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2-Hydroxyisocaproic acid: A novel in vitro protease activity modulator with potential benefits for arthritis treatment

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ABSTRACT

Osteoarthritis affects approximately half a billion people worldwide and creates a significant economic burden, accounting for up to 2.5% of the national gross domestic product. Despite extensive research, a disease-modifying osteoarthritis drug remains unavailable. 2-hydroxyisocaproic acid is a physiological substance and the 2-hydroxy analogue of the essential amino acid leucine. This study investigates the potential of 2-hydroxyisocaproic acid to down-regulate key proteases involved in articular cartilage degradation. Specifically, we explore the use of 2-hydroxyisocaproic acid to inhibit the activity of matrix metalloproteinase 13 and a disintegrin and metalloproteinase with thrombospondin motifs 5, aiming to reduce the degradation of type II collagen and aggrecan, respectively, in osteoarthritis. Our findings demonstrate that 2-hydroxyisocaproic acid modulates and reduces the activity of both matrix metalloproteinase 13 and a disintegrin and metalloproteinase with thrombospondin motifs 5. Notably, 2-hydroxyisocaproic acid's reversible inhibition of these enzymes does not involve covalent bonding, positioning it as an enzyme modulator or down-regulator rather than a direct inhibitor.

Introduction

Arthritis manifests in over 100 distinct forms, among which osteoarthritis (OA) is predominant. This condition is characterized by structural impairments in hyaline articular cartilage (AC), compromised subchondral bone integrity, synovial tissue hypertrophy, enhanced vascularity, and instability of tendons and ligaments.[1,2] The etiology of OA, however, remains not fully elucidated.[3]

Globally, OA was identified as the fourth leading cause of disability in 2020.[4] By 2021, over 22% of individuals aged over 40 were suffering from knee OA, affecting an estimated 500 million people worldwide.[5] In the United States alone, there were 54 million diagnosed cases and an additional 66 million self-reported cases in 2019.[6] Beyond being a prevalent ailment second only to back pain, OA accounts for an annual financial impact of $460 billion in medical expenses in the U.S.[6] Beyond personal hardship, the socioeconomic implications in developed nations are profound, accounting for 1.0% to 2.5% of the gross domestic product.[7]

Emerging evidence suggests that the primary pathological events in OA involve changes in AC, leading to subsequent synovitis and subchondral bone degradation.[8] It was not until the 1980s that the conceptual understanding of OA evolved from mere mechanical "wear and tear" degradation to a disease characterized by specific biochemical pathways causing AC damage.[9-11] OA is now recognized as a final common pathway influenced by multiple factors, most notably age, genetic predispositions, joint trauma, altered biomechanics, and obesity.[12-15] This pathogenesis is likely triggered by trauma, leading to inflammation and the release of matrix-degrading enzymes,[9] with altered biomechanical stress contributing to changes in chondrocyte metabolism, degradation of the specialized cartilage extra cellular matrix (ECM), and apoptosis of articular chondrocytes.[16] Inflammation is now strongly linked to the pathogenesis of OA, with synovitis often arising as a secondary response to cartilage damage, serving as a crucial link in the initiation and progression of the disease.[12]

Experimental studies, such as a single ex vivo impact to human cartilage, have shown cell death at the impact site, immediate release of inflammatory cytokines like interleukine-6 (IL-6) and tumor necrosis factor-α (TNF-α), radial progression of apoptosis, and cartilage degeneration extending to adjacent non-impacted areas.[17] This disruption of chondrocytes' resting state may be viewed as an injury response that triggers developmental programs, leading to matrix remodeling, inappropriate hypertrophic maturation, and cartilage calcification.[18]

The initial loss of aggrecan is a critical early event in OA, beginning at the joint surface and extending to deeper zones, followed by collagen fibril degradation and mechanical tissue failure.[19] Although proteoglycans are more remodeled under physiological conditions than collagens,[20] the sequence of degradation in the cartilage matrix components during OA development remains complex; collagen degradation typically follows the loss of aggrecan. Aggrecan loss can be reversed, but collagen degradation is irreversible, though cartilage cannot be repaired once collagen is lost.[21] Eventually, a feedforward loop ensues as AC fragments, broken down by chondrocyte-derived proteinases, irritate the synovium, leading to secondary synovitis and further increases in proteases and inflammatory cytokines from synovial cells, thus perpetuating the disease cycle.[22] OA is also associated with low-grade systemic and joint inflammation.[18,23,24]

The metalloproteinase families, particularly a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) and matrix metalloproteinases (MMPs), are key mediators of cartilage destruction in OA.[22]

The hallmark of OA pathology is extensive AC damage.[25] MMPs, a family of calcium-dependent and zinc-containing endoproteinases, vary in substrate specificity, cellular localization, activation,

and inducibility, belonging to the metzincin protease superfamily.[1] They possess broad substrate specificities, degrading not only ECM and basement membranes (BM) but also various bioactive non-matrix proteins, with 23 different types of MMPs encoded by 24 human genes.[26] MMPs also process and release cytokines, chemokines, insulin-receptor, and growth factors from their precursors or cryptic sites.[27,28]

A principal collagenolytic enzyme, MMP-13 targets mature AC for degradation and is predominantly expressed in connective tissue.[29] It degrades type II collagen in cartilage, as well as proteoglycans, types IV and IX collagens, osteonectin, and perlecan in AC.[30] Clinical studies have associated high MMP-13 expression with AC destruction.[31] MMP-13's role in early OA development is significant, being five to ten times more effective at degrading type II collagen than other collagenases.[22] It plays a dual role in ECM destruction, degrading both collagen and the proteoglycan aggrecan.[32]

The ADAMTS family of secreted zinc metalloproteinases comprises nineteen members known for their ability to bind and degrade extracellular cartilage matrix components.[30] ADAMTS-4 and -5 are the primary mediators of aggrecan cleavage in situ, playing significant roles in AC degradation.[33,34]

LD-2-Hydroxy-isocaproic acid (HICA, also known as leucic acid), an organic acid with the chemical equation $C_6H_{12}O_3$ and a molecular weight of 132.2 g/mol, is an end product of leucine metabolism in human muscle and connective tissue, naturally occurring in mammalian organisms. During fasting, serum concentrations of HICA increase.[35]

2-Hydroxyisocaproic acid, classified within the 2-hydroxycarboxylic acid group of amino acid metabolites, is part of the broader category of carboxylic acids, characterized by a carboxyl group (-COOH). This compound, among other carboxylic acids, has been reported to inhibit various MMPs, particularly with derivatives of glutamine inhibiting MMP-8.[36] Some amino acid derivatives have shown in vitro effects on inhibiting connective tissue breakdown, and some molecules have been patented for treating degenerative joint diseases, though none are currently in clinical use.[37]

In OA, the loss of aggrecan facilitates MMP-13 mediated degradation of exposed type II collagen. Both ADAMTS-5 and MMP-13 remain compelling therapeutic targets, provided that their side effects on physiological functions can be minimized.[38] Despite the absence of a disease-modifying osteoarthritis drug (DMOAD), the broad-spectrum, low-grade inhibition of MMPs could address the multifactorial nature of the disease more effectively than targeting a single enzyme, which might cause uncontrollable systemic damage.[39]

Demonstrating efficacy in inhibiting a range of proteases, HICA has been effective against human MMP-2, -8, and -9, as well as neutrophil elastases and cathepsin G in vitro.[40,41] This study aims to explore HICA's impact on key AC-degrading enzymes, offering a new potential therapeutic approach for OA.

