**Use of Undenatured Type II Collagen in the Treatment of Rheumatoid Arthritis**

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**ABSTRACT:** Rheumatoid arthritis is a debilitating chronic disease that lacks an effective treatment. It is the leading cause of disability in the United States. Rheumatoid arthritis is an inflammatory response believed to involve T cells reacting to an antigen within the joints and articular cartilage. Over-the-counter pain relievers and anti-inflammatory medications, such as aspirin, acetaminophen, and ibuprofen, are commonly used for preventive measures, but these products only treat the symptoms, not the cause, and may also produce serious side effects. A growing body of evidence indicates that type II collagen is a major structural protein responsible for tensile strength and toughness in the cartilage and also a potential autoantigen in people who have rheumatoid arthritis. If the activity of T cells that release joint-destroying factors could be reduced, outcomes for patients with rheumatoid arthritis could be improved. One method of achieving this is termed oral tolerance, a concept that is proving useful in the treatment of autoimmune diseases. Oral tolerance describes a state of immune hyporesponsiveness following the oral ingestion of a protein. It is, therefore, a method by which a peripheral immune tolerance (down regulation) to a particular antigen may be induced by presenting specific amounts of that antigen to the gastrointestinal system. Several clinical studies have demonstrated the effectiveness and usefulness of undenatured collagen II in ameliorating the symptoms of rheumatoid arthritis with no serious adverse effects. Thus its administration may demonstrate therapeutic efficacy by inducing oral tolerance for the treatment of this disease.

**Introduction**

Arthritis is one of the most prevalent chronic health problems in the United States, affecting nearly 43 million people. Although it is often thought of as a disease that predominantly affects the elderly, it is the number 1 cause of disability affecting those over the age of 15. In fact, more than half of those affected by arthritis are under the age of 65, and almost 300,000 of those affected are children. Each year arthritis is responsible for 44 million outpatient visits and almost 1 million hospitalizations, and it is second only to heart disease in terms of its effect on days lost from work. As might be imagined from these statistics, the toll that arthritis takes on the healthcare industry is substantial, costing the United States approximately $65 billion each year in health-related expenses. Unfortunately, the incidence of arthritis does not appear to be decreasing, and by the year 2020 the Centers for Disease Control (CDC) predicts that almost 60 million Americans will suffer from some form of this disease.

While the term arthritis may bring to mind a simple condition characterized by painful joints and difficulty performing certain tasks, it actually encompasses more than 100 different diseases. Of these different forms of arthritis, osteoarthritis (OA) and rheumatoid arthritis (RA) are the most common.

**Osteoarthritis.** OA currently affects 20 million Americans. It is a degenerative joint disease in which the cartilage covering the ends of bones deteriorates, resulting in pain, stiffness, and loss of movement. This form of arthritis generally begins after the age of 40 and develops slowly over many years. People usually report pain beginning in joints on only one side of the body, in contrast to RA. While inflammation may be present, joint pain in OA is typically not accompanied by the amount or severity of inflammation observed in those with RA. Weight-bearing joints such as the knee and hip tend to be more affected than non-weight-bearing joints such as the elbow or shoulder. The general feeling of sickness that can accompany other forms of arthritis does not usually accompany OA.

**Rheumatoid Arthritis.** RA is not a "new" disease. One of the first descriptions of a disease resembling RA can be found in the Charaka Samhita, a medical text from India that dates back as far as 500 BC. Another ancient reference to the disease dates to 100 BC: the Roman Scribonius Largus described a polyarthritic condition occurring mainly in elderly women that closely resembled what we now understand to be RA. Rheumatoid arthritis currently affects approximately 2 million Americans and about 1% of the world's population. However, the pathology and progression of RA is somewhat different from that of OA. It often develops...
suddenly, within weeks or months, and generally begins between the ages of 25 and 50. Non-weight-bearing joints such as the hands, shoulders, and elbows are usually affected bilaterally, and a significant amount of redness, tenderness, swelling, and inflammation is often present. RA often results in a feeling of sickness and fatigue and may be accompanied by weight loss as well as fever. Morning joint stiffness lasting for an hour or longer is relatively common. Subcutaneous nodules that form over bones may also be present. Interestingly, 3 times as many women as men are afflicted with rheumatoid arthritis. Some patients will experience a monophasic course of the disease that may abate within 2 years, while others will experience a polyclonal, or progressive, course. Of all the forms of arthritis, RA tends to be one of the most serious and disabling; 16% of those who have had the disease for 12 or more years become completely disabled. Lifespan has been shown to be shortened by approximately 7 years in men and 3 years in women, which is equivalent to the increased mortality observed in those with diabetes and Hodgkin’s disease.

