

Novel Formulation of THIAA & Berberine Offers Selective Inhibition of Matrix Metalloproteinase Expression within the Extracellular Matrix

SUMMARY

*Matrix metalloproteinases (MMPs) are proteolytic enzymes that have the ability to degrade most components of the extracellular matrix (ECM), which includes the collagens that compose tendons, ligaments, and cartilage.¹⁻⁹ Nutrigenomic research has yielded the discovery of agents with a high degree of predicted safety derived from *Humulus lupulus* (hops) and berberine that help reduce MMP-inducing cytokines and transcription factors.¹⁰⁻¹⁸ These inflammatory mediators can initiate processes that foster greater expression of specific MMPs that lead to cartilage and collagen degradation.^{1,5,7,8,19,26} Addressing precursors to MMP expression through the application of a targeted nutrient combination may help reduce the excess breakdown of the ECM that can lead to ECM-implicated diseases.*

COLLAGEN DEGRADATION

The ECM is the mesh-like extracellular milieu that distinguishes animal tissue and provides support and anchorage for cells. This dynamic structure—composed of glycosaminoglycans, collagen, elastin, chondrocytes, osteoblasts, and more—allows for the body's ability to adapt to gross structural and physiological stressors, such as mechanical loading.³ The ECM also plays a key role in regulating intercellular communication, and is responsible for the circulation of nutrients and the removal of cellular waste products.³

ECM proteins are regulated by a family of more than 20 zinc-binding MMP enzymes that are further subdivided into groups (e.g., gelatinases, collagenases, stromelysins).^{2-4,8,9,19} MMPs are key in the normal turnover activities in ECM components—similar to bone remodeling—and are also networked to inflammatory and immune processes.^{2,4,7,9,27} In lower concentration, MMPs are beneficial to normal growth, tissue repair, and reproduction.^{2-4,7,9,19} But in higher levels of expression, MMPs have been implicated in numerous degenerative pathologies.^{1-7,9,28,29} Elevated MMP expression has also been associated with tendon

pathologies (acute tendon injuries, tendonitis, torn rotator cuffs), degenerative discs, and sites of repeated injury or mechanical strain.³⁰⁻³⁴

Each MMP works on a variety of substances within the ECM, and—as a group—MMPs can collectively degrade all ECM components.^{3,4,7,9,19,20} MMP-13, sometimes referred to as collagenase-3, exhibits the broadest specificity of the collagenases, with the highest activity against type II collagen, the primary collagen found in cartilage, and has been expressed in pathologies associated with excessive ECM degradation.^{3,4,7,19,20} (MMP-13 also degrades types I, III, IV, X, and XIV collagen, along with aggrecan core protein.)^{3,4} MMP-13 plays a role in activation of MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B), which can degrade basement membrane components and are thought to play an important role in final collagen degradation following MMP-13-mediated damage.^{4,7,9}

MMPs are produced by structural cells (fibroblasts, endothelial/epithelial cells) and inflammatory cells (macrophages, lymphocytes, neutrophils, eosinophils).⁷ The following mediators are pivotal in the regulation of MMP production, expression, and activity:

- Proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), oncostatin M (OSM), and interleukin-1 β (IL-1 β)^{3,9,20,24,25,27,35,36}
- Oxidative stress and reactive oxygen species (ROS), such as hydrogen peroxide^{7,8,16,18,37,38}
- Transcription factors, such as nuclear factor- κ B (NF- κ B), activator protein-1 (AP-1), and runt-related transcription factor 2 (Runx-2)^{7,9,17,20,22,23,35,36}
- Mitogen-transducing signal proteins, including protein kinase C (PKC)^{7,9,36}
- Protein kinase B (PKB/Akt) and protein kinase A (PKA)⁹
- Mitogen-activated protein kinase (MAPK) pathways, including p38 MAPK, the Jun kinase

pathway (JNK), and the extracellular signal-regulated kinases 1 and 2 pathway (ERK 1/2)^{4,7-9,20,22,23}