Material and Methods

This experiment was carried out to demonstrate inhibition of recombinant human a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5/Aggrecanase 2, #CC1034, Chemicon, International. Inc.) and human matrix metalloproteinase 13 (MMP-13, #30100802, BioTeZ, Berlin-Buch, Berlin, Germany) mediated break down of aggrecan (CC1890, Sigma-Aldrich, Darmstadt, Germany) by 10% rasemic D,L-2-hydroxy-isocaproic acid (HICA, Leios OŰ, Tallinn, Estonia) at pH 7.4. As positive inhibitor controls Ilomastat (Ilo, #CC1010, Millipore) and chlorhexidine (CHX, Corsodyl®, [2 mg/ml] (GlaxoSmithKline, Brøndby, Denmark) were used and 50 mM Tris-HCl-buffer (TNC): pH 7.5; 0.2 M NaCl; 1.0 mM $CaCl_2$) was used as neutral assay buffer solution in this study.[42]

Briefly, MMP-13 was preincubated 1 hour at 37°C with p-aminophenylmercuric acetate (APMA, A9563, Sigma-Aldrich) to optimally activate (pro)MMP-13. As substrate, aggrecan was added and then ADAMTS-5 or with APMA activated MMP-13 as catalytic/proteolytic enzyme, TNC, as neutral control, and then 10% HICA, CHX or Ilo as protease/MMP-inhibitors were added. After that, they all were incubated together to find out the effects of different inhibitors on time function at a temperature of +37°C from 1 hour (h) to 2 days (d). Thus, the reactions were individually terminated by boiling with modified Laemmli's buffer. When the last incubation time was finished the samples were electrophoresed by 8% SDS-PAGE. After the electrophoresis the defragmentation of aggrecan was visualized using Silver Stain Pierce® (#24612, Thermo Fisher Scientific Inc., Vantaa, Finland). More details about the volumes of samples used in the experiment are described in the figure legends.

In experiment two MMP-13 activity was measured by catalytic activity assay as described previously.[43] In brief, MMP-13 was captured from assay buffer solution using MMP-13-spesific monoclonal antibody-coated 96-well plates. The wells were washed three times with PBS-T (phosphate buffered saline solution containing 0.05% [v/v] Tween-20 and incubated with 125-µl assay buffer (5O mM Tris-HCl, pH 7.6, 150 mM NaCl, 5 mM $CaCl_2$, 1 µM $ZnCl_2$ and 0,01% [v/v] Brij-35), to which 15 µl (50 µg/ml) modified pro-urokinase (UKcol) and 10 µl (6 mM stock) chromogenic substrate S-2444 was added. Color development was recorded by measurement of A_{405} using a Titertek Multiskan 8-channel photometer. For measurement of total activity (already active plus latent MMP-13) in biological fluid, the immobilized MMP-13 was incubated With assay buffer containing 0.5 mM APMA for 2h, after which UKcol and S-2444 were added and activity was recorded.[43]

Results

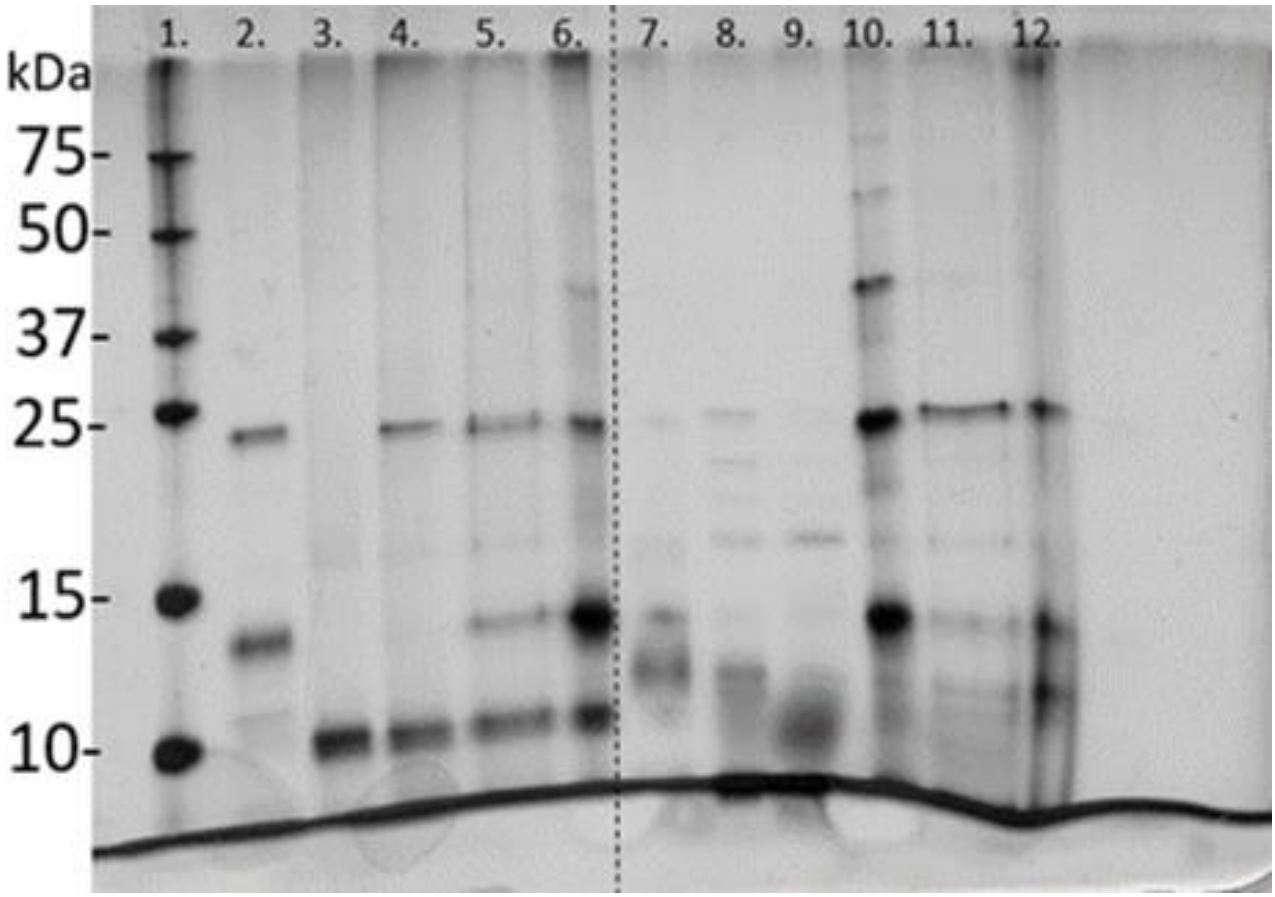
In the first experiment, we show that 10% D,L-2-hydroxy-isocaproic acid (HICA) inhibited the breakdown of aggrecan by both ADAMTS-5 and MMP-13 proteases (lanes 5 and 11, respectively). Figure 1 shows that HICA prevents break down of aggrecan. Therefore, both ADAMTS-5 and MMP-13 may be considered to be modulated enzyme activities by HICA rather than prevent enzyme activity like by chlorhexidine (CHX) in our study (Figure 1).

