Prophylactic and therapeutic activity of alkaline phosphatase in arthritic rats: single-agent effects of alkaline phosphatase and synergistic effects in combination with methotrexate

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Alkaline phosphatase (AP) is a gate-keeper of innate immune system responses by detoxifying inflammation triggering moieties released from endogenous and external sources. We examined whether AP's broad mechanism of action constitutes a safe therapeutic, either as single agent or combined with methotrexate (MTX), for chronic inflammatory disorders, for example, rheumatoid arthritis (RA). A rat model for RA was used with repeated intra-articular methylated bovine serum albumin (mBSA) injections in 1 knee ("arthritic" knee), with the contralateral knee serving as internal control. Recombinant human placental AP (200 μg, subcut) was administered before mBSA injections (*prophylactic* setting) or after arthritis induction (*therapeutic* setting) or combined with MTX (0.3 mg/kg or 1 mg/kg; intraperitoneally). As end point of treatment outcome, macrophage infiltration in knees, liver, and spleen was assessed by immunohistochemistry (ED1 and ED2 expression), immunofluoresence (macrophage marker folate receptor-β (FRβ)), and (^{18}F)fluoro-polyethylene glycol-folate positron emission tomography (PET) (macrophage imaging) and ex vivo tissue distribution. Single-agent AP treatment and combinations with MTX were well tolerated. Both prophylactic and therapeutic AP markedly reduced synovial macrophage infiltration in arthritic knees (ED1: 3.5- to 4-fold; ED2: 3.5- to 6-fold), comparable with MTX treatment. AP-MTX combinations slightly improved on single agent effects. PET monitoring and ex vivo tissue distribution studies corroborated the impact of AP, MTX, and AP-MTX on reducing synovial macrophage infiltration. Beyond localized articular effects, AP also revealed systemic anti-inflammatory effects by a 2-fold reduction of ED1, ED2, and FRβ$^+$ macrophages in liver and spleen of arthritic rats. Collectively, single-agent AP and AP combined with MTX elicited local and systemic anti-arthritic activity in arthritic rats. (Translational Research ■■;■■:■■–■■)

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Abbreviations: AP = alkaline phosphatase; DMARD = disease modifying anti-rheumatic drug; EULAR = European league against rheumatism; FR = folate receptor; hRESCAP = human rescue alkaline phosphatase; i.a = intra-articular; ID/g = injected dose/gram; i.p = intraperitoneally; mBSA = methylated bovine serum albumin; MTX = methotrexate; PEG = polyethylene glycol; PET-CT = positron emission tomography-computed tomography; RA = rheumatoid arthritis; s.c = subcutaneously

AT A GLANCE COMMENTARY

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Background

Inflammation triggering moieties (ITMs) such as adenine nucleotides (AMP, ADP, ATP) when released extracellularly, provoke a pro-inflammatory response. Alkaline phosphatase (AP) functions as a gate-keeper of innate immune system responses by detoxifying, that is, dephosphorylating ITMs.

Translational Significance

AP may fulfill a novel, unique, and unmet niche in rheumatoid arthritis (RA) treatment by combining different, yet synergistic mode of actions with MTX and other chemical and biological disease-modifying drugs. AP as anti-inflammatory protein could be positioned as a promising new RA treatment either as stand-alone therapy or in combination with MTX.

INTRODUCTION

Rheumatoid arthritis (RA) is an auto-immune disease with hallmarks of synovial and systemic inflammation which, when left untreated, leads to progressive bone and joint destruction.[1-3] Current treatment options for RA include chemical disease modifying anti-rheumatic drugs (cDMARDs) with methotrexate (MTX) and glucocorticoids as predominant initial treatments.[4,5] MTX has an established place in RA treatment based on its low cost, safety profile, efficacy, and long-standing clinical experience.[6,7] Still, a considerable fraction (30%–40%) of RA patients is faced with MTX intolerance or inefficacy early on in the treatment or loss of efficacy during chronic treatment.[8-11] At this stage, treatment with biological DMARDs (bDMARDs) is indicated,[1] but these come with higher costs and are also prone to development of resistance.[10,12] Therefore, identification of novel modalities that could reinforce longer-lasting efficacy of MTX, or have alternative mechanisms of action, deserve continued interest.

AP, a glycosylphosphatidylinositol-anchored protein, is a member of the family of ecto-phosphatase proteins.[13-17] At least 4 different enzyme isoforms of AP are differentially expressed in various tissues (eg, liver, intestine, placenta, bone) and immune cells (eg, macrophages) from where AP may be released in soluble form by glycosylphosphatidylinositol-specific phospholipase D.[16,18] As ectophosphatases act extracellularly by dephosphorylating inflammation triggering moieties (ITMs), AP is thought to have a gate-keeper function in the innate immune system by detoxifying well-known ITMs from external and internal sources such as lipopolysaccharides, CpG oligodeoxynucleotides, and nucleotide phosphates (adenosine tri-, di-, and mono-phosphates [ATP, ADP, and AMP], respectively).[19-25] Whereas increased concentrations of extracellular nucleotide phosphates exert pro-inflammatory signals,[26,27] their ectophosphatase-mediated conversion to adenosine conveys a well-recognized anti-inflammatory effect via interactions with adenosine receptors.[28] This mechanism of action is reminiscent of the dominant anti-arthritic mechanism of action of MTX,[8-10,29] which involves the non-lytic extracellular release of adenosine and extrusion of adenine nucleotides (AMP, ADP, ATP) that are converted to adenosine by the action of ectophosphatases CD39 and CD73 on immune-competent cells.[30] Recent evidence indicates that downregulation of CD39 on regulatory T cells during MTX treatment inhibits local generation of adenosine, thereby conferring MTX resistance in RA.[31] Under conditions of attenuated CD39 and CD73 function, AP may compensate for their function. AP therapeutic interventions have been studied in preclinical and clinical models of local and systemic inflammatory diseases. In fact, in animal models, exogenous ectophosphatase interventions by AP resulted in near-complete inhibition of systemic tumor necrosis factor-alpha (TNFα), interleukin (IL6), and IL8 response after a systemic inflammatory insult with lipopolysaccharide.[23,32] In humans, AP administration prevented the induction of these pro-inflammatory cytokines or chemokines peri- and postsurgically in patients undergoing open heart surgery.[33,34] In the RA setting, AP intervention is relatively unexplored. As a preliminary account, a safety assessment (phase 1/2a) study of multiple injections with bovine intestinal AP (twice daily, 2000 U, sc, for 3 days) in 6 RA patients (treatment-resistant, severe active RA, including anti-TNF failures) with 3 months' follow-up showed no safety events, and 1 sustained 1 transient clinical response with temporary improvement of DAS response (A Hammond, data on file). Bovine AP, however,