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An anti-inflammatory agent, 2-Hydroxyisocaproic Acid, prevents Del-1 fragmentation in vitro

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ABSTRACT

Inflammation, a critical aspect of the immune system's defense and recovery processes, manifests in two primary forms: acute and chronic. Understanding and controlling these responses are vital for managing various inflammatory conditions. 2-Hydroxyisocaproic acid is a physiological substance and the 2-hydroxy-analogue of the essential amino acid leucine. This research focuses on the interaction of 2-Hydroxyisocaproic acid with key inflammatory mediators, matrix metalloproteinase 8, and Developmental Endothelial Locus 1. Matrix metalloproteinase 8 plays a significant role in the inflammatory process. Developmental Endothelial Locus 1 acts as a crucial immunomodulator, maintaining tissue homeostasis. Our findings reveal that 2-Hydroxyisocaproic acid inhibits the fragmentation of Developmental Endothelial Locus 1 by dose-dependently modulating and reducing the matrix metalloproteinase 8 activity. Notably, 2-Hydroxyisocaproic acid's reversible inhibition of matrix metalloproteinase 8 does not eventually involve covalent bonding, positioning it as an enzyme modulator or down regulator rather than a direct inhibitor. This property of active matrix metalloproteinase 8 reduction by 2-Hydroxyisocaproic acid opens new avenues for therapeutic intervention, particularly in managing excessive inflammatory responses, such as the "cytokine storm" observed in lung tissue inflammation or arthritic joints like in osteoarthritis.

Introduction

Inflammation constitutes an integral component of our innate defense and recuperative mechanisms, intricately orchestrated by the immune system. It is the intricate orchestration wherein the immune system discerns and eradicates deleterious foreign entities while concurrently facilitating the regenerative process. This phenomenon manifests as either an acute, immediate response or a chronic, enduring condition, often eluding clear demarcation.[1,2]

The immune system demonstrates precise control over inflammatory processes to prevent the occurrence of excessive immune reactions, which have the potential to precipitate an over-exuberant host response, ultimately culminating in the detriment of the subject in question. It is worth noting that local tissues do not merely serve as passive receptors of immune surveillance; rather, they exert a substantial influence over the initiation and regulation of both immune and inflammatory responses.[3] Homeostatic factors originating from specific tissue sources play a pivotal role in governing the inception and cessation of immune responses. These factors meticulously modulate the recruitment and activation of immune cells, as well as the functional adaptability of both tissue-resident and recruited immune cells.[3]

In this intricate orchestration, Developmental Endothelial Locus-1 (DEL-1) emerges as a key immunomodulator, serving as the linchpin in restoring and preserving the homeostatic equilibrium within the microenvironment of tissues. DEL-1 operates by striking a delicate balance between inflammatory responses and the mechanisms of immune regulation, thus facilitating the reestablishment and maintenance of the harmonious state in the tissue microenvironment.[4] It is also expressed in joint tissues.[5]

A glycoprotein of 52 kilodaltons, DEL-1 is mainly secreted by endothelial cells, demonstrating an affinity for association with both the endothelial cell surface and the extracellular matrix. In humans, it is encoded by the EDIL3 gene.[6] This multifaceted protein is expressed in the endothelial cell population, as well as within a select subset of macrophages. Thus, is not surprise that DEL-1 exerts a regulatory influence over crucial biological processes, namely angiogenesis, apoptosis, as well as cell adhesion and migration.[7] DEL-1 has shown also to act in an anti-inflammatory and protective fashion.[7]

