Control of Rheumatoid Arthritis by Oral Tolerance

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Objective. Previous randomized controlled trials for treatment of rheumatoid arthritis (RA) with acid-soluble chicken and bovine type II collagen (CII) have produced conflicting results. This randomized, double-blind, controlled trial examined the therapeutic effect of bovine CII tablets in RA.

Methods. CII tablets were prepared by adsorption onto a lactose base. Patients with a duration of RA of ≥2 years and who had failed treatment with at least 1 slow-acting drug were recruited, provided that they had active arthritis. Patients were randomly assigned to receive either 0.05 mg, 0.5 mg, or 5 mg of CII or placebo daily for 6 months. All slow-acting drugs were stopped at least 4 weeks before starting CII, although prednisolone was permitted at dosages <10 mg/day. Clinical assessments were performed at screening and at 0, 1, 4, 8, 12, 16, 20, and 24 weeks of treatment.

Results. Fifty-five patients were recruited. Initially, there were no significant differences in mean Disease Activity Scores between groups. At 24 weeks, there was a significant difference (P = 0.041, by Kruskal-Wallis analysis of variance); the major components of this difference were attributable to relatively large decreases in the 0.5 mg CII group (19% of initial values) and to minimal decreases in patients receiving placebo (3% of initial values). Twenty patients had American College of Rheumatology 20% responses; 11 of these were in the 0.5 mg CII group and 3 were in each of the other groups, a significant difference (χ² = 14.6, P = 0.002). There was no significant difference in any clinical measure between the placebo, 0.05 mg CII, and 5 mg CII groups. There were no side effects associated with CII treatment.

Conclusion. Treatment with 0.5 mg/day of bovine CII is well tolerated and produces small, but significant, disease improvement in RA. However, the therapeutic window is narrow. The difference between our results and those of other trials may relate to the dose, species, and formulation of the CII.

Complex immune mechanisms contribute to the pathology of rheumatoid arthritis (RA). Conventional therapies suppress the immune system nonspecifically and are associated with significant side effects, including infections. In RA, the arthritogenic antigen remains unknown. Type II collagen (CII) of the articular cartilage is one of the candidate autoantigens and some RA patients demonstrate immunity against CII (1). In rodents and primates, CII is arthritogenic; collagen-induced arthritis (CIA) is derived from the injection of CII in adjuvant, as described originally by Trentham et al (2) and subsequently by many others (3–5). Using CIA as a model for arthritis, investigators in our group found that feeding CII to rats by gavage would protect them against the development of CIA (6,7). This is the phenomenon of oral tolerance that has been well documented in other, nonautoimmune situations (8). Interestingly, oral feeding of CII can also suppress disease in antigen-induced arthritis (9), in which collagen is not the antigen; this phenomenon is known as bystander tolerance.

These experiments in rodents have provided the basis for human clinical trials. In RA, the initial study (10) showed a clinical improvement in patients treated daily with chicken-derived CII (0.1 mg for 1 month, then 0.5 mg for 2 months, diluted in orange juice) compared with patients given placebo. No side effects were seen.
Disappointingly, a study from a different group, using bovine CII, failed to demonstrate any significant difference between the active treatment and placebo groups, although only a minority of patients showed a response (11). Recently, a larger study using chicken collagen (12) showed significant clinical improvement in the 0.02 mg/day group based on the Paulus 20% improvement criteria (13), but not the American College of Rheumatology (ACR) 20% improvement (ACR20) criteria (14). The reason for this discrepancy in the results is unknown. We conducted a double-blind placebo-controlled trial to evaluate the use of orally delivered bovine collagen in an inert tablet base to control RA, since we believe that this approach would produce a standard preparation and hence more reliable results.

**PATIENTS AND METHODS**

**Patients.** RA patients ages ≥18 years and fulfilling the 1987 ACR (formerly, the American Rheumatism Association) criteria for the diagnosis of RA (15) were entered into the study after giving their written informed consent. Patients must have had the disease ≥2 years and have failed treatment with at least 1 slow-acting antirheumatic drug (SAARD). SAARDs were stopped at least 1 month before starting the trial treatment. All of the patients had to have active arthritis which was defined by the presence of 3 of 4 clinical criteria: ≥3 swollen joints, ≥6 tender joints, early morning stiffness ≥45 minutes, and erythrocyte sedimentation rate (ESR) ≥28 mm/hour. Oral steroid treatment was permitted if the dosage was <10 mg/day. Vegetarians and patients receiving proton pump inhibitors were excluded. The study was approved by the local ethics committees.