**Etiology**

While RA is classified as an autoimmune disease, the exact causes that result in its development remain a mystery. What is known is that many different and complex factors are involved. While some cases of OA may be the result of years of “wear and tear” on joint structures, other forms can be traced to an injury, infection, or metabolic disorder. RA, however, does not result from overuse of a joint or from injury, but rather from an autoimmune problem in which the body attacks and damages its own tissue. The damage that occurs in RA appears to be propagated by cytokines secreted by T cells in response to certain autoantigenic stimuli within the joint. Various immunological factors are involved, including but not limited to CD4-inducer lymphocytes, CD4 memory cells, macrophages, neutrophils, and tumor necrosis factor. One of the most likely candidates for this autoantigenic stimulus is the collagen component of cartilage, specifically type II collagen. Some researchers believe that an infection may trigger the initial inflammation in a joint through molecular mimicry or other mechanisms, which in turn initiates the autoimmune response. Genetics may also play a role, as may other factors, including stress.

The cartilage in joints allows for flexibility and motion and provides a cushion against the impact of various forces on the bone. The detailed structure of cartilage is complex but can be simplified into 2 major components: collagen and proteoglycans. Proteoglycans are large protein molecules attached to large carbohydrate chains called glycosaminoglycans. These proteoglycans help to provide a matrix in which the collagen as well as water can coexist. There are a number of different types of collagen, but type II accounts for the major part of cartilage. It is composed of 3 identical chains (termed α-1 chains) that form a triple helix. This interconnected network of collagen and proteoglycans is crucial in maintaining joint flexibility and resistance to stress and fracture. In RA, autoantigenic responses, most likely triggered by type II collagen, ultimately result in the progressive inflammation, pain, and destruction characteristic of this disease.

Regardless of the initial cause, a progressive degeneration of the structure and function of the joint takes place, making normal activities of life increasingly difficult. In the case of RA, the body is unable to recognize its collagen as normal and in turn attacks it as if it were a foreign invader. A novel approach to treatment, termed oral tolerance, in which small amounts of type II collagen are presented to the gastrointestinal tract, has been the focus of significant positive scientific research. What is achieved is a downregulation of the body’s ability to destroy its own collagen, resulting in improvement in symptoms and slowing the progression of the disease. However, to fully appreciate the use of oral tolerance in the treatment of RA, it is important to understand the typical treatment options currently in use.

**Current Treatment Options**

The treatment options for those with rheumatoid arthritis are typically nonsteroidal anti-inflammatory drugs (NSAIDs), alone or in combination with what are known as disease-modifying antirheumatic drugs (DMARDs). As is well known, chronic use of NSAIDs, especially in the elderly, is linked to numerous side effects, including gastrointestinal bleeding and renal malfunction. Even the newer generation of COX-II inhibitors such as rofecoxib (a furanone derivative), celecoxib (a 1,5-diaryl substituted pyrazole), and infliximab (a monoclonal antibody) are not without their own problems. While these drugs do reduce inflammation, they do not address the underlying causes of the arthritis and therefore cannot alter the progression of the disease. Furthermore, rofecoxib and celecoxib have been contraindicated for use by patients suffering from hypersensitivity, asthma, urticaria, or allergic reactions. All of them can cause bleeding, ulceration, perforation of the stomach and intestines, and anaphylactoid reactions. In addition, rofecoxib has recently been associated with a possible increased risk of heart attack and stroke. Prolonged use of these newer COX-II inhibitors in the elderly can result in side effects similar to those seen with traditional NSAIDs. DMARDs attempt to address the underlying pathology of RA more thoroughly by slowing
the progression of the pathology. One of the components of this disease, in addition to inflammation, is microvascular injury coupled with the formation of new capillaries. Many of the DMARDs attempt to inhibit the formation of new capillaries as well as address the underlying inflammation. This category of drugs includes azathioprine, corticosteroids, gold, hydroxychloroquine, methotrexate, sulfasalazine, and a number of newer medications such as leflunomide and etanercept. While these medications have been shown to offer clinical improvement to those with rheumatoid arthritis, they can also be associated with significant toxicity and side effects, including myelosuppression, lymphoproliferative disorders, macular damage, thrombocytopenia, osteoporosis, hyperglycemia, and hepatotoxicity. Another factor that should be taken into account is cost: the monthly cost for etanercept and infliximab, both of which must be injected, can be more than $1000. Also, according to a recent report by the FDA, Remicade (infliximab) has been associated with tuberculosis infection, nerve damage, and risk of cancer lymphoma in patients. In severe cases of joint damage, surgery is often necessary, but this is also costly and involves a lengthy recovery time.