This effect of DEL-1 is expresses by a ligand for lymphocyte function-associated antigen (LFA)-1. Del-1 exerts its inhibitory effect on neutrophil recruitment during inflammation by counteracting LFA-1-dependent adhesion.[7] Actually, Del-1 competes with intercellular adhesion molecule 1 (ICAM)-1 for binding to LFA-1 on leukocytes, inhibiting their adhesion to the endothelium and subsequent migration into the tissue, thereby playing a critical role in modulating inflammation.[5] Additionally, Del-1 extends its regulatory impact to encompass the restraint of complement-dependent phagocytosis in macrophages.[8] Thus, acting as a modulator of macrophage-1 antigen (Mac-1) function, particularly in Mac-1-mediated complement-dependent phagocytosis, Del-1 aligns with its proposed role as an endogenous homeostatic agent contributing to tissue equilibrium. Thus, this regulatory function holds potential significance in the context of inflammatory, infectious diseases, and tissue injury.[8]

The majority of the genetically distinct but structurally related matrix metalloproteinases (MMPs) primarily function outside cells and are commonly associated with processing extracellular matrix (ECM) proteins. However, the belief that MMPs exclusively target ECM proteins is a misconception. These enzymes also degrade various non-ECM bioactive substrates, including cytokines, chemokines, serpins, adhesion molecules, complement components, growth factors, and even receptors such as the insulin receptor. Particularly, collagenases MMP-1, MMP-8, and MMP-13 stand out for their ability to cleave core matrisome proteins and non-matrix bioactive substrates.[9] Notably, collagenolytic MMPs are increasingly expressed in osteoarthritis (OA) cartilage. Interestingly, MMP-8 can be found and is de-novo expressed in OA lesions even in the absence of neutrophils, challenging the traditional perception of MMP-8 as solely a neutrophil collagenase. Under the influence of proinflammatory cytokines like TNF-α and IL-1β, chondrocytes or synovial cells demonstrate the capability to inductively de-novo express and produce MMP-8 de-novo.[10] Correspondingly inductive de-novo expression has been evidenced by synovial fibroblasts and endothelial cells.[11]

2-Hydroxyisocaproic acid (HICA), also known as leucic acid, 2-hydroxy-4-methylvaleric acid, or 2-hydroxy-4-methylpentanoic acid, possesses a molecular weight of 132.16 g/mol.[12] HICA, being the 2-hydroxy-analogue of the essential amino acid leucine, is a physiological substance. It emerges as a by-product of the leucine-acetyl-CoA pathway, precisely as an end product of leucine metabolism within human tissues, most notably in muscle and connective tissue.[13,14] It has been demonstrated to exhibit safety when administered within recommended dosages, and it continues to be available in the market as a dietary supplement designed for the enhancement of performance.[15-17,18 unpublished data] HICA exhibited cytostatic properties at all concentrations tested, while showing no cytotoxicity up to 10 mg/mL regardless of exposure time. It demonstrated no genotoxic effects below 5 mg/mL. HICA maintained approximately 70% of its osteogenic differentiation potential at 1 mg/mL.[19,20] It's Na-salt LD50 have been tested with mouse, and it was noted to 650mg/kg iv.[21] Naturally occurring within mammalian organisms, HICA exhibits elevated concentrations in serum during periods of fasting.[13] In circulation HICA is unbound and exhibits a plasma concentration in healthy adults ranging from 0.1 to 0.25 µmol/l, with a mean value of 0.25±0.02 µmol/l.

In the pathogenesis of OA, inflammation has been identified as a pivotal factor.[22] Synovitis, identified as a secondary process triggered by innate immune activation subsequent to cartilage damage, assumes a critical role in both initiating and perpetuating OA.[23] Several studies consistently correlate the presence of synovitis in OA with increased pain and joint dysfunction. Furthermore, synovitis exhibits potential as a predictive factor for accelerated rates of cartilage loss in specific patient cohorts.[22] Hence, addressing inflammation promptly in

OA may offer the potential for a disease-modifying therapeutic agent.

Nieminen and colleagues in 2014,[24] first unveiled evidence of the impact of HICA on DEL-1. In their in vivo research revealed that the expression of DEL-1 significantly increased following HICA treatment. Inflammation plays a vital role in physiological functions and significantly impacts various processes. However, within clinical contexts, a pivotal objective involves devising a compelling strategy to mitigate inflammation specifically in OA, which is acknowledged as the leading cause of disability.[25] The current study proposes the application of compounds like HICA to strategically manipulate and down-regulate the exaggerated immune and proteolytic response at specific loci and stages of inflammation. This approach aims to limit hyperactive immune and proteolytic responses, offering a targeted strategy in the treatment of various inflammatory disorders including such as OA.

