A PILOT TRIAL OF ORAL TYPE II COLLAGEN IN THE TREATMENT OF JUVENILE RHEUMATOID ARTHRITIS

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Objective. To evaluate the efficacy of oral chicken type II collagen (CCII) in the treatment of juvenile rheumatoid arthritis (JRA).

Methods. Ten patients with active JRA were treated with CCII for 12 weeks. Efficacy parameters, which included swollen and tender joint count and score, grip strength, 50-foot walking time, duration of morning stiffness, and patient and physician global scores of disease severity, were assessed monthly.

Results. All patients completed the full course of therapy. Eight patients had reductions in both swollen and tender joint counts after 3 months of CCII. The mean changes from baseline in swollen and tender joint counts for the 8 responders at the end of the study were $-61\%$ and $-54\%$, respectively. Mean values for other efficacy parameters also showed improvement from baseline. There were no adverse events that were considered to be treatment related.

Conclusion. Oral CCII may be a safe and effective therapy for JRA, and its use in this disease warrants further investigation.

Juvenile rheumatoid arthritis (JRA) affects an estimated 65,000-70,000 children in the US (1). While it has been suggested that JRA has a better prognosis than adult rheumatoid arthritis (RA) (2), more recent data show that $\sim45\%$ of children with JRA have active disease at 10-year followup (3).

Current treatment options for JRA are often unsatisfactory, because of both limited efficacy and concern about toxicity. These include antiinflammatory agents such as aspirin, naproxen, tolfenamic acid, ibuprofen, antimalarial agents, gold, and methotrexate, as well as physical therapy. In a minority of patients, rapidly progressive disease is refractory to these therapies and leads to permanent joint destruction with physical incapacitation. Systemic corticosteroids are relatively contraindicated in the treatment of JRA, except in patients with severe polyarthritis or severe systemic disease that has failed to respond to more conservative treatment. In addition to multiple other toxicities, growth suppression is a major deterrent to the use of steroids in the treatment of JRA. A multicenter study of D-penicillamine and hydroxychloroquine in the treatment of severe JRA showed that, when given in conjunction with a nonsteroidal antiinflammatory drug (NSAID), neither agent was superior to placebo (4). Methotrexate has been shown to be an effective treatment of refractory JRA (5), but parents and physicians alike remain concerned about possible long-term side effects. The toxic-to-therapeutic ratio of cytotoxic agents, such as cyclophosphamide, is even more narrow. Moreover, reports of malignancy either during or after therapy with immunosuppressive drugs have precluded their use in all but the most severely ill patients.

The evidence that sensitized T cells participate in provoking inflammation in RA and other rheumatic diseases (6) provides direction to the search for treatment modalities based on specific immunosuppression, which would be both highly effective and minimally toxic. The ability to induce antigen-specific peripheral immune tolerance by oral administration of antigens has been recognized for some time (7). It is presumed that the physiologic interaction of proteins with the gut immune system has evolved to prevent systemic immune responses to ingested proteins. Hypersensitivity reactions to food proteins are rare, and the mechanism of oral tolerance is based on this unique immunologic system. Given in low doses,
orally administered antigens induce active immunosuppression, whereas high antigen doses lead to clonal anergy (8).

Oral administration of type II collagen has been shown to ameliorate arthritis in two animal models of RA, one induced by immunization with type II collagen (9,10) and the other induced by Freund's complete adjuvant (11). In addition, a placebo-controlled, phase II study in 60 adults with severe, active RA demonstrated significant ($P < 0.03$) improvement in tender and swollen joint counts after 3 months of treatment (12). A multicenter, double-blind, dose-ranging study of oral chicken type II collagen (CCII) in adult RA has recently been completed (Barnett ML et al: manuscript in preparation). The present open study of oral CCII in the treatment of JRA was undertaken based on these earlier results.