- Phosphatidylinositol 3-kinase signaling pathway (PI3K/Akt)^{9,25,27}
- Poly (ADP-ribose) polymerase (PARP)³⁹⁻⁴¹
- Cyclooxygenase-2 (COX-2) and prostaglandins (PGE₂)^{9,22}
- Elevated homocysteine^{8,21,37}

In addition to the factors above that impact MMP expression, MMPs are capable of inducing the expression of other MMPs (e.g., MMP-13 can activate MMP-2 and vice versa).¹⁹ MMP enzymes also have the ability to produce or modulate precursors to proinflammatory cytokines and transcription factors, thereby contributing to inflammation and further MMP expression.^{4,19} Inhibition of signaling pathways, transcription factors, and associated cytokines has therefore been suggested in a growing amount of scientific evidence as a therapeutic approach to conditions associated with MMP overexpression.^{1-4,6,9,15,17,19,23,25,27,29,31-33,35, 40-43}

NATURAL AGENTS FOR DOWN-REGULATING MMP ENZYMES

The following nutrients and derivatives have been shown to support selective inhibition of collagen-damaging expression of MMPs:

THIAA. In an extensive screen for kinase activity at the MetaProteomics[®] Nutrigenomics Research Center—the proteomics research facility of Metagenics[®], Inc.—tetrahydro iso-alpha acids (THIAA, derived from hops), favorably modulated PKC beta and gamma, two important kinases involved in cell inflammatory processes.¹⁰

Berberine. This plant alkaloid has a long history of use in Ayurvedic and traditional Chinese medicine, as well as in a variety of applications in modern clinical use.^{12,14-18,44,45} Collected research indicates that berberine can down-regulate the activity of MMP-1 (which also acts against type II collagen and activates MMP-2) and MMP-9, as well as modulating the expression or activity associated with ROS, IL-1 β , IL-6, NF- κ B, AP-1, TNF- α , and kinase pathways.¹³⁻¹⁸

Selenium. This essential trace element provides defense against ROS and inflammation, and has also been shown to reduce MMP-2 and MMP-9 expression

through modulation of ROS and NF- κ B, as well as through possible interference with the p38 MAPK pathway.⁴⁵⁻⁴⁷

Zinc. Evidence suggests that zinc supplementation may be effective in reducing spontaneous cytokine release and inflammatory activity. Furthermore, unlike pharmaceutical agents, zinc helps improve immune response rather than suppress it.^{48,49} In a recent study, an increase in circulating zinc (among subjects who started with low or borderline-normal levels) favorably modulated IL-6 and monocyte chemoattractant protein-1 activity (MCP-1, a marker associated with inflammation), as well as natural killer cell activity.⁴⁹

Biotin. Research suggests that the status of this vitamin necessary for cell growth may play a role in inflammatory disease. A deficiency in biotin has been suggested to up-regulate TNF- α production.⁵⁰ One clinical study suggests that biotin supplementation modulates IL-1 β and IL-2 expression.⁵¹

Niacinamide. The role of PARP activity in cellular apoptosis—which can induce MMP expression—is well known.³⁹ Niacinamide has been shown in animal, in vitro, and human studies to modulate PARP expression with a corresponding decrease in activity of transcription factors and cytokines.³⁹⁻⁴¹ Another in vitro study demonstrated the effectiveness of niacinamide in inhibiting IL-1 β -induced cartilage degeneration.⁵²

Folic acid and vitamins B₆ and B₁₂. Interventions with folic acid, alone or in combination with vitamins B₆ and B₁₂, have been shown to reduce hyperhomocysteinemia.^{8,43,53,54} Elevated homocysteine levels—associated with inflammatory conditions and the disturbance of collagen synthesis—show a positive association with elevated MMP-2 and MMP-9 expression, which has corresponding inverse association with B vitamin status.^{7,8,21,43,55} In a 6-week clinical trial with folic acid supplementation, subjects whose elevated homocysteine levels normalized also had a significant reduction in MMP-9 levels.⁴³ Some evidence suggests that homocysteine triggers the ERK 1/2 pathway that regulates MMP-9 expression.⁸

NUTRIGENOMIC TESTING¹⁰

Extrapolating this nutritional research, a strategic combination of these nutrients was proposed with the following objectives:

- Modulate inflammatory processes via:
 - Suppressing inflammatory kinase expression
 - Providing anti-inflammatory (and immune-enhancing) nutrient support
- Modulate demonstrated MMP activity via:
 - Inhibiting MMP-13 in human chondrosarcoma cells
 - Providing nutrient support for homocysteine reduction

Key formula ingredients were tested separately in cell cultures, and then the entire combination as a whole underwent both cell line and clinical testing to evaluate its potential.