Material and Methods

This study was performed to test the inhibition of the MMP-8 mediated DEL-1 fragmentation by both isomers of HICA dextro (D) and levo (L). The degradation of DEL-1 by active MMP-8 was determined essentially by using SDS-PAGE described.[26,27] Briefly: first recombinant human MMP-8 enzyme (#30100702, Proteaimmun) was preincubated 1 hour at 37°C with the optimal organomercurial proMMP-8 activator p-aminophenylmercuric acetate (APMA, #A9563, Sigma-Aldrich) and used inhibitor candidates different HICA isomers (D-HICA, #CAS 20312-37-2, Santa Cruz Biotechnology; L-HICA, #219827 Sigma-Aldrich), 2-hydroxy-isovaleric acid both D- and L-forms (HMB, #379093 Sigma-Aldrich) at indicated concentration. Then the recombinant human DEL-1 (#6046-ED-050, R&D System) at indicated concentration was added as a substrate and after that all let incubate overnight at +37°C. Using 11% SDS-PAGE with Silver Stain Pierce® (#1610449, Bio-Rad, Finland) methods, the inhibition process was visualized. In this study, Ilomastat (Ilo, #CC1010, Millipore) were used as a positive synthetic inhibitor and TNC-buffer, 50 mM Tris-HCl-buffer: pH 7,5; 0,2 M NaCl; 1,0 mM $CaCl_2$ as negative controls.[28]
In this first experiment, the volumes have been constant of substrate (Del-1), enzyme (MMP-8), and activator (APMA) as well as various inhibitor candidates (HICAs/HMBs). Thus, minimizing the changing factors in the experiment, wanting to find out which of the HICA forms would best inhibit MMP-8 activity. There is an illustrative table below the Figure 1 A about this experimental setup.

In the second experiment, the inhibition effects studied with increased concentration of D-HICA with above mentioned the methods. The inhibitory effect of the 10% D-HICA form was investigated by adding it amounts to 2 µl gradually to 8 µl. Otherwise, the amounts of substrate, enzyme and activator were constant and the same as in the first experiment, keeping in mind the reliability of the results. The observation table from this experiment is also below the Figure 1 B.

Results

In the first experiment, we compared the ability of different forms of HICA, all at the same concentrations, to prevent MMP-8 from fragmenting the DEL-1 substrate under otherwise identical experimental conditions. The results of this experiment revealed that D-HICA was the most effective inhibitor of DEL-1 fragmentation by suppressing MMP-8 activity (Figure 1A). The L-HICA form also exhibited some degree of substrate protection, albeit less effectively than the D-HICA form.

In the second experiment, we sought to investigate whether there was a dose-response relationship in the inhibitory potential of D-HICA. This second experiment demonstrated a dose-dependent enhancement of the inhibitory effect of D-HICA under the same experimental conditions as in the first experiment, with DEL-1 as the substrate and MMP-8 as the enzyme (Figure 1B).

HICA's reversible inhibition of MMP-8 does not eventually involve covalent bonding and should be regarded as an enzyme modulator or down-regulator rather than a direct inhibitor.

Discussion

There is a need for a pharmaceutical solution to finely modulate the host's immune and exaggerated destructive proteolytic responses, facilitating pathogen clearance, mitigating exorbitant immune response, and expediting recovery while preserving tissue integrity. We presented an invention which aims to introduce compounds tailored for precise immune response adjustments at distinct host loci and stages. Thus, a conclusion could be drawn that HICA, through MMP-8 activity alteration, can maintain and also promote DEL-1's and MMP-8's homeostatic potentials.[29] This could also include tempering over-exuberant responses such as cytokine storms in specific tissues, thus averting undue organ damage and enabling a swift return to physiological equilibrium and homeostasis.