Figure 1. Illustrates the inhibition of recombinant human a disintegrin metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) and human matrix metalloproteinase 13 (MMP-13) mediated break-down of aggrecan by 10% rasemic D,L-2-hydroxy-isocaproic acid (HICA). Molecular weight markers are indicated left. Figure 1 shows that 10% HICA inhibited the breakdown of aggrecan by both ADAMTS-5 and MMP-13 proteases (lines 5 and 11, respectively). Figure 1 shows that HICA prevents break down of aggrecan. Therefore, both ADAMTS-5 and MMP-13 may be considered to modulate enzyme activity rather than prevent enzyme activity like chlorhexidine in our study. Chlorhexidine (CHX). Ilomastat (Ilo)

Sample with concentrations in lines:

1. 0,25 µl molecular weight standards, Bio-Rad
2. 4,2 µl Aggrecan (0,5 µg/µl)
3. 4 µl ADAMTS-5 (0,1 µg/µl)
4. 4,2 µl Aggrecan + 4 µl ADAMTS-5 + 6 µl TNC-buffer, inc: 2d
5. 4,2 µl Aggrecan + 4 µl ADAMTS-5 + 6 µl 10% HICA, inc: 2d
6. 4,2 µl Aggrecan + 4 µl ADAMTS-5 + 6 µl CHX (2 mg/ml), inc: 2d
7. 1 µl MMP-13 (0,2 µg/µl) + 3 µl 2 mM APMA + 6 µl TNC
8. 4,2 µl Aggrecan + 1 µl MMP-13 (0,2 µg/µl) + 3 µl 2 mM APMA + 6 µl TNC, inc: 1h

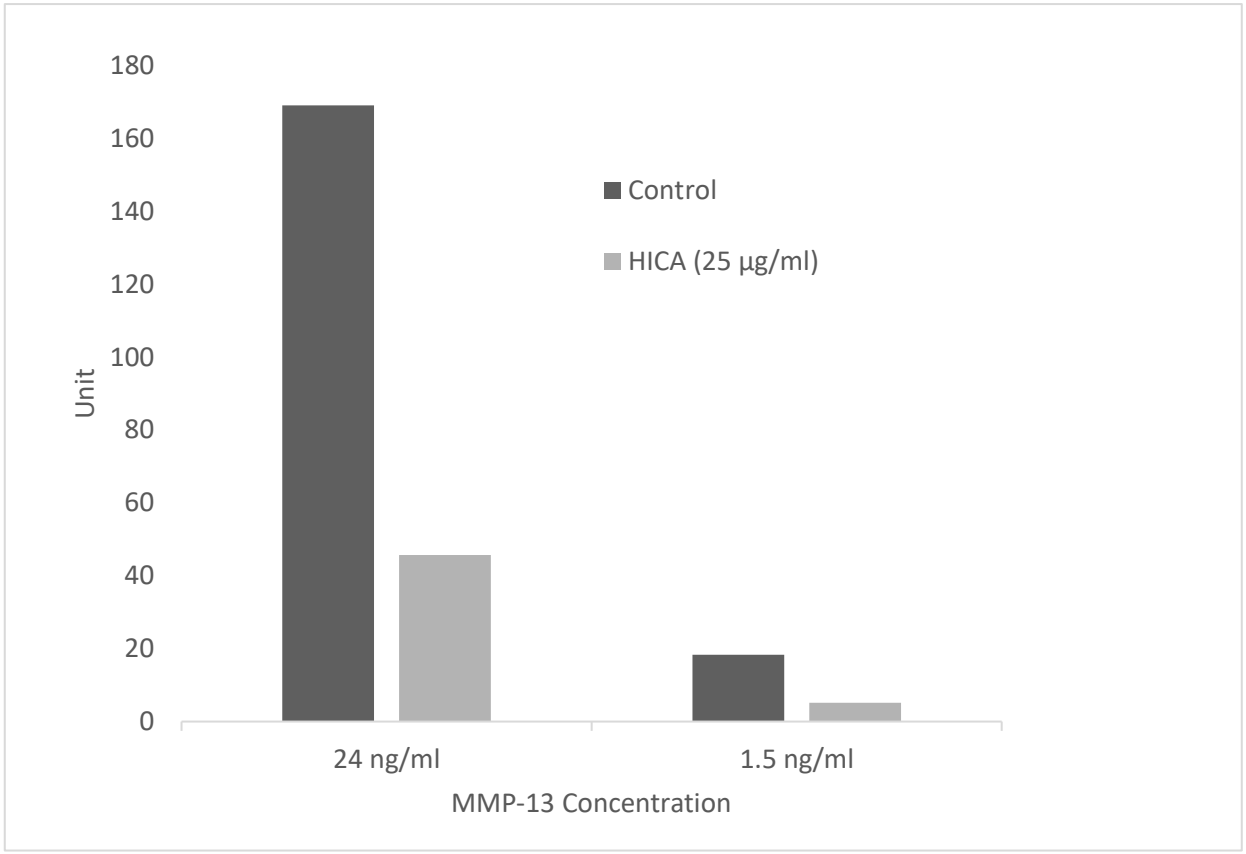
9. 4,2 µl Aggrecan + 1 µl MMP-13 (0,2 µg/µl) + 3 µl 2 mM APMA + 6 µl TNC, inc: 2d
10. 4,2 µl Aggrecan + 1 µl MMP-13 (0,2 µg/µl) + 3 µl 2 mM APMA + 6 µl CXH, inc: 2d
11. 4,2 µl Aggrecan + 1 µl MMP-13 (0,2 µg/µl) + 3 µl 2 mM APMA + 6 µl 10% HICA, inc: 2d
12. 4,2 µl Aggrecan + 1 µl MMP-13 (0,2 µg/µl) + 3 µl 2 mM APMA + 6 µl Ilo. inc: 2d



HICA's reversible inhibition of ADAMTS-5 and MMP-13 does not eventually involve covalent bonding and should thus be regarded as an enzyme modulator or down-regulator rather than a direct inhibitor.