THIAA and berberine have both been tested for their effects on MMP-13 expression in human chondrosarcoma cells (SW1353, a fibroblast-like cell line) at MetaProteomics. Both compounds—tested individually and together—were able to decrease in a dose-dependent manner both TNF- α - and IL-1 β -induced MMP-13 expression in SW1353 cells. Furthermore, a combination formula (containing a 1:1 ratio of THIAA to berberine, along with biotin, folic acid, niacinamide, selenium, zinc, and vitamins B₆ and B₁₂) was also found to reduce TNF- α - and IL-1 β -induced MMP-13 expression in a dose-dependent manner (Figures 1 and 2).

These cell studies indicate that the formula contains potent actives to support the ECM by reducing activity of MMP-13, one of the main MMPs found in cartilage.

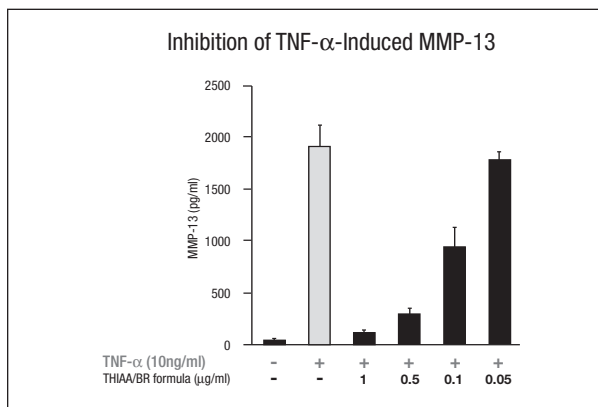


Figure 1. THIAA/berberine formula inhibits TNF- α -induced MMP-13 expression in SW1353 cells.

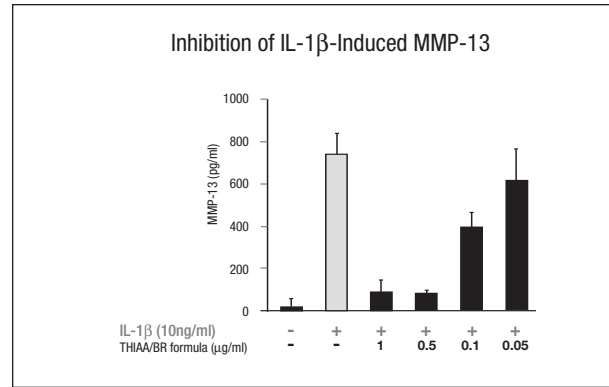


Figure 2. THIAA/berberine formula inhibits IL-1 β -induced MMP-13 expression in SW1353 cells.

CLINICAL OBSERVATIONS¹¹

Recently, the Functional Medicine Research CenterSM—the clinical research arm of Metagenics—measured the efficacy of the THIAA/berberine combination formula in a small, open label, case study series that was conducted with offsite practitioners who were asked to select subjects with a selected clinical history and exam:

- Patients for whom bodywork had only been of brief help, previously requiring repeated adjustments
- Patients with active inflammatory challenges, including chronic and acute pain states
- Patients with poor tissue integrity secondary to chronicity of symptoms, fibrosis (fibromyalgia), and hypothyroidism

Subjects (n=12) took 2 tablets of the THIAA/berberine formula 1 hour before bodywork, and then 1 to 2 tablets as needed 3 times daily up to 10 tablets per day. Questionnaires were administered at baseline prior to bodywork and administration of formula, and at specific time points: immediately after bodywork and then 1 hour, 6 hours, 24 hours, and 7 days afterward. Subjects were asked to score the severity of their pain and lack of flexibility using Likert psychometric scales of 1-10. On the pain scale, a score of 10 represented the highest level of pain. On the flexibility scale, a score of 1 represented the least level of flexibility.