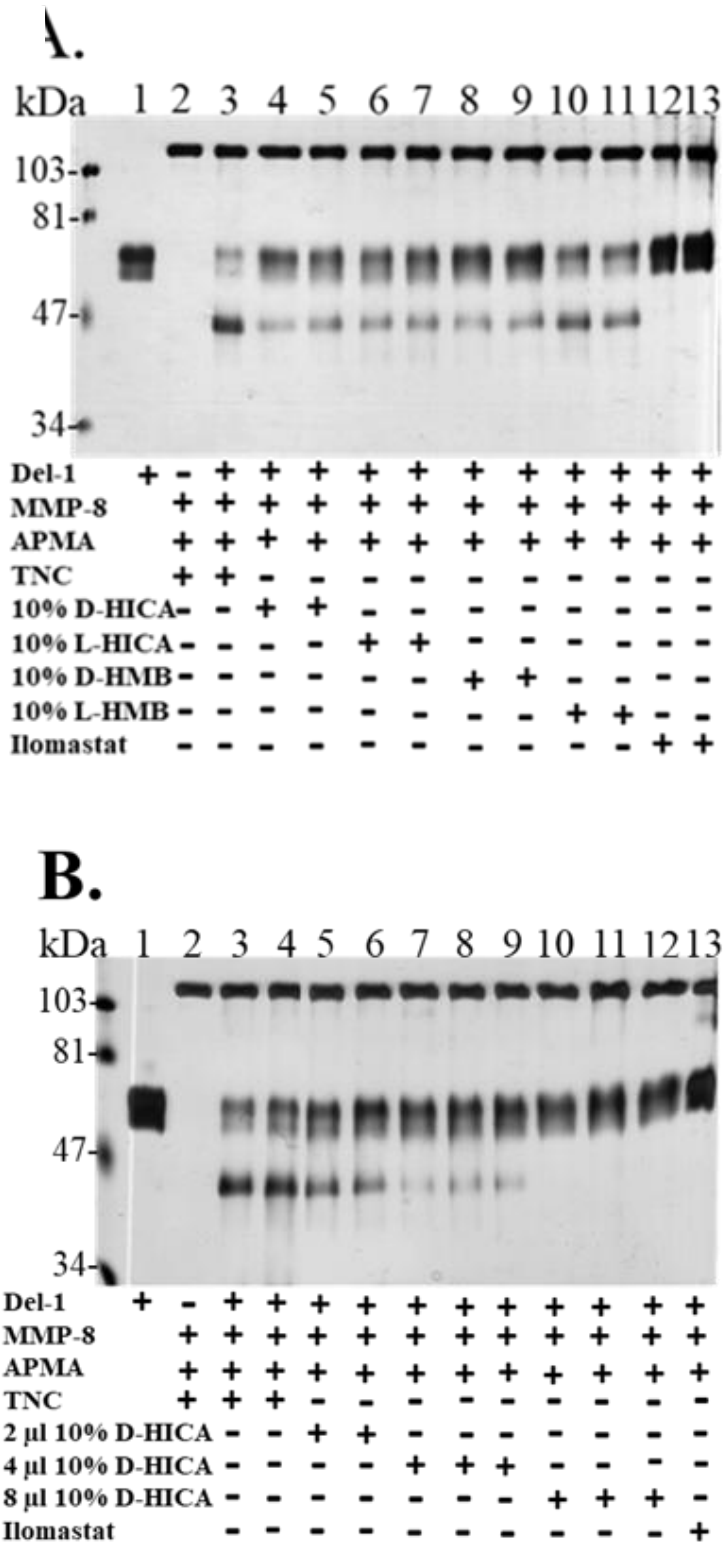

Figure 1. D,L-2-hydroxy-isocaproic acid (HICA) prevents human matrix metalloproteinase 8 (MMP-8) mediated fragmentation of recombinant human developmental endothelial locus-1 (DEL-1). Both HICA isomers relatively inhibit MMP-8 (A), but D-HICA isomer seams to inhibit the fragmentation of DEL-1 effective as L-HICA (A), and there is dose dependent inhibition noted with D-HICA (B). Molecular weight markers are indicated left. The labels + and – mean that the experiment has performed with or without about that reagent, respectively.