In the second experiment, we demonstrated an inhibition of MMP-13 mediated break-down of collagen by rasemic D,L-2-hydroxy-isocaproic acid (HICA), which is shown in Figure 2. HICA was found to exert effective inhibition of the activity of MMP-13 at a concentration level 25 µg/ml. At 24 ng/ml MMP-13 concentration there was 73% decreased activity noted and with 1.5 ng/ml concentration 72% decrease (Figure 2).

Figure 2. Inhibition of matrix metalloprotease 13 (MMP-13) activity by rasemic D,L-2-hydroxy-isocaproic acid (HICA) using modified pro-urokinase and chromogenic S2444 as substrate. MMP-13 activity was measured as described previously.[44] For a more detailed description, please refer to the Material and Methods section. HICA was found to effectively inhibit the activity of MMP-13 at a concentration of 25 µg/ml. At an MMP-13 concentration of 24 ng/ml, a 73% decrease in activity was noted, and at a concentration of 1.5 ng/ml, there was a 72% decrease.



Discussion

The aim of this study was to evaluate if HICA can inhibit key proteases involved in AC degradation. HICA was found to relatively inhibit or to down-regulate the human ADAMTS-5 aggrecanase when aggrecan served as the substrate. Similarly, human MMP-13 collagenase activity was also relatively inhibited, both when aggrecan and collagen were used as substrates.

Since the late 1970s, over 56 MMP inhibitors have been explored as clinical candidates, but only three have been clinically evaluated for cardiovascular indications: doxycycline for acute coronary syndrome and periodontal disease, Batimastat for coronary stent restenosis, and PG-116800 for preventing post-ischemic left ventricular dilation.[45] To date, no MMP-13 inhibitor has reached clinical practice.[46] Since 2005, MMP inhibitors have been targeted for cancer (24 drugs), arthritis (27 drugs), and cardiovascular disease (10 drugs).[45]

Inhibitors of matrix metalloproteinases MMPs could be effective in preventing the destruction of AC. Carboxylic acids, particularly tetrahydroisoquinoline-3-carboxylates, have shown promise in inhibiting MMP-8.[47] Nakai and colleagues from Scripps Research Institute identified potent MMP-13 inhibitors through high-throughput screening and activity-based protein profiling.[48] Adhikari and colleagues designed glutamine derivatives that initially showed potential in inhibiting MMPs.[49]

Limited bioavailability and lack of enzyme selectivity have hindered the development of MMP inhibitors. Peptidomimetic compounds with a hydroxamic acid moiety were noted as very potent MMP inhibitors in the early phase of OA but still exhibited poor bioavailability and little specificity for targeted MMPs. Additionally, hydroxamic acid inhibitors are rapidly metabolized by the liver's first-pass metabolism and require frequent administration to maintain therapeutic plasma levels. More selective compounds are needed due to the overlap of function among individual enzymes and the complex inter-relationships involved in the expression, activation, and natural inhibition within the MMP family, which may necessitate multienzyme inhibition to ensure efficacy for targeted diseases.[50] Although PF152 seemed to be a very promising molecule that dose-dependently and selectively inhibited MMP-13 in experimental models, it was shown to cause nephrotoxicity by affecting the organic anion transporter 3.[51,52]

Amino acid derivatives such as L-2-hydroxyisocaproate (HICA), a leucine analog, have been reported to influence protein synthesis similarly to L-leucine, exhibiting multiphasic responses that include inhibition of protein degradation at concentrations as low as 0.1 mM.[53] HICA has also been identified as an inhibitor of other proteinases[54] and is considered an anti-catabolic agent in clinical and experimental studies.[55-64] HICA is classified as "anti-catabolite" and is widely used in the body building community.[64]

A physiological substance, HICA has a plasma concentration in healthy adults of 0.25±0.02 μM, and in circulation, it is not bound to plasma proteins.[35] Normal child serum concentration is 0.71±0.51 μM and range 0.02-2.04 (n=10).[65] It is found in human plasma, saliva, urine, amniotic fluid, and breast milk.[65-69] Foods such as certain cheeses, sourdough, beer, radish, wines, and soy sauce also contain HICA.[70-79]

Effectively absorbed through active transport in the human bowel, HICA can bypass the liver's first-pass metabolism.[80-82] It can also be assumed that HICA is also passively absorbed from alimentary canal by diffusion.[62,63,83] Thus, HICA does reach the target tissue, the systemic circulation, when administrated orally.[84,85] Topically, HICA is non-toxic even at high doses and HICA is not cytotoxic nor genotoxic at concentrations <10 mg/ml, regardless of the exposure time, although it was cytostatic at all tested concentrations. HICA retained about 70% of the osteogenic differentiation potential at 1 mg/ml.[86,87]

Aggrecan, the predominant proteoglycan in cartilage, is composed of chondroitin sulphate and keratan sulphate binding to the linear core protein hyaluronic acid backbone. It provides AC structural rigidity, compressibility, and collagen elasticity.[88,89] Aggrecan gives a low coefficient of friction on the joint surfaces and gives the cartilage protective resiliency.[22]

Two major families within the metalloproteinase class, namely a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) and the matrix metalloproteinases (MMPs), are principal agents of cartilage destruction in arthritic conditions.[22] Specifically, ADAMTS-4 and -5 are pivotal in the cleavage of aggrecan in situ, significantly contributing to cartilage degradation.[33,34] In healthy joints, distinct locations within the cartilage are mapped by MMP and aggrecanase neoepitopes, indicating that these proteolytic enzyme groups operate at different sites during the normal turnover of aggrecan.[19]

The degradation of AC in arthritic diseases is primarily mediated by MMPs and ADAMTSs, with MMP-13 being a major collagenolytic enzyme in this process (Vincenti and Brinckerhoff 2002). It is critical for the progression of OA and is a prime target for pharmacological intervention.[25]

Population growth, especially among the elderly, and rising obesity rates do not entirely account for the sharp increase in total knee replacements observed in recent decades. It is reasonable to assume that additional factors are at play. A

notably high increase in knee replacements among younger individuals is likely linked to an increase in adolescent knee injuries and broader criteria for undergoing this procedure.[90] Globally, joint replacement surgeries are expanding at an annual rate of 10%, with 95% performed on patients with OA.[91] Addressing therapeutic targets at various OA stages is critically needed.[4]

Despite successful anterior cruciate ligament (ACL) repairs, which involve reconstruction of torn ligaments, 45%-50% of these patients will eventually develop OA.[92-94] Studies comparing OA prevalence between reconstructed and healthy, operated knees show a higher incidence of OA.[95] This suggests that ACL reconstruction does not prevent OA, with a threefold increase in OA prevalence observed in knees post-reconstruction compared to their healthy counterparts.