**Study design.** Patients were randomly assigned to 1 of 4 groups that received either a low dose (0.05 mg daily) or moderate dose (0.5 mg daily) or high dose (5.0 mg daily) of bovine CII or placebo. Treatment was given for 6 months. Randomization was carried out using blocks of 12 patients.

**Collagen preparation.** Collagen from bovine nasal cartilage was solubilized with pepsin and acetic acid according to standard procedures (6). The purity of the final acid-soluble product was checked by polyacrylamide gel (6). Silver staining showed a single band. The arthritogenicity and tolerogenicity of bovine CII were confirmed in rodents. The acid-soluble CII product was checked by polyacrylamide gel (6). Silver staining showed a single band. The arthritogenicity and tolerogenicity of bovine CII were confirmed in rodents. The acid-soluble CII product was checked by polyacrylamide gel (6). Silver staining showed a single band. The arthritogenicity and tolerogenicity of bovine CII were confirmed in rodents. After dialysis into distilled water, sterilization by filtration, and freeze dried. Tablets were prepared by mixing a dispersion of the CII with lactose and drying the subsequent granules at 30°C. Povidone was used as a tablet-binding agent, maize starch as a disintegrant, and magnesium stearate as a tablet lubricant. A record of the patients’ compliance was kept by counting the number of returned tablets.

**Disease assessment.** Clinical assessments were performed at weeks −1, 0, 1, 4, 8, 12, 16, 20, and 24. Both the patient and the assessor were blinded to the nature of the treatment. Assessments were based on the ACR (16) and European League Against Rheumatism (17) core data sets and included duration of early morning stiffness, fatigue score, the number of tender and swollen joints (28-joint score), visual analog scale for pain, patient’s and physician’s global assessment of disease activity, ESR, and modified Health Assessment Questionnaire. Full blood cell counts and biochemical evaluations were performed at weeks 0, 12, and 24.

**Statistical analysis.** Results were analyzed on an intention-to-treat basis with last observation carried forward. The primary outcome was the modified Disease Activity Score (DAS) (18). The DAS is a validated composite score based on the ESR, patient’s global assessment of disease activity, and tender and swollen joint counts. Demographic data were compared using chi-square tests. Results were examined by analysis of variance and 2-tailed unpaired parametric and nonparametric tests for comparisons between the active treatment groups and placebo. The Bonferroni method was used to correct for multiple comparisons. The ACR20 response criteria were also used to analyze the response to treatment through a 4 × 2 chi-square table.

**RESULTS**

**Demographic data.** Demographic characteristics (age, sex, and disease duration), the initial DAS, and other assessments of disease activity were similar in each of the 4 groups (Tables 1 and 2). The number of patients who did not complete the trial was 8, 4, 4, and 5 in the placebo, 0.05 mg, 0.5 mg, and 5 mg CII groups, respectively. All withdrawals were due to continuing activity of RA despite treatment.

**Changes in the DAS.** The DAS, which was the primary outcome measure, changed with treatment. The

<table>
<thead>
<tr>
<th>Table 1. Demographic details of the patients*</th>
<th>Oral type II collagen</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>No. of patients</td>
<td>15 11 14 15</td>
<td>15 11 14 15</td>
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<tr>
<td>Age, mean ± SD years</td>
<td>49.5 ± 17 57.5 ± 12 55.5 ± 14 57.4 ± 13</td>
<td>15 11 14 15</td>
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<tr>
<td>Sex ratio, no. male/no. female</td>
<td>3:12 3:8 4:10 2:13</td>
<td></td>
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<tr>
<td>Disease duration, mean ± SD years</td>
<td>11.2 ± 10 15.7 ± 12 9.3 ± 7 11.6 ± 10</td>
<td>15 11 14 15</td>
</tr>
<tr>
<td>Rheumatoid factor positive, no. patients</td>
<td>7 8 12 10</td>
<td></td>
</tr>
<tr>
<td>Erosive disease, no. patients</td>
<td>10 9 9 11</td>
<td></td>
</tr>
<tr>
<td>NSAIDs, no. patients</td>
<td>12 10 12 12</td>
<td></td>
</tr>
<tr>
<td>Steroids, no. patients</td>
<td>4 3 3 2</td>
<td></td>
</tr>
<tr>
<td>No. of previous slow-acting drugs, mean ± SD</td>
<td>2.6 ± 1.6 3.9 ± 2.4 3 ± 2.1 3.1 ± 1.8</td>
<td>15 11 14 15</td>
</tr>
<tr>
<td>Nodules, no. patients</td>
<td>7 7 8 5</td>
<td></td>
</tr>
<tr>
<td>Initial Disease Activity Score, mean ± SD</td>
<td>6.7 ± 0.9 6.8 ± 1.3 6.2 ± 1.0 7.1 ± 1.0</td>
<td>15 11 14 15</td>
</tr>
</tbody>
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* NSAIDs = nonsteroidal antiinflammatory drugs.
mean changes in the DAS are shown in Figure 1. The average decrease in DAS score during 6 months of treatment was highest (19% of initial values) in patients receiving 0.5 mg collagen and lowest (3% of initial values) in patients receiving placebo. Comparing all groups by Kruskal-Wallis one-way analysis of variance (ANOVA) showed no significant differences in the initial DAS scores. At 24 weeks, the DAS scores were significantly different between groups ($P = 0.041$). In addition, the areas under the curve between 1 week and 24 weeks and the percentage change between the initial and week-24 DAS scores both showed significant differences between groups ($P = 0.041$ and $P = 0.048$, respectively). The major component of this difference was attributable to the difference between placebo and 0.5 mg collagen. In further support of these results, a comparison of these groups by Mann-Whitney U test showed no significant differences in initial DAS scores. However, comparisons of 24-week DAS scores, areas under the curve between 1 week and 24 weeks, and the percentage change between initial and week-24 DAS scores all showed significant differences ($P = 0.01$ in all 3 cases) between the placebo and 0.5 mg groups.