A physiological substance, HICA, exerts immune response modulation by preventing fragmentation of DEL-1 in a dose-dependent manner. Its beneficial immunomodulatory effect may be achieved through the inhibition of MMP-8 at the loci. MMPs, a family of calcium and zinc-dependent proteolytic enzymes, have a broader substrate range than initially thought, encompassing in addition ECM molecules also numerous non-matrix bioactive molecules.[30] HICA's reversible inhibition of MMP-8 does not eventually involve covalent bonding and should be regarded as an enzyme – eventually a bit "leaky" – modulator or down-regulator rather than efficient inhibitor, let alone "enzyme killer".[31]

The compound has been isolated from plasma,[32] urine,[33] saliva,[34] and amniotic fluid.[35] Elevated HICA

concentrations are detected during extended fasting, post-exercise periods when proteins are utilized as an energy source, and in cases of diabetic ketoacidosis, as demonstrated by Liebich and Först.[36] HICA quantification is feasible from human plasma in both adults and children.[37] Furthermore, trace amounts, on the order of 17 µg/l, have been ascertained within human breast milk as well.[38]

In selected food products produced via fermentation, such as certain cheeses, dairy items, wines, and soy sauce, HICA is also present.[39-44] Its noted properties encompass anti-inflammatory, antiproteolytic, anticatabolic, and antimicrobial attributes.[45-47] Its wide-ranging antibacterial activity extends to both obligately and facultatively anaerobic gram-positive and gram-negative bacterial species.[48]

While the immune system's reliance on MMPs had been previously hypothesized, our in vitro study substantiated their pivotal role. Recent perspectives on matrix metalloproteinases (MMPs) suggest their regulation of diverse inflammatory and reparative mechanisms, implying a potential foundational role in the evolutionary progression of the immune system.[49]

Targeting a wide array of biological entities, MMPs include proteinases, inhibitors, clotting factors, chemotactic molecules, latent growth factors, growth factor-binding proteins, cell surface receptors, cell-cell adhesion molecules, and nearly all structural extracellular matrix immunomodulatory proteins.[50] A prevailing theme in MMP function is their involvement in inflammation, evident in various diseases displaying altered MMP levels associated with inflammatory conditions.[49] Our work presents evidence of MMP proteolysis may modulate chemokine activity, finding is concordant with previous studies, specifically through the direct control of chemokine activity by certain MMPs via cleavage.[51]

Also recognized as collagenase 2 or neutrophil collagenase, MMP-8 is primarily secreted by polymorphonuclear (PMN) granulocytes during inflammatory responses. While collagen type 1 remains its primary substrate, previous studies highlight its activity on various bioactive non-matrix substrates, including signaling molecules, receptors, growth factors, and cytokines.[52-55] MMP-8 has also a critical role of in cardiovascular, diabetic and periodontal pathogenesis.[29,55,56]

Owing to their pivotal significance in human physiological processes, an ideal inhibitor of MMPs should selectively intervene as needed, analogous to "cutting the tip of the iceberg". Regulating the activation of "harmful" cytokines or relevant molecules when their overproduction occurs. MMPs, akin to all secreted proteinases, undergo regulation at various stages: gene expression, compartmentalization, pro-enzyme activation, and enzyme inactivation. Further control is exercised through substrate availability and affinity.[49]

Nieminen et al.[24] first unveiled evidence of the impact of HICA on DEL-1. Their research revealed that the expression of DEL-1 significantly increased following HICA treatment.