Bluteau et al.[96] observed a significant surge in MMP-13 and MMP-3 expression following ACL surgery, with no change in the expression of tissue inhibitors of MMPs (TIMP). The expression of MMP-13 genes rose sharply post-surgery and remained elevated. Similarly, a marked increase in MMP-13 gene expression was noted immediately after ACL transection, which continued at high levels. Corresponding increases in MMP-13 mRNA and protein were observed in the synovium and meniscus. The joint cavity releases substantial amounts of MMPs into the joint fluid, potentially disrupting the balance between tissue synthesis and degradation, thus impeding ACL healing and exacerbating osteoarthritis.[97]

The inhibition of ADAMTS-5 may safeguard against cartilage damage and aggrecan loss following OA induction via surgical destabilization of the medial meniscus.[98] It is noteworthy that blocking ADAMTS-5 activity might also reduce pain, according to preclinical studies.[88]

Conclusion

Proteases play a crucial role in normal physiological processes, making their complete inhibition unfeasible. Despite the incomplete understanding of the arthritic disease and its destructive progression, it is essential to modulate excessive protease activity to restore physiological balance. Although significant efforts have been made to develop effective therapies or disease-modifying drugs for osteoarthritis and its progression, success has been limited. This study suggests that D,L-2-hydroxy-isocaproic acid, with its ability to modulate enzyme activity and inhibit key proteinases, may offer therapeutic benefits across all stages of OA. The aim of this study was to evaluate the potential of HICA to inhibit key proteases involved in AC degradation. HICA was found to significantly inhibit or down-regulate human ADAMTS-5 aggrecanase activity when aggrecan was used as the substrate. Similarly, human MMP-13 collagenase activity was also significantly inhibited.

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 Ltd Oy. The authors have no other competing interests such as 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 Patents, 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 proteinases relevant to arthritis especially osteoarthritis.

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References:

1. Huang Z, Ding C, Li T, Yu SP. Current status and future prospects for disease modification in osteoarthritis. *Rheumatology (Oxford)*. May 1 2018;57(suppl_4):iv108-iv123. doi:10.1093/rheumatology/kex496

2. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. *Lancet*. Apr 27 2019;393(10182):1745-1759. doi:10.1016/S0140-6736(19)30417-9

3. Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. *Arthritis Rheum*. Jun 2001;44(6):1237-47.

4. Yao Q, Wu X, Tao C, et al. Osteoarthritis: pathogenic signaling pathways and therapeutic targets. *Signal Transduct Target Ther*. Feb 3 2023; 8(1):56. doi:10.1038/s41392-023-01330-w

5. Quicke JG, Conaghan PG, Corp N, Peat G. Osteoarthritis year in review 2021: epidemiology & therapy. *Osteoarthritis Cartilage*. Feb 2022;30(2): 196-206. doi:10.1016/j.joca.2021.10.003

6. Lo J, Chan L, Flynn S. A Systematic Review of the Incidence, Prevalence, Costs, and Activity and Work Limitations of Amputation, Osteoarthritis, Rheumatoid Arthritis, Back Pain, Multiple Sclerosis, Spinal Cord Injury, Stroke, and Traumatic Brain Injury in the United States: A 2019 Update. *Arch Phys Med Rehabil*. Jan 2021;102(1):115-131. doi:10.1016/j.apmr.2020.04.001

7. Hiligsmann M, Cooper C, Arden N, et al. Health economics in the field of osteoarthritis: an expert's consensus paper from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Semin Arthritis Rheum*. Dec 2013;43(3):303-13. doi:10.1016/j.semarthrit.2013.07.003

8. Honorati MC, Bovara M, Cattini L, Piacentini A, Facchini A. Contribution of interleukin 17 to human cartilage degradation and synovial inflammation in osteoarthritis. *Osteoarthritis Cartilage*. Oct 2002; 10(10):799-807. doi:10.1053/joca.2002.0829

9. Anderson DD, Chubinskaya S, Guilak F, et al. Post-traumatic osteoarthritis: improved understanding and opportunities for early intervention. *J Orthop Res*. Jun 2011;29(6):802-9. doi:10.1002/jor.21359

10. Huskisson EC, Dieppe PA, Tucker AK, Cannell LB. Another look at osteoarthritis. *Annals of the rheumatic diseases*. Oct 1979;38(5):423-8. doi:10.1136/ard.38.5.423

11. Dingle JT. Articular Damage in Arthritis and Its Control. *Annals of Internal Medicine*. 1978;88 (6):821-826. doi:10.7326/0003-4819-88-6-821

12. Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther Adv Musculoskelet Dis*. Apr 2013;5(2):77-94. doi:10.1177/1759720X12467868

13. Lawrence RC, Felson DT, Helmick CG, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum*. Jan 2008;58(1):26-35.

14. Barter M, Bui C, Young D. Epigenetic mechanisms in cartilage and osteoarthritis: DNA methylation, histone modifications and microRNAs. *Osteoarthritis and cartilage*. May 2012;20(5):339-349. doi:10.1016/j.joca.2011.12.012

15. Loughlin J. The genetic epidemiology of human primary osteoarthritis: current status. *Expert Rev Mol Med*. May 24 2005;7(9):1-12. doi:10.1017/S1462399405009257

16. Glyn-Jones S, Palmer A, Agricola R, et al. Osteoarthritis. *The Lancet*. Jul 25 2015;386(9991): 376-387. doi:10.1016/S0140-6736(14)60802-3

17. Bajaj S, Shoemaker T, Hakimiyan AA, et al. Protective effect of P188 in the model of acute trauma to human ankle cartilage: the mechanism of action. *Journal of orthopaedic trauma*. Sep 2010; 24(9):571. doi:10.1097/BOT.0b013e3181ec4712

18. Goldring MB, Marcu KB. Cartilage homeostasis in health and rheumatic diseases. *Arthritis Res Ther*. 2009;11(3):224. doi:10.1186/ar2592

19. Nagase H, Kashiwagi M. Aggrecanases and cartilage matrix degradation. *Arthritis Res Ther*. 2003;5(2):94.

20. Karsdal MA, Madsen SH, Christiansen C, Henriksen K, Fosang AJ, Sondergaard BC. Cartilage degradation is fully reversible in the presence of aggrecanase but not matrix metalloproteinase activity. *Arthritis Res Ther*. 2008;10(3):R63. doi:10.1186/ar2434

21. Troeberg L, Nagase H. Proteases involved in cartilage matrix degradation in osteoarthritis. *Biochim Biophys Acta*. Jan 2012;1824(1):133-45. doi:10.1016/j.bbapap.2011.06.020

22. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. *Front Biosci*. Jan 1 2006;11(1):529-543. doi:10.2741/1817

23. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol*. Jan 2011;7(1):33-42. doi:10.1038/nrrheum.2010.196

24. Wojdasiewicz P, Poniatowski LA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm*. 2014;2014:561459. doi:10.1155/2014/561459

25. Wang M, Sampson ER, Jin H, et al. MMP13 is a critical target gene during the progression of osteoarthritis. *Arthritis Res Ther*. Jan 8 2013;15(1):R5. doi:10.1186/ar4133

26. Murphy G, Nagase H. Progress in matrix metalloproteinase research. *Molecular aspects of medicine*. 2008;29(5):290-308.