**PATIENTS AND METHODS**

A total of 10 patients with JRA were enrolled in the study. To be eligible, patients had to meet the American College of Rheumatology criteria for JRA (13). In addition, patients had to be between the ages of 8 and 14 years and had to have active arthritis, as defined by the presence of $\geq 3$ swollen joints and $\geq 6$ tender joints. Patients with any onset subtype were eligible provided that they had the required number of inflamed joints at the time of enrollment. Thus, a patient who had involvement of $\leq 4$ joints within the first 6 months of disease (and who would therefore be classified as having pauciarticular onset) would nonetheless be eligible for enrollment in this study provided there were $\geq 3$ swollen and $\geq 6$ tender joints at the time of study entry. Patients were excluded if they were unable to discontinue treatment with disease-modifying antirheumatic drugs (DMARDs), if they had structural damage to the joints that was not considered to be amenable to physical rehabilitation if inflammation were to subside, or if they had serious concurrent medical problems.

During the course of the trial, patients were permitted to continue treatment with NSAIDs or low-dose corticosteroids (no more than the equivalent of 10 mg prednisone/day), provided that the doses remained stable during the treatment period. Increases in NSAID or prednisone dosage or initiation of any other antirheumatic therapy represented protocol violations. Patients were required to discontinue DMARDs at the start of the trial, with no mandated washout period.

Patients who met all entry criteria were enrolled and began treatment with CCII for a 3-month period. All patients and their parents were required to sign an informed consent form detailing protocol procedures, possible risks and benefits, etc. Treatment consisted of 100 $\mu$g/day of CCII for the first month and 500 $\mu$g/day thereafter. CCII was provided as a liquid suspension in 0.1M acetic acid at 4°C and added to cold orange juice immediately prior to ingestion. Doses and technique were the same as those used in the previous trial in adults (12). Patients were required to return for monthly visits, at which time safety and efficacy measurements were obtained. Patients who exhibited an initial positive response but subsequent worsening after the initial 3-month treatment period were considered for further treatment with the study medication, on a case-by-case basis.

Clinical efficacy was assessed by ascertaining painful and swollen joint counts and joint scores according to the method of Weinblatt et al (14), evaluating a total of 54 diarthrodial joints for pain/tenderness and 52 joints for swelling, duration of morning stiffness, grip strength, 50-foot walking time, and patient/parent and physician global scores of disease activity at each visit. Laboratory data, including complete blood cell count (CBC), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) level, and serum IgG antibodies to type II collagen (12), were recorded at baseline and after 3 months of therapy.

**RESULTS**

All 10 patients who enrolled and began study medication completed the full 3 months of treatment. There were 5 girls and 5 boys, with a mean age of 10.9 years and a mean disease duration of 4.3 years. The disease onset type was polyarticular in 3 patients, pauciarticular in 3 patients, and systemic in 4 patients. Four patients had previously been treated with DMARDs, and 1 had been treated by his parents with a variety of herbal medications. Patient 6 discontinued azathioprine 1 day prior to beginning therapy with CCII, but no other patients were taking DMARDs at the time of enrollment. Six of the 10 patients received concomitant stable doses of NSAIDs and/or low-dose prednisone during the study period (along with acetaminophen in 1); 1 patient continued to take acetaminophen, and 3 patients took no concomitant medications for their arthritis. Eight of the 10 patients were in Steinbrocker functional class II (15) at study entry, and the remaining 2 patients (patients 2 and 9) were in class III. HLA typing was not performed. Demographic and clinical features of the patients are presented in Table 1.

Eight patients had reductions in both swollen and tender joint counts after receiving CCII for 3 months. The mean changes from baseline in swollen and tender joint counts for the 8 responders at the end of the study were $-61\%$ and $-54\%$, respectively. Six patients had $>33\%$ reduction in both swollen and tender joint counts. Individual patient values for swollen and tender joint counts at baseline and after 3 months of therapy are shown in Figure 1. The time to onset of response for the 10 patients was variable. In patient 1, almost all of the improvement was achieved within 1 month of the initiation of treatment, but the
response occurred more slowly in other patients. On average for all 10 patients, the percentage of total improvement in swollen and tender joint counts achieved after only 1 month of treatment was 35% and 49%, respectively.