In inflammatory and autoimmune disorders, an imbalance favoring IL-17 disrupts DEL-1 expression, exacerbating inflammation and promoting neutrophil recruitment. This reciprocal interaction between DEL-1 and IL-17 may play a role in hyperinflammation associated with conditions such as COVID-19 and Kawasaki disease. Notably, autoantibodies targeting DEL-1, particularly prevalent in Kawasaki disease, contribute to IL-17-mediated hyperinflammation, suggesting a humoral neutralization of DEL-1 function, allowing uncontrolled IL-17 activity.[6] Research conducted by Sohn et al.[57] unveiled that IL-17 levels are heightened not only in OA synovial fluid but also in the sera, although not to the extent observed in rheumatoid arthritis (RA) patients.

The primary sources of IL-17 are CD+4 effector T cells and the Th-17 lineage.[58] Moreover, IL-17 possesses the ability to induce chemokine production in osteoarthritic chondrocytes, a process effectively suppressed by the introduction of anti-IL-17 monoclonal antibodies.[59] IL-17 plays a pivotal role in stimulating the secretion of various cytokines and may contribute to the activation of multiple catabolic pathways, leading to cartilage and tissue damage in osteoarthritis.[60] IL-17 activity inhibition may prevent chondrocyte apoptosis.[61] DEL-1 protects chondrocytes against apoptosis triggered by intrinsic/extrinsic pathway activators and anoikis.[62,63]

Moreover, DEL-1's presence is constitutively observed in various tissues, including the lung, brain, and bone. Nonetheless, its expression is notably prominent within the domains of endothelial cells, macrophages, and neurons. It is noteworthy that this glycoprotein is not merely limited to intracellular functions; rather, it also follows a secretion pathway and forms associations with either the cell surface or the extracellular matrix. [3]

Wang et al.[64] proposed DEL-1 as a potential therapeutic avenue for inflammatory arthritis, highlighting its ability to interfere with arthritogenic processes both locally and systemically. Their findings demonstrate DEL-1's efficacy in restraining arthritis by limiting inflammatory cell recruitment to the joints. Surprisingly, DEL-1 also exerts a systemic effect, downregulating the induction of arthritogenic antibody responses in the lymph nodes.[64] This dual mode of action is founded on a localized mechanism within the joints, aligning with the previously elucidated anti-leukocyte recruitment role of endothelial cell-derived DEL-1. Simultaneously, a systemic mechanism comes into play, associated with the capacity of stromal cell-derived DEL-1 to temper the responses of T follicular helper (Tfh) cells and germinal center B (GC-B) cells within the lymph nodes.

The enrichment of DEL-1 in the synovial fluid of rheumatoid arthritis (RA) patients with low-level inflammation, as opposed to those with high-level inflammation,[65] implies a potential protective role for DEL-1 in human RA.[64] The remarkable ability of DEL-1 to disrupt arthritogenic processes both locally and systemically lends credence to the notion that this molecule may indeed emerge as a propitious option for rheumatoid arthritis (RA) treatment. Their investigation not only demonstrated DEL-1's efficacy in restraining arthritis but also unveiled its capacity to curtail local recruitment of inflammatory cells to the joints.[64]

Serving as an endogenous inhibitor of the prominent leukocyte adhesion receptor LFA-1, DEL-1 effectively prevents the adhesion of leukocytes to the endothelium. Unlike numerous adhesion molecules, such as selectins and members of the Ig superfamily, which actively promote leukocyte adhesion to the endothelium, DEL-1, in contrast, exerts a unique function by actively impeding the binding process between leukocytes and the endothelium.[7] In doing so, it effectively suppresses the infiltration of leukocytes into inflamed tissues.[66]

The protein might selectively regulate Macrophage Migration Inhibitory Factor (MIF), a proinflammatory cytokine crucial in controlling leukocyte recruitment, innate and adaptive immunity, and tumor progression under conditions of low-grade inflammation or in resting cells. These findings suggest that Del-1 functions as a moderator of inflammation by suppressing NF-κB-dependent (nuclear factor kappa-light-chain-enhancer of activated B cells) proinflammatory cytokine production in monocytes and macrophages.[67] Alterations in human MIF expression due to genetic changes are linked to the severity of conditions such as asthma, cystic fibrosis, and rheumatoid arthritis.[68] The study by Yuh et al.[69] demonstrated that DEL-1 has the capacity to activate a 3 integrin–FAK–ERK1/2–RUNX2 pathway within osteoprogenitor cells, consequently stimulating new bone formation in mice. These results imply a potential therapeutic leverage of DEL-1 in the restoration of bone loss attributed to periodontitis and potentially other osteolytic disorders.