27. Vilen S-T, Salo T, Sorsa T, Nyberg P. Fluctuating roles of matrix metalloproteinase-9 in oral squamous cell carcinoma. *The Scientific World Journal*. 2013;2013

28. Lauhio A, Farkkila E, Pietilainen KH, et al. Association of MMP-8 with obesity, smoking and insulin resistance. *Eur J Clin Invest*. Sep 2016;46(9):757-65. doi:10.1111/eci.12649

29. Vincenti MP, Brinckerhoff CE. Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Res*. 2002;4(3):157-64. doi:10.1186/ar401

30. Shiomi T, Lemaitre V, D'Armiento J, Okada Y. Matrix metalloproteinases, a disintegrin and metalloproteinases, and a disintegrin and metalloproteinases with thrombospondin motifs in non-neoplastic diseases. *Pathol Int*. Jul 2010;60(7):477-96. doi:10.1111/j.1440-1827.2010.02547.x

31. Roach HI, Yamada N, Cheung KS, et al. Association between the abnormal expression of matrix-degrading enzymes by human osteoarthritic chondrocytes and demethylation of specific CpG sites in the promoter regions. *Arthritis Rheum*. Oct 2005;52(10):3110-24. doi:10.1002/art.21300

32. Mehana E-SE, Khafaga AF, El-Blehi SS. The role of matrix metalloproteinases in osteoarthritis pathogenesis: An updated review. *Life sciences*. Oct 1 2019;234:116786. doi:10.1016/j.lfs.2019.116786

33. Sandy JD, Flannery CR, Neame PJ, Lohmander LS. The structure of aggrecan fragments in human synovial fluid. Evidence for the involvement in osteoarthritis of a novel proteinase which cleaves the Glu 373-Ala 374 bond of the interglobular domain. *J Clin Invest*. May 1992;89(5):1512-6. doi:10.1172/JCI115742

34. Lohmander LS, Neame PJ, Sandy JD. The structure of aggrecan fragments in human synovial fluid. Evidence that aggrecanase mediates cartilage degradation in inflammatory joint disease, joint injury, and osteoarthritis. *Arthritis Rheum*. Sep 1993;36(9):1214-22. doi:10.1002/art.1780360906

35. Hoffer LJ, Taveroff A, Robitaille L, Mamer OA, Reimer ML. Alpha-keto and alpha-hydroxy branched-chain acid interrelationships in normal humans. *J Nutr*. Sep 1993;123(9):1513-21. doi:10.1093/jn/123.9.1513

36. Wishart DS, Guo A, Oler E, et al. HMDB 5.0: the Human Metabolome Database for 2022.

Nucleic Acids Res. Jan 7 2022;50(D1):D622-D631. doi:10.1093/nar/gkab1062

37. Broadhurst MJ, Brown PA, Johnson WH, Lawton G, inventors; Hoffmann-La Roche Inc, Nutley, N.J., assignee. Amino acid derivatives. patent 5,304,549. patent application 823,212. 1994.

38. van den Berg WB. Osteoarthritis year 2010 in review: pathomechanisms. *Osteoarthritis Cartilage*. Apr 2011;19(4):338-41. doi:10.1016/j.joca.2011.01.022

39. Rowan AD, Litherland GJ, Hui W, Milner JM. Metalloproteases as potential therapeutic targets in arthritis treatment. *Expert Opin Ther Targets*. Jan 2008;12(1):1-18. doi:10.1517/14728222.12.1.1

40. Westermarck HW, Hietala P, Jaarma M, Sorsa T, Vaara M, inventors; Oy Extracta Ltd, assignee. Antimicrobial and anti-inflammatory compounds. EPO patent EP0871438B1. patent application EP0871438A1. 1996.

41. Nieminen MT, Hernandez M, Novak-Frazer L, et al. DL-2-hydroxyisocaproic acid attenuates inflammatory responses in a murine Candida albicans biofilm model. *Clin Vaccine Immunol*. Sep 2014;21(9):1240-1245. doi:10.1128/CVI.00339-14

42. Tervahartiala T, Ingman T, Sorsa T, Ding Y, Kangaspunta P, Konttinen YT. Proteolytic enzymes as indicators of periodontal health in gingival crevicular fluid of patients with Sjogren's syndrome. *Eur J Oral Sci*. Feb 1995;103(1):11-6. doi:10.1111/j.1600-0722.1995.tb00004.x

43. Hanemaaijer R, Sier CF, Visser H, et al. MMP-9 activity in urine from patients with various tumors, as measured by a novel MMP activity assay using modified urokinase as a substrate. *Ann N Y Acad Sci*. Jun 30 1999;878:141-9. doi:10.1111/j.1749-6632.1999.tb07680.x

44. Hanemaaijer R, Visser H, Konttinen YT, Koolwijk P, Verheijen JH. A novel and simple immunocapture assay for determination of gelatinase-B (MMP-9) activities in biological fluids: saliva from patients with Sjogren's syndrome contain increased latent and active gelatinase-B levels. *Matrix Biol*. Dec 1998;17(8-9):657-65. doi:10.1016/s0945-053x(98)90116-0

45. Peterson JT. The importance of estimating the therapeutic index in the development of matrix metalloproteinase inhibitors. *Cardiovasc Res*. Feb 15 2006;69(3):677-87. doi:10.1016/j.cardiores.2005.11.032

46. Karila T, Tervahartiala T, Cohen B, Sorsa T. The collagenases: are they tractable targets for preventing cartilage destruction in osteoarthritis? *Expert Opin Ther Targets*. Feb 2 2022:1-13. doi:10.1080/14728222.2022.2035362

47. Matter H, Schudok M, Schwab W, et al. Tetrahydroisoquinoline-3-carboxylate based matrix-metalloproteinase inhibitors: design, synthesis and structure-activity relationship. *Bioorg Med Chem*. Nov 2002;10(11):3529-44. doi:10.1016/s0968-0896(02)00215-8

48. Nakai R, Salisbury CM, Rosen H, Cravatt BF. Ranking the selectivity of PubChem screening hits by activity-based protein profiling: MMP13 as a case study. *Bioorg Med Chem*. Feb 1 2009;17(3):1101-8. doi:10.1016/j.bmc.2008.03.018

49. Adhikari N, Halder AK, Mallick S, Saha A, Saha KD, Jha T. Robust design of some selective matrix metalloproteinase-2 inhibitors over matrix metalloproteinase-9 through in silico/fragment-based lead identification and de novo lead modification: Syntheses and biological assays. *Bioorg Med Chem*. Sep 15 2016;24(18):4291-4309. doi:10.1016/j.bmc.2016.07.023

50. Johnson LL, Dyer R, Hupe DJ. Matrix metalloproteinases. *Curr Opin Chem Biol*. Aug 1998;2(4):466-71. doi:10.1016/s1367-5931(98)80122-1