Methylsulfonylmethane, chondroitin, and glucosamine, widely used for treatment of OA, are known to help rebuild proteoglycans and reduce inflammation but are unable to help in the process of inactivating "killer" T cells to ameliorate rheumatoid arthritis.

Oral Tolerance

When the immune system is functioning properly, it recognizes and identifies foreign substances in order to help eliminate them from the body. One type of immune cell that is particularly important in this process is the T cell, which can be classified in a number of different categories, depending on function. "Helper" T cells have the function of releasing factors that help increase or decrease the immune response. These have been further classified into Th-1 and Th-2 subsets. Th-1 cells amplify proinflammatory responses; Th-2 cells limit such responses. "Killer" T cells attack and destroy antigens. The B cell is also crucial to the functioning of the immune system, as it is responsible for the production of antibodies. In a normal individual, the immune system does not seek out and destroy healthy tissue due in part to the fact that T cells that have specificity for antigens on normal tissue are either suppressed or destroyed prior to being released into circulation. In the case of rheumatoid arthritis, however, T cells with self-antigens for type II collagen are not properly destroyed or suppressed, resulting in the damage that is a characteristic hallmark of this disease.

By decreasing the activity of T cells that are releasing joint-destroying factors, the outcome for patients with RA can be improved, and one such method to achieve this is oral tolerance. The concept of oral tolerance has existed since 1911, and traditional medical literature is filled with papers describing this mechanism and how it might benefit those with autoimmune diseases.

Recent studies have shown that small doses of type II collagen derived from chicken cartilage produce oral tolerance and work with the immune system to prevent the body from attacking its joints.

Oral tolerance can be induced by 2 major mechanisms, bystander suppression and clonal anergy, depending on the dose of antigen that is presented. Throughout the small intestine, there are patches of gut-associated lymphoid tissue (GALT). Within the GALT can be found tissue that consists of nodules (Peyer's patches) that contain organized assemblages of T and B lymphocytes, macrophages, and dendritic cells and are the primary area within the gastrointestinal tract where immune responses are generated. This immune tissue is designed to protect the host from ingesting pathogens as well as to prevent the host from reacting to ingested proteins. In fact, scientists have attempted to use the GALT as a route for administering vaccines but have been deterred by systemic hyporesponsiveness. Nonetheless, the generation of immune responses within the GALT is the primary mechanism by which orally ingested proteins can suppress systemic immunity.

Bystander Suppression

This form of oral tolerance is achieved by presenting small amounts of antigen to the GALT, which in turn generates a T-cell response. After the antigen (in this case, type II collagen) is consumed, regulatory Th2 and Th3 cells migrate from the GALT through the lymphatic system and then into peripheral circulation. When they encounter an antigen similar to that which was ingested, they secrete cytokines, including transforming growth factor-beta, interleukin-4, and interleukin-10, that result in the down regulation of activated helper Th1 cells. It is these activated helper T cells that are, in part, involved in producing the inflammation and destruction of collagen in RA. If this activity against healthy collagen can be decreased, the progression of the disease can be altered. It should be noted that the oral antigen does not need to enter the systemic circulation in order to induce a response, as the regulatory T cells are induced as a result of the interaction between the antigen and the GALT.