A constituent of the cell-associated matrix, Del-1 is predominantly concentrated in the superficial zone of AC. This matrix potentially serves as a protective microenvironment surrounding the chondrocytes located in the superficial zone, thereby contributing to the safeguarding of the AC surface. Furthermore, Del-1 is significantly enriched within this region. Chondrocytes, when isolated from the superficial zone of articular cartilage via protease and collagenase treatment, maintain their association with the matrix, wherein Del-1 is a principal element. This cell-associated matrix is posited to augment the protective capabilities of the AC surface by establishing a specialized microenvironment around the superficial zone chondrocytes.[70]

The notion that synovial inflammation may play a pivotal role in the etiology of osteoarthritis (OA) gains compelling support from an array of investigations. Elevated levels of serum C-reactive protein (CRP) have been closely linked to the progression of OA.[71-73] Furthermore, it has been revealed that mechanical forces can directly trigger the production of inflammatory mediators within the cartilage and synovium.[74] A comprehensive analysis of OA synovial fluid identified 108 proteins, shedding light on the presence of low-grade inflammation in OA.[57] Some of these plasma proteins possess the ability to activate Toll-like receptor 4 (TLR4), thereby inciting the production of a spectrum of inflammatory cytokines, including those associated with OA upregulation. The implications of these findings suggest that plasma proteins, whether exuded from the plasma or produced by synovial tissues, may function as damage-associated molecular patterns, thereby contributing to the low-grade inflammation observed in OA.[57] Identifying inflammatory mediators provides potential targets for therapeutic interventions aimed at alleviating symptoms and mitigating structural joint damage in OA.[22]

Notably, in certain instances, the degree of synovitis in OA and rheumatoid arthritis (RA) tissues can be remarkably similar, posing difficulties in distinguishing between the two.[75] Recent research indicates the presence of low-grade inflammation in OA.[76] Moreover, current studies demonstrate that indications of mild synovial inflammation impact 50-80% of patients, influencing disease advancement and symptomatic manifestation.[77]

Intriguingly, OA can instigate systemic inflammation, and the notably high levels of cytokines in OA sera are primarily attributed to overproduction of these cytokines within the joint. This is further corroborated by the correlation between high-sensitivity CRP levels in the serum of OA patients and the degree of inflammatory infiltrate within their joints.[57,72,78]

Inflammation notably increases in individuals with the early OA.[79] A compelling distinction between early and late OA lies in the heightened mononuclear cell infiltration and the overexpression of inflammatory mediators in the early stages of the disease. This surge in inflammation in the early phases of OA often translates into more pronounced symptomatic presentations, signifying a natural course that diminishes over time. Therefore, the management of inflammation assumes particular relevance in the early stages of OA, contributing to patient compliance with ongoing treatment. Early interventions in OA may be as an optimal approach, capitalizing on a window of opportunity during which disease-modifying strategies targeting inflammatory processes may prove most efficacious for OA prevention and treatment.[23]

Similar to RA, OA exhibits elevated serum IL-17 levels in patients with early knee OA, implicating a potential pathogenic role in the disease [80,81]. The correlation between serum IL-17 and the severity of knee OA-related pain further underscores its potential as a marker for early intervention. Notably, among the IL-17 family cytokines, IL-17A stands out as the most extensively studied, with a demonstrated capacity to induce the most potent changes within the transcriptome of synovium and chondrocytes in OA patients.[82]