51. Settle S, Vickery L, Nemirovskiy O, et al. Cartilage degradation biomarkers predict efficacy of a novel, highly selective matrix metalloproteinase 13 inhibitor in a dog model of osteoarthritis: confirmation by multivariate analysis that modulation of type II collagen and aggrecan degradation

peptides parallels pathologic changes. *Arthritis Rheum*. Oct 2010;62(10):3006-15. doi:10.1002/art.27596

52. Cai H, Agrawal AK, Putt DA, et al. Assessment of the renal toxicity of novel anti-inflammatory compounds using cynomolgus monkey and human kidney cells. *Toxicology*. Apr 5 2009;258(1):56-63. doi:10.1016/j.tox.2009.01.006

53. Mortimore GE, Pösö AR, Kadowaki M, Wert JJ, Jr. Multiphasic control of hepatic protein degradation by regulatory amino acids. General features and hormonal modulation. *J Biol Chem*. Dec 5 1987;262(34):16322-7. doi:https://doi.org/10.1016/S0021-9258(18)49257-5

54. Stockel-Maschek A, Stiebitz B, Koelsch R, Neubert K. Novel 3-amino-2-hydroxy acids containing protease inhibitors. Part 1: Synthesis and kinetic characterization as aminopeptidase P inhibitors. *Bioorg Med Chem*. Aug 15 2005;13(16): 4806-20. doi:10.1016/j.bmc.2005.05.040

55. Walser M, inventor; Composition for promotion of protein synthesis and suppression of urea formation in the body utilizing alpha-hydroxy-acid analogs of amoni acids. US patent 1 444 621. patent application 32122/73. 30. April 1973 1973.

56. Walser M, inventor; The Johns Hopkins University, Baltimore, Md., assignee. Therapeutic compositions comprising alpha-hydroxy analogs of essential amino acids and their administration to humans for promotion of protein synthesis and suppression of urea formation. U.S. patent 4,100,160. patent application 669,588. Mar. 23, 1976 1978.

57. Boebel KP, Baker DH. Comparative utilization of the alpha-keto and D- and L-alpha-hydroxy analogs of leucine, isoleucine and valine by chicks and rats. *J Nutr*. Oct 1982;112(10):1929-39. doi:10.1093/jn/112.10.1929

58. Tischler ME, Desautels M, Goldberg AL. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? *J Biol Chem*. Feb 25 1982;257(4):1613-21.

59. Lindgren S, Jonsson U, Sandberg G, Granelli K, inventors; Kabivitrum AB, assignee. Energy substrate containing hydroxycarboxylic acid and a glycerol ester. patent EP 367734 A1. patent application 89850290.1. 06.09.1989 1990.

60. Lindgren S, Sandberg G, Enekull U, Werner T, inventors; Kabivitrum Ab, assignee. Energy substrate containing hydroxycarboxylic acid. patent EP 363337 A1. patent application 89850292.7. 1990.

61. Hietala P, Karila TAM, Seppälä TA, Tähtivuori K, inventors; Nutrient supplement and use of the same. Finland patent application PCT/FI2005/050365. 2005.

62. Westermarck HW, Hietala P, Jaarma M, Sorsa T, Vaara M, inventors; Exracta Oy, assignee. Use of alpha-hydroxy acids in the manufacture of a medicament for the treatment of inflammation. patent application PCT/FI96/00363. 1997.

63. Westermarck HW, Apajalahti J, Hietala P, Jaarma M, inventors; Oy Extracta Ltd, assignee. Use of hydroxy acid or a product containing the same in animal feed. patent US 6,203,835 B1. patent application 08/981,108. 2001.

64. Lang CH, Pruznak A, Navaratnarajah M, et al. Chronic alpha-hydroxyisocaproic acid treatment improves muscle recovery after immobilization-induced atrophy. *Am J Physiol Endocrinol Metab*. Aug 1 2013;305(3):E416-28. doi:10.1152/ajpendo.00618.2012

65. Mamer OA, Laschic NS, Scriver CR. Stable isotope dilution assay for branched chain alpha-hydroxy-and alpha-ketoacids: serum concentrations for normal children. *Biomed Environ Mass Spectrom*. Oct 1986;13(10):553-8. doi:10.1002/bms.1200131007

66. Lancaster G, Mamer OA, Scriver CR. Branched-chain alpha-keto acids isolated as oxime derivatives: relationship to the corresponding hydroxy acids and amino acids in maple syrup urine

disease. *Metabolism*. Mar 1974;23(3):257-65. doi:10.1016/0026-0495(74)90064-x

67. Jakobs C, Sweetman L, Nyhan WL. Hydroxy acid metabolites of branched-chain amino acids in amniotic fluid. *Clin Chim Acta*. Jul 16 1984;140 (2):157-66. doi:10.1016/0009-8981(84)90340-1

68. Ehling S, Reddy TM. Direct analysis of leucine and its metabolites β-hydroxy-β-methylbutyric acid, α-ketoisocaproic acid, and α-hydroxyisocaproic acid in human breast milk by liquid chromatography–mass spectrometry. *Journal of agricultural and food chemistry*. Sep 2 2015;63(34):7567-7573. doi:10.1021/acs.jafc.5b02563

69. Ward ME, Politzer IR, Laseter JL, Alam SQ. Gas chromatographic mass spectrometric evaluation of free organic acids in human saliva. *Biomed Mass Spectrom*. Apr 1976;3(2):77-80. doi:10.1002/bms.1200030207

70. Ehling S, Reddy TM. Investigation of the presence of beta-hydroxy-beta-methylbutyric acid and alpha-hydroxyisocaproic acid in bovine whole milk and fermented dairy products by a validated liquid chromatography-mass spectrometry method. *J Agric Food Chem*. Feb 19 2014;62(7):1506-11. doi:10.1021/jf500026s

71. Yamamoto A. STUDIES ON FLAVORS OF SAKE. 2. SEPARATION AND IDENTIFICATION OF HYDROXY CARBONIC ACID. *NIPPON NOGEI KAGAKU KAISHI*. 1961;35(7):619.

72. Van Wyk CJ, Kepner RE, Webb AD. Some Volatile Components of Vitis Vinifera Variety White Riesling. 2. Organic Acids Extracted from Wine. *Journal of Food Science*. 2006;32(6):664-668. doi:10.1111/j.1365-2621.1967.tb00859.x

73. Begemann WJ, Harkes PD, inventors; Process for enhancing a fresh cheese flavour in foods U.S. patent US3853996. 1974b.

74. Smit BA, Engels WJ, Wouters JT, Smit G. Diversity of L-leucine catabolism in various microorganisms involved in dairy fermentations, and identification of the rate-controlling step in the formation of the potent flavour component 3-methylbutanal. *Appl Microbiol Biotechnol*. Apr 2004;64(3):396-402. doi:10.1007/s00253-003-1447-8

75. Tressl R, Kossa T, Renner R, Koeppler H. Gas chromatographic-mass spectrometric investigation of volatile compounds of hops, wort, and beer and their formation. I. Organic acids in wort and beer. *Monatsschrift fuer Brauerei*. 1975; 28(5):109-118. doi:10.1007/BF01274254

76. Broadbent JR, Gummalla S, Hughes JE, Johnson ME, Rankin SA, Drake MA. Overexpression of Lactobacillus casei D-hydroxyisocaproic acid dehydrogenase in cheddar cheese. *Appl Environ Microbiol*. Aug 2004;70(8):4814-20.