Clonal Anergy

Another mechanism by which an orally administered protein can induce a down regulation of an immune response is via a mechanism called clonal anergy. This
situation results from the ingestion of high doses of an antigen, which in turn induces a state of unresponsiveness from overactive Th1 cells. These cells are not deleted but are rendered incapable of responding to a specific antigen. In essence, they are turned off, or “anergized,” and will no longer recognize the antigen as a target for destruction.

Clinical Studies

Via the mechanism of oral tolerance, type II collagen has been studied for its ability to benefit those with RA. This makes sense, because type II collagen is the most abundant structural protein present in cartilage. Numerous animal models of arthritis have demonstrated significant benefit from orally administered, native (undenatured) type II collagen. Its administration has been able to suppress almost all experimentally inducible forms of RA in animals, including antigen-induced arthritis, adjuvant arthritis, type II collagen-induced arthritis, streptococcal cell-wall arthritis, and silicone-induced arthritis. These impressive results led to the investigation of native type II collagen supplementation in humans with RA. In 1993, an open-label pilot trial and a phase II trial in humans were conducted at Harvard Medical School. In the pilot trial, 10 patients diagnosed with RA had their immunosuppressive and disease-modifying drugs discontinued and were given 0.1 mg of native type II collagen daily for 1 month, followed by 0.5 mg of native type II collagen for the next 2 months. Six of the 10 subjects experienced a significant improvement (defined as >50% compared with baseline) in swollen and tender joint counts, as well as morning stiffness, 50-foot walk time, grip strength time, and erythrocyte sedimentation rate. One subject who had previously been treated with methotrexate experienced complete remission, which continued for 26 months. No adverse effects were noted. Because of these observed improvements, a placebo-controlled phase II follow-up trial was performed consisting of 60 subjects with severe, active RA. Participants were randomly assigned to groups taking either a placebo or a daily dose of 0.1 mg native type II collagen for 1 month, then 0.5 mg for 2 months. At 1, 2, and 3 months, the collagen group experienced significant improvement ($P < 0.05$) in the number of swollen joints, the number of painful and tender joints ($P = 0.06$ at 2 months), and 50-foot walk time. Four patients in the collagen group, compared with no patients in the placebo group, experienced complete remission of the disease. One of the most notable findings was the lack of side effects as a result of the treatment, an important issue given the side effects that can be present with various DMARDs and NSAIDs. Importantly, a recent independent report has also confirmed the effectiveness of type II collagen in juvenile RA, a disease affecting almost 300,000 children. Ten patients between the ages of 8 and 14 years who had active RA were treated orally with type II collagen for 3 months. Eight of the 10 patients had a reduction in both swollen and tender joints at the end of 3 months. One patient in this study also achieved complete remission. It was concluded that oral treatment with native type II collagen may be a safe and effective form of treatment for juvenile RA.

A fourth study of native type II collagen supplementation in RA reported significant improvement in subjects who met Paulus criteria (morning stiffness, joint tenderness, joint swelling, and erythrocyte sedimentation rate). After 24 weeks, 39% of those taking type II collagen versus 19% taking placebo experienced significant improvement. While 19% may appear to be a large response in the placebo group, it is not unusual to observe this type of response in studies of arthritis. The impressive finding was the high degree of improvement in the group treated with undenatured, type II collagen as compared to the group taking the placebo. An interesting observation in this study was that subjects with a presence of serum IgA and IgG antibodies to collagen at the beginning of the study had a significantly better response to treatment than those lacking such antibodies.

In a fifth double-blind, placebo-controlled study performed in Germany, 90 subjects with early RA were divided into groups receiving daily doses of 1 mg collagen, 10 mg collagen, or placebo. At the end of the study, 3 patients in the 10 mg group, 1 patient in the 1 mg group, and no patients in the placebo group had experienced marked improvement. While these results may not appear very impressive, the authors were surprised by the degree of benefit given the small subset of patients. In another German study, daily doses of 1 mg or 10 mg of undenatured type II collagen resulted in reduced type II collagen antibody titres in patients showing a clinical response. This study also suggested that 10 mg was a more effective dose than 1 mg. These studies provide the basis and rationale for the use of native type II collagen as a safe and effective modality of treatment for those suffering from RA.

