target in synovial membrane

We first confirmed the presence of P2X<sub>7</sub>, protein expression in both the lining layer and interstitial regions of human RA synovial tissues (Supplementary Fig. 1). Similar patterns of expression were observed in synovial tissues derived from rats with established (day 6) SCW-induced arthritis (Supplementary Fig. 1).

To explore the functional importance of such expression, we used a novel, highly specific inhibitor of human P2X<sub>7</sub>, namely AZD9056. AZD9056, a member of an adamantine amide series of compounds (Patent WO2004/074224) (24), was identified by high-throughput screening (25) and was further optimised to a highly potent and selective P2X<sub>7</sub>, receptor antagonist. AZD9056 inhibited, in a concentration-dependent manner, IL-1β (Fig. 1a) and IL-18 (17, 19, 20). The latter are up-regulated in rodent models of arthritic inflammation and in RA synovial membrane wherein they exhibit potent inflammatory properties, providing a novel therapeutic target. We present in vitro and rodent in vivo model evidence for P2X<sub>7</sub>, receptor expression and effector function.

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Competing interests:

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model using BzATP-stimulated human primary RA synovial cells, AZD9056 inhibited IL-1β release in a concentration-dependent manner (Fig. 1d). These in vitro results suggested that the presence of P2X, tissue expression could be of functional importance in sensing danger and in regulating effector cytokine production in RA.

**P2X, antagonism ameliorates articular inflammation and damage in vivo**

To explore the in vivo significance of this finding, we administered graded doses of the specific, orally bioavailable antagonist of rat P2X7, AZ11657312, for 6 days after the induction of SCW arthritis in previously sensitised rats. Peak (2-hour) plasma concentrations for 10, 30 and 60 mg/kg treatment groups were 469±90, 1410±653 and 3363±105 ng/mL, respectively, representing satisfactory inhibitory concentrations (6-, 17.5- and 42-fold the pA2 for this compound, respectively). Trough (16-hour) plasma concentrations were markedly lower (30, 21, 35±19 and 69±118 ng/mL, respectively). Whereas a slight reduction in calliper ankle swelling (vs. vehicle control group; Fig. 2a), which primarily reflects oedema, was noted in AZ11657312 treatment groups, substantial and significant reduction in synovial membrane inflammation was evident by day 3 but particularly by day 6. Compared with controls, recipients of 30 or 60 mg/kg AZ11657312 exhibited reduction in synovitis, inflammation of synovial sub-lining, chondronecrosis and sub-chondral bone resorption, mainly in granulocytic precursors (Fig. 2b). Moreover, suppression of tissue inflammation and damage was dose-dependent. Consistent with this, radiographical examination of the tibial-tarsal compartment indicated reduced articular damage in the 60 mg/kg group compared with controls (Fig. 2c). Importantly, we also observed dose-dependent inhibition of mechanical hyperalgesia (von Frey threshold) with 65% inhibition in the 60 mg/kg group (p<0.05, area under the curve vs. vehicle control; Fig. 2d), suggesting that the tissue and radiographical changes were clinically meaningful. No effect on plasma levels of the acute-phase reactant α1-acid glycoprotein (data not shown) was observed, consistent with predominant effects at a local tissue level. When administration of AZ11657312 was deferred until 1 day after onset of arthritis, we observed reduction in histologic scores compared with vehicle treated controls; these were generally of lesser magnitude and achieved significance only for the 60 mg/kg group for the synovial inflammation score, but not for chondronecrosis, or bone resorption. Consensurate with this radiographic damage scores were reduced but not by the same magnitude as noted with prophylactic dosing (data not shown). Together, these data indicate that P2X7 mediates pro-inflammatory activity in the joint. It may also play a role in pain sensing in the joint although such effects cannot be separated from other direct central or peripheral effects operating via P2X7-mediated pain pathways. Inhibition of this pathway however mediates optimal clinical benefit when initiated early.

The P2X7 receptor is an ATP-gated ion channel primarily expressed on cells of the immune system. In vitro, it regulates monocyte release of pro-inflammatory IL-1 family cytokines via the NALP3 inflammasome (see Supplementary Fig. 2). Several effector cytokines of the IL-1 superfamily are present in RA synovium that exhibit pro-inflammatory potential in ex vivo model systems including IL-1, IL-18 and IL-33. In particular, IL-1β is a potent activator of synovial fibroblasts, chondrocytes and osteoclasts, and exhibits immune-regulatory activity especially in expanding Th17 cells that are also implicated in RA pathogenesis. However, IL-1 targeting alone, using a variety of biological inhibitors, has
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not been clinically effective for reasons that are unclear. We hypothesised that the broader cytokine regulatory role of P2X<sub>7</sub>, beyond IL-1, to include IL-18 and perhaps IL-33, might confer therapeutic utility in RA. This is a controversial area since intracellular inhibition of IL-1 family processing by caspase targeting has been disappointing. We considered that there remained merit in exploring additional approaches to modification of the IL-1 family in vivo, not least because caspase does not play an equally important role in processing distinct cytokine family members.

Furthermore, whereas P2X7 would not likely directly influence TNF or IL-6 expression in monocyte series, indirect effects may occur to modify cytokines of proven therapeutic utility - P2X<sub>7</sub> expression on synovial fibroblasts was recently implicated in IL-6 release (26). Here, using a novel, specific antagonist of the human P2X<sub>7</sub> receptor AZD9056, which is a first-in-class agent, to target this ion channel, we provide evidence consistent with the notion that targeting the P2X<sub>7</sub> receptor could yield clinically meaningful improvement in a human autoimmune disorder.

SCW arthritis is a well-characterised model of inflammatory arthritis that integrates innate and adaptive immune components to drive articular damage. Due to exquisite species specificity of AZD9056, we used a synthologue in inhibitor of rat P2X<sub>7</sub> to define the therapeutic potential of P2X<sub>7</sub> targeting in preclinical studies. P2X<sub>7</sub> antagonism suppressed synovial inflammation and radiographical damage and reduced mechanical hyperalgesia. Despite these significant effects on synovial tissues, no effect on the systemic acute-phase response was observed. These data are
consistent with known functions of IL-1, IL-18 and IL-33, including regulation of pathways integral to promoting synovial inflammation, articular matrix damage and nociception (19, 27) but limited effects on systemic IL-6 regulation. It was notable, however, that effects were diminished when inhibition was delayed until after the onset of substantial disease in the model. This is a rapid induction, aggressive monoarthritis model. The partial responses observed upon delayed inhibitor administration may reflect the multitude of inflammatory effector pathways operating in established disease, but indicate that P2X7 at that stage mediates at least some, albeit reduced, contribution to events.

Recent advances in the treatment of RA have been remarkable, not least in the advent of biological agents that deliver substantial disease suppression and radiographical protection. There remains, however, considerable need for the development of novel SMRs that might reproduce the effects of biological cytokine blockade with advantages of convenience, tolerability or cost. These preclinical data provide evidence that were deemed sufficient to support targeting the P2X7 pathway in human clinical trials (28).

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