77. Axel C, Brosnan B, Zannini E, Furey A, Coffey A, Arendt EK. Antifungal sourdough lactic acid bacteria as biopreservation tool in quinoa and rice bread. *Int J Food Microbiol*. Dec 19 2016;239 :86-94. doi:10.1016/j.ijfoodmicro.2016.05.006

78. Loh LX, Ng DHJ, Toh M, Lu Y, Liu SQ. Targeted and Nontargeted Metabolomics of Amino Acids and Bioactive Metabolites in Probiotic-Fermented Unhopped Beers Using Liquid Chromatography High-Resolution Mass Spectrometry. *J Agric Food Chem*. Nov 24 2021;69 (46):14024-14036. doi:10.1021/acs.jafc.1c03992

79. Mei S, He Z, Zhang J. Identification and analysis of major flavor compounds in radish taproots by widely targeted metabolomics. *Front Nutr*. 2022;9:889407. doi:10.3389/fnut.2022.889407

80. Friedrich M, Murer H, Sterchi E, Berger EG. Transport of L-leucine hydroxy analogue and L-lactate in human small intestinal brush border membrane vesicles. *Eur J Clin Invest*. Feb 1992;22 (2):73-8. doi:10.1111/j.1365-2362.1992.tb01939.x

81. Mann GE, Pearson JD, Sheriff CJ, Toothill VJ. Expression of amino acid transport systems in cultured human umbilical vein endothelial cells. *J Physiol*. Mar 1989;410:325-39. doi:10.1113/jphysiol.1989.sp017535

82. Abumrad NN, Wise KL, Williams PE, Abumrad NA, Lacy WW. Disposal of alpha-ketoisocaproate: roles of liver, gut, and kidneys. *Am J Physiol*. Aug 1982;243(2):E123-31. doi:10.1152/ajpendo.1982.243.2.E123

83. Sumi K, Sakuda M, Munakata K, Nakamura K, Ashida K. alpha-Hydroxyisocaproic Acid Decreases Protein Synthesis but Attenuates TNFalpha/IFNgamma Co-Exposure-Induced Protein Degradation and Myotube Atrophy via Suppression of iNOS and IL-6 in Murine C2C12 Myotube. *Nutrients*. Jul 13 2021;13(7)doi:10.3390/nu13072391

84. Schauder P, Matthaei D, Henning HV, Scheler F, Langenbeck U. Blood levels of branched-chain amino acids and alpha-ketoacids in uremic patients given keto analogues of essential amino acids. *Am J Clin Nutr*. Jul 1980;33(7):1660-6. doi:10.1093/ajcn/33.7.1660

85. Matthews DE, Harkin R, Battezzati A, Brillon DJ. Splanchnic bed utilization of enteral alpha-ketoisocaproate in humans. *Metabolism*. Dec 1999;48(12):1555-63. doi:10.1016/s0026-0495(99)90245-7

86. Selis D, Pande Y, Smoczer C, et al. Cytotoxicity and Genotoxicity of a New Intracanal Medicament, 2-hydroxyisocaproic Acid-An In Vitro Study. *J Endod*. May 2019;45(5):578-583. doi:10.1016/j.joen.2019.01.012

87. Smoczer C, Park YK, Herrington JB, et al. A Potential Intracanal Medicament, 2-Hydroxyisocaproic Acid (HICA): Cytotoxicity, Genotoxicity, and Its Effect on SCAP Differentiation. *Dentistry Journal*. 2023;11(12):270. doi:10.3390/dj11120270

88. Malfait A, Tortorella M. The "elusive DMOAD": Aggrecanase inhibition from laboratory to clinic. *Clin Exp Rheumatol*. 2019;37(Suppl 120):S130-34.

89. Guilak F, Nims RJ, Dicks A, Wu C-L, Meulenbelt I. Osteoarthritis as a disease of the cartilage pericellular matrix. *Matrix Biology*. 2018;71:40-50.

90. Losina E, Thornhill TS, Rome BN, Wright J, Katz JN. The dramatic increase in total knee replacement utilization rates in the United States cannot be fully explained by growth in population size and the obesity epidemic. *J Bone Joint Surg Am*. Feb 1 2012;94(3):201-7. doi:10.2106/JBJS.J.01958

91. Little CB, Hunter DJ. Post-traumatic osteoarthritis: from mouse models to clinical trials. *Nat Rev Rheumatol*. Aug 2013;9(8):485-97. doi:10.1038/nrrheum.2013.72

92. Cinque ME, Dornan GJ, Chahla J, Moatshe G, LaPrade RF. High Rates of Osteoarthritis Develop After Anterior Cruciate Ligament Surgery: An Analysis of 4108 Patients. *Am J Sports Med*. Jul 2018;46(8):2011-2019. doi:10.1177/0363546517730072

93. Oiestad BE, Holm I, Aune AK, et al. Knee function and prevalence of knee osteoarthritis after anterior cruciate ligament reconstruction: a prospective study with 10 to 15 years of follow-up. *Am J Sports Med*. Nov 2010;38(11):2201-10. doi:10.1177/0363546510373876

94. Oiestad BE, Holm I, Engebretsen L, Risberg MA. The association between radiographic knee osteoarthritis and knee symptoms, function and quality of life 10-15 years after anterior cruciate ligament reconstruction. *Br J Sports Med*. Jun 2011;45(7):583-8. doi:10.1136/bjsm.2010.073130

95. Barenius B, Ponzer S, Shalabi A, Bujak R, Norlen L, Eriksson K. Increased risk of osteoarthritis after anterior cruciate ligament reconstruction: a 14-year follow-up study of a randomized controlled trial. *Am J Sports Med*. May 2014;42(5):1049-57. doi:10.1177/0363546514526139

96. Bluteau G, Gouttenoire J, Conrozier T, et al. Differential gene expression analysis in a rabbit model of osteoarthritis induced by anterior cruciate

ligament (ACL) section. *Biorheology*. 2002;39(1-2):247-58.

97. Malemud CJ, Islam N, Haqqi TM. Pathophysiological mechanisms in osteoarthritis lead to novel therapeutic strategies. *Cells Tissues Organs*. 2003;174(1-2):34-48. doi:10.1159/000070573

98. Glasson SS, Askew R, Sheppard B, et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature*. 2005;434(7033):644.

99. Heim-Riether A, Taylor SJ, Liang S, et al. Improving potency and selectivity of a new class of non-Zn-chelating MMP-13 inhibitors. *Bioorg Med Chem Lett*. Sep 15 2009;19(18):5321-4. doi:10.1016/j.bmcl.2009.07.151