Swollen and tender joint scores decreased from baseline in 9 of the 10 patients. The mean reductions for all 10 subjects in swollen and tender joint scores after 3 months of therapy were 43% and 51%, respectively. These results are shown in Figure 2.

Mean values for morning stiffness and 50-foot walking time showed improvement from baseline. Clinical efficacy results for these parameters are presented in Figure 3. Although grip strength is not considered to be a reliable measure in children, mean values in right and left grip strength for the 10 patients did show a slight improvement from baseline to 3 months (data not shown). In addition, mean patient and physician global assessment scores also improved compared with baseline. One patient (patient 4) had total resolution of arthritis by the end of treatment and has subsequently been able to discontinue all medications with no return of symptoms during a 14-month followup period. No significant trends in any hematologic parameters, including CBC and ESR, were noted during the study. None of the patients tested positive for RF or collagen antibodies prior to or on completion of treatment.

CCII was well tolerated. Mild, transient skin rashes were noted in 4 patients during the study; in 3, the rash did not seem to be related to the study medication, and in no instance did the rash prompt
interruption of therapy. In 1 patient, an erythematous, pruritic rash was present on the legs at the time of study entry. This rash appeared to worsen during the first month of the trial, but it then resolved without specific therapy while the patient continued to take CCII. Two other patients reported transient erythematous rashes (not observed by the investigator) which were believed to be related to new soap or new laundry detergent. Facial flushing, which occurred 20 minutes after ingestion of CCII and lasted 1–2 hours, was noted by 1 patient during the initial 2 weeks of treatment, but subsequently resolved spontaneously.

Patient 6 had a history of chronic hepatitis C at the time of study entry. During the second month of the trial period, the findings on routine blood tests performed by his personal physician were notable for elevated transaminase levels. His only symptom at that time was an increase in fatigue. One week later, when his transaminase levels were found to have risen further, he underwent a liver biopsy. This revealed mild chronic active hepatitis similar to that exhibited on a previous biopsy performed in 1991, and it was decided that his dosage of oral corticosteroids should be increased. Repeat liver function tests (LFTs) performed the day prior to the initiation of high-dose prednisone treatment demonstrated spontaneous improvement in his transaminase values to <50% of their peak levels, but this test result became available only after the patient had taken one 20-mg dose of prednisone. The patient discontinued high-dose prednisone after this single dose, and his LFT findings returned to normal within 1 week and subsequently remained

Figure 2. Swollen and tender joint scores for individual patients. Swollen (A) and tender (B) joint scores for each individual patient at baseline and after 3 months of treatment with chicken type II collagen are shown.

Figure 3. Secondary efficacy parameters (A, morning stiffness; B, 50-foot walking time) at baseline and after 3 months of treatment with chicken type II collagen. Individual patient numbers are shown on the graphs next to their respective plot markers.
normal for the duration of the study. At no time during this period was his CCII therapy interrupted, and this transient rise in LFT values was not believed to be related to the study medication. Of note, since the conclusion of the trial, the patient has had another similar episode of transient transaminitis while not taking CCII.

After conclusion of the study protocol, a second 3-month course of CCII was requested for and provided to 4 patients (patients 1, 2, 7, and 8). Patient 4 was examined 14 months after study completion, at which time it was confirmed that she remained completely free of any symptoms of arthritis with no medications, had no tender or swollen joints, and had normal laboratory values.

DISCUSSION

Oral tolerization is a well-recognized phenomenon in which the oral administration of antigen induces peripheral immune tolerance to the fed antigen (7). The utility of oral tolerization as a treatment modality for a variety of autoimmune diseases, including RA (12), multiple sclerosis (16), type 1 diabetes mellitus (17), and uveitis (18), is currently under active investigation. To date, no significant adverse events have been noted in any animal or human study of oral tolerance, and the simplicity and apparent safety of this form of treatment make it extremely appealing in these chronic, disabling diseases.