The Importance of a Native (Undenatured) Form of Collagen

To confer oral tolerance, type II collagen must be used in its undenatured, 3-dimensional, triple-helical structure. Unfortunately, most products on the market containing type II collagen do not contain the undenatured form. In these products it has undergone harsh chemical or high-temperature manufacturing procedures which denature it, thus rendering it inactive and incapable of eliciting an immune response once administered. In fact, no peer-reviewed studies exist to support
the use of denatured type II collagen in RA, and one study has shown denatured type II collagen to have no impact on the severity of the disease.\textsuperscript{29} In order to insure that undenatured type II collagen is present, highly sensitive ELISA assays must be performed to confirm that the collagen is biologically active.

**Source of Undenatured Type II Collagen**

It is well understood that type II collagen can be obtained from all types of animals, including mice, rats, chickens, pigs, and dogs, as well as from humans. However, an ideal commercial source would be to obtain it in a cost-effective way from animals housed and maintained in a germ-free environment. Chickens raised in a controlled environment with ambient temperature and purified air, free from bacteria, viruses, fungi, and other microorganisms, are currently the best source of commercial undenatured type II collagen.

**Dose**

Clinical studies support the use of native, undenatured type II collagen and recommend that it be taken with water at bedtime. Furthermore, studies have shown that small doses (typically 10 mg or less) derived from chicken cartilage work with the human immune system to promote healthy joints and improve mobility and flexibility, as well as attenuating the symptoms of RA. The ideal situation is to ingest undenatured collagen II on an empty stomach when the acid content in the stomach is low. Generally, protein absorption in a human body may take from 4 to 8 hours.

**Potential Use of Undenatured Type II Collagen in Osteoarthritis**

Therapeutic interventions that work rapidly for RA, such as NSAIDs or cortisone injections, are also palliative for OA. Unfortunately, drugs that work rapidly for OA do not, in general, provide sufficient reduction of inflammation or pain relief on their own in rheumatoid arthritis. OA therapies in this category include NSAIDs, hylan g-f 20, and most probably glucosamine and chondroitin sulfate. Presumably, this dichotomy relates to the much more substantial degree of inflammation present in RA versus OA.

OA is a wear and tear phenomenon usually associated with aging; the disease progresses with rigorous exercise when muscles and bones are already weakened due to aging (exercise also causes muscle and bone damage in aged patients with RA). It is also characterized by an inflammatory synovial response that leads to joint wear and tear.\textsuperscript{28} As RA will effectively cause gradual deterioration and inflammation of certain joints due to immune disorders, OA will cause wear and tear due to the normal aging process and an increase in enzymatic activity. In the absence of significant and disfiguring inflammation (which is characteristic of rheumatoid arthritis), wear and tear activity may be misdiagnosed as OA rather than RA and treated accordingly. In some cases, OA is added as an additional diagnosis simply because wear and tear persist and exists normally. The biochemical markers associated with OA inflammation, such as various cytokines (interleukin-4 and interleukin-10), tumor necrosis factor-alpha, and interferons are also associated with RA inflammation.\textsuperscript{22,30} Therefore, therapies used to treat OA inflammation are also used to treat severe OA inflammation. Earlier research demonstrates that type II collagen suppresses T-cell-mediated inflammation, which is characterized by cytokines interleukin-4 and interleukin-10 and is seen in the synoviums of both OA and RA patients. Another benefit of type II collagen is that it contains small amounts of glucosamine and chondroitin, which are good for joint mobility and flexibility. In light of these facts, it may be postulated that undenatured type II collagen may also provide benefit to a significant population of OA patients as well as those with RA.

**Conclusion**

Finding an effective cure for RA is a major challenge for health professionals. Over-the-counter pain relievers, NSAIDs and other anti-inflammatory drugs, and monoclonal antibody and COX-II inhibitors have major adverse side effects, including liver disease, gastritis, vomiting, cardiovascular dysfunctions, and, possibly, tuberculosis. Furthermore, infliximab, rofecoxib, and celecoxib are very expensive drugs for regular use. Another expensive alternative is surgery, which involves a long recovery time. Several human clinical trials have demonstrated the effectiveness and usefulness of undenatured type II collagen in significantly reducing the painful symptoms of RA with no adverse side effects.

**References**

8. Trentham DE, Dynesius-Trentham RA, Orav EJ, et al. Effects of oral admin-