Pain scores over the course of 7 days were dramatically decreased (range: 2-4) relative to the baseline median score of 7. The median improvement in pain averaged 71% immediately after treatment

and 70% for 24 hours after bodywork and formula administration. The median flexibility score of 3 at baseline improved immediately after treatment to a score of 7, and was maintained at nearly that level 24 hours later. One week later, the median score for pain remained at a 43% reduction from initial score, suggesting lasting benefits from the combination of bodywork and the continuation of nutritional supplementation. (Figures 3-5.)

Overall tolerance of the product was good. Two subjects noted some gastrointestinal (GI) discomfort after taking the product on an empty stomach, which was addressed by taking the tablets with food. One subject had more persistent GI discomfort, including a presumed episode of gastrointestinal reflux disease.

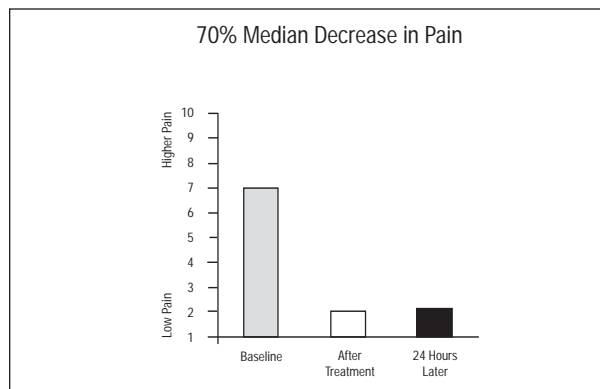


Figure 3. Subjects were reported to have a median decrease in pain of 71% immediately after treatment and 70% after 24 hours.

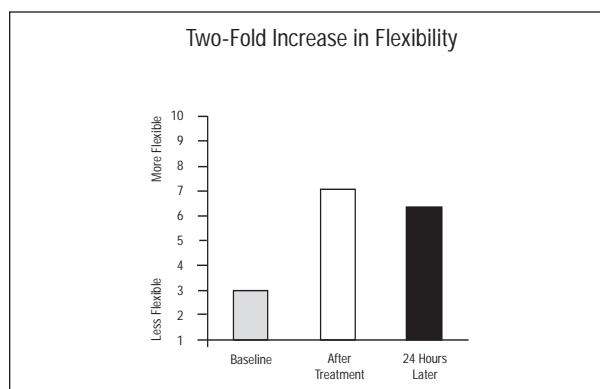


Figure 4. Participants had a median increase on flexibility of over 230% immediately following treatment, which was maintained at nearly that level 24 hours after bodywork with continued supplementation.

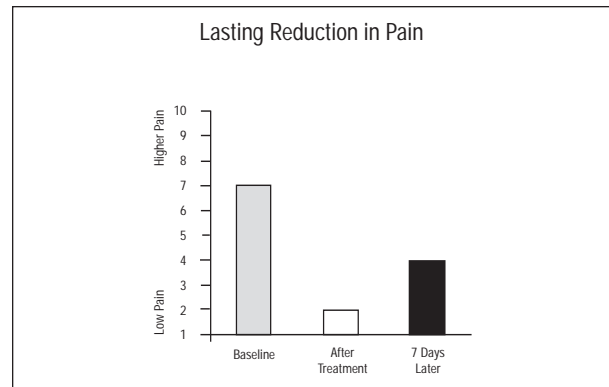


Figure 5. After 1 week, subjects reported a median reduction in pain of 43% following initial bodywork and continued supplementation.

CONCLUSION

The collective results from cell line testing and clinical observations suggest that the combination THIAA/berberine formula may offer nutritional support to help modulate cartilage-damaging MMP expression and provide a complement to bodywork to produce noticeable changes in pain and flexibility. This formula may serve as a potential adjunctive approach to therapies specific to the ECM (chiropractic/osteopathic adjustments), as well as inflammatory conditions associated with cartilage degradation, to help enhance mobility.

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