Based on results of animal studies, the mechanism responsible for oral tolerance varies depending on the dose of fed antigen, with low doses inducing active suppression and high doses resulting in clonal anergy (8). The regulatory cells that orchestrate active suppression act via the secretion of suppressive cytokines, such as transforming growth factor β and interleukin-4 (19). Experiments in animals support the notion of the generation of regulatory lymphocytes in Peyer’s patches which subsequently migrate to mesenteric lymph nodes and spleen (20). Secretion of regulatory cytokines by these cells in vitro is dependent on antigen-specific stimulation with the fed antigen (21). Thus, it is presumed that active suppression of inflammation by these regulatory lymphocytes requires further migration of these cells to a local microenvironment, where the fed antigen is present.

Because the regulatory cells generated by oral tolerization are primed in an antigen-specific manner but suppress in a non–antigen-specific manner, they mediate “bystander suppression” when they encounter the fed autoantigen at other sites. This phenomenon of bystander suppression has been demonstrated in experimental autoimmune encephalomyelitis (EAE), a cell-mediated autoimmune disease that serves as an animal model for multiple sclerosis. EAE can be induced by immunization with myelin basic protein (MBP) or proteolipid protein (PLP). Oral administration of MBP has been shown to suppress both MBP- and PLP-induced EAE (22). Similarly, oral administration of type II collagen has been shown to ameliorate RA induced in animal models by immunization with either Freund’s complete adjuvant (11), CCII (9,10), or methylated bovine serum albumin (23). Thus, it may not be necessary to identify the target autoantigen for a given disease. It is necessary only to orally administer a protein which is present at the site of inflammation and which is capable of inducing regulatory cells to secrete suppressive cytokines. These findings have important implications for the use of oral tolerance as a therapeutic approach for the treatment of T cell–mediated inflammatory autoimmune diseases in humans in which the inciting autoantigen is unknown or in which there is autoreactivity to multiple autoantigens in the target tissue.

Alternatively, a dominant pathway for oral tolerance may involve T cell anergization (24,25). In this case, the induction of oral tolerance would be presumed to result in disease suppression only when the fed antigen is also the target autoantigen for the disease under study. The demonstration of a sustained remission of arthritis in 1 of our 10 patients might arguably be more consistent with this latter view, based on the longevity of her response. However, this would imply that type II collagen was the disease-specific autoantigen in her case, and while collagen reactivity can be demonstrated in some patients with RA, it is unknown whether this is actually involved in the primary pathogenesis of the disease or merely reflects tissue degradation.

The present study demonstrates that oral CCII may be a safe and effective form of treatment for JRA. The most remarkable improvements in clinical parameters of arthritis were noted in patients 1 and 4, both of whom were girls with relatively recent onset of disease. Patient 1 had polyarticular onset, whereas patient 4 had systemic features of fever and rash in addition to polyarticular joint involvement at onset. Of note, of the 3 boys with pauciarticular onset of disease, 2 experienced minimal, if any, benefit from collagen (patients 5 and 7). As mentioned above, HLA typing was not performed, but it would be of interest
to know whether these patients were HLA-B27 positive. If this were the case, it might suggest that type II collagen is ineffective in the treatment of juvenile spondylarthopathies.

In an open-label study, one must always be concerned about the contribution of the placebo effect, and this may be even more true in a pediatric population. Therefore, conclusions regarding efficacy based on this pilot trial would be premature, but nonetheless, these preliminary data support the assertion that further study of oral CCII in the treatment of JRA is warranted. The observation that 1 patient achieved a complete remission of her arthritis is especially compelling in this regard and is similar to the experience observed in a minority of adults treated with CCII (12).

More importantly, as pertains to this pilot study, oral CCII appears to be extremely well tolerated in this pediatric patient population. The only adverse event noted during the study that was believed to be related to the study medication was transient facial flushing, which occurred in 1 patient for ~2 weeks after collagen treatment was begun. The elevated transaminase levels noted in patient 6 during the second month of the study resolved without interruption of collagen therapy and were believed to be related to his underlying chronic hepatitis C. The combination of favorable safety data and promising clinical results in this pilot trial strongly indicate that there should be further studies of this novel therapeutic agent in the treatment of JRA.

REFERENCES