Post-traumatic osteoarthritis (PTOA) resembles early OA in its inflammatory processes. Following an injury, the immune system acts as an initial responder at the injury site, aiding in debris removal and crucial tissue remodeling for repair. Cytokines released by immune cells stimulate tissue reconstruction, including differentiation of stem cells, development of blood vessels, activation of resident tissues, and synthesis of ECM. The specific tissue environment after trauma influences how the immune system reacts. Both tissue-specific senescence-associated secretory phenotypes (SASPs) and resulting immune responses likely determine subsequent tissue repair, fibrosis, or progression to chronic disease. Joint trauma triggers an immune response through signaling molecules like damage-

associated molecular patterns (DAMPs). Interestingly, addressing mechanical injuries or instability does not seem to prevent the development of PTOA, indicating additional biological mechanisms beyond mere wear and tear or pure inflammation.[83,84]

A body of research consistently suggests that the presence of synovitis within the context of OA correlates with more intense pain and greater joint dysfunction. Additionally, it may serve as a predictive marker for accelerated rates of cartilage loss within specific patient populations.[22] Given this nuanced understanding of OA pathogenesis, it becomes apparent that these inflammatory regulators represent potential targets for therapeutic interventions aimed at mitigating both symptomatic manifestations and the structural deterioration of the affected joints.[75]

Regrettably, though our current understanding of DEL-1's roles in cartilage and its potential involvement in OA remains largely unexplored, as underscored by Rosenthal, Gohr, et al.[85] in 2011. Though, a compelling challenge persists in our quest for a pharmaceutical intervention capable of finely tuning the host's immune response, aiding in the efficient eradication of pathogens, mitigating excessive immune reactions, and expediting the recuperative process following an immune response, all while curtailing collateral tissue damage. Consequently, our work and the present invention sheds more light to the above and endeavor to furnish compounds and their application to artfully manipulate the host's immune response at distinct and precise locations, as well as various stages. This includes the strategic limitation of hyperactive responses such as the dreaded "cytokine storm" occurring, for instance, within lung tissue or inflamed arthritic joint. The overarching aim is to avert harm to vital tissues and organs, thereby facilitating a swift return from such pathological states to a state of physiological equilibrium and homeostasis.

Conclusion

Current understanding of developmental endothelial locus 1's roles in articular cartilage and its potential involvement in arthritis remains largely unexplored. A compelling challenge persists in our quest for a pharmaceutical intervention capable of finely tuning the host's immune response. There is a critical need to skillfully manipulate the host's immune response at distinct and precise locations, as well as at various stages, including the strategic limitation of hyperactive responses such as the dreaded "cytokine storm." Our present findings reveal that 2-Hydroxyisocaproic acid in vitro inhibits the fragmentation of DEL-1 by dose-dependently modulating and reducing matrix metalloproteinase-8 activity. Notably, the reversible inhibition of MMP-8 by 2-Hydroxyisocaproic acid does not involve covalent bonding, positioning it as an enzyme modulator or down-regulator rather than a direct inhibitor. This property of active matrix metalloproteinase-8 reduction by 2-Hydroxyisocaproic acid opens new avenues for therapeutic intervention, particularly in managing excessive inflammatory responses, such as the "cytokine storm" observed in lung tissue inflammation or in arthritic joints like those affected by osteoarthritis.

Disclosure of potential conflicts of interest

The authors are named as inventors on patents (FI129515B) and U.S. Patent No. 11,793,779 both of which were filed by Salarusta Ltd, these patents are related to this work and the patents cover the use of a substance on a treatment of arthritis. The authors have an ownership interest in Salarusta Oy. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants, or patents received or pending, or royalties.

The data presented herein was previously disclosed in part in the aforementioned Patent FI12951B and U.S. Patent No. 11,793,779. In this study, we further explore the findings initially disclosed in the Patent, where we presented the preliminary data on 2-hydroxyisocaproic acid's effects on various proteinases. This paper expands upon these findings by more practical approach to tackle exuberant inflammation.

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