**Changes in the ACR20 responses.** We also analyzed ACR20 responses. Twenty patients had an ACR20 response at 3 months and 35 did not. The distribution of the ACR20 responders showed significant differences between groups. Three responders were observed in the placebo group, 3 in the 0.05 mg CII group, 11 in the 0.5 mg CII group, and 3 in the 5 mg CII group ($\chi^2 = 14.6; P = 0.002$).

Differences in individual core data set measures among the 4 treatment groups are shown in Table 2. All of the variables showed a trend favoring 0.5 mg collagen. Only the swollen joint count showed a significant difference between groups at 24 weeks, and the Kruskal-Wallis one-way ANOVA showed a significant difference ($P = 0.026$). Analysis of individual groups by Mann-Whitney U tests showed a significant difference between 0.5 mg CII and placebo ($P = 0.027$).

**DISCUSSION**

Although oral treatment with CII has been shown to prevent and suppress disease in CIA (19) and antigen-induced arthritis (9), its efficacy in RA has not been...
convincingly demonstrated. There are conflicting results from 3 placebo-controlled trials of chicken and bovine CII. Initially, Trentham et al showed that chicken collagen suspension in orange juice suppressed disease in RA patients who had just stopped their SAARD (10). One criticism of that study was the lack of a SAARD-washout period. A subsequent larger study (12) involving 274 patients treated with either placebo or 0.02, 0.1, 0.5, or 2.5 mg/day of chicken CII for 24 weeks showed a statistically significant improvement in the 0.02 mg/day group using the Paulus 20% improvement criteria only, but not the ACR20 criteria. However, a smaller study in RA involving 90 patients who were administered bovine collagen showed no significant disease improvement (11). Bovine CII was also ineffective when given as adjuvant to SAARDs with or without prednisone (20). Data from our study could provide a possible explanation for these conflicting results.

One of the reasons for the difference between our results and those of previous studies may be the dose and the formulation of CII. Both the data from Barnett et al (12) and our data showed that the therapeutic dose range for chicken CII is small; delivering the correct dose is critical. This is supported by data from animal models in which high and low doses of oral antigen mediated different effects (21). High doses of antigen induced tolerance by the deletion of antigen-specific T cells. In contrast, low doses of antigen suppressed disease through release of cytokines such as transforming growth factor β by bystander tolerance, which can be effective using an antigen from relevant target tissue even if it is not the disease-causing antigen (21). Since collagen is not the major or only autoantigen in RA, depletion of collagen-specific T cells is not our treatment objective. Therefore, the use of a low dose of CII may be more effective in immunomodulation of RA. In any case, the different effects of different doses can explain the narrow therapeutic window and the variation between different preparations.

Another possible confounder is the use of nonsteroidal antiinflammatory drugs and inhibition of cyclooxygenase-2 (COX-2). Recent research has shown that COX-2 may have an important role in the development of oral tolerance (22). All current nonsteroidal antiinflammatory drugs (NSAIDs), as well as prednisone, inhibit COX-2. Since most of our patients were taking NSAIDs and some were taking low-dose steroids (Table 1), this may have a negative effect on the development of tolerance. Unfortunately, the small numbers of patients studied preclude separate analysis of patients on different drug regimens. Similarly, this may explain why previous studies of bovine CII as adjuvant to NSAIDs, SAARDs, and/or prednisone did not produce significant disease improvement (20,23).

Since the dose of CII is so important in determining efficacy, the treatment formulation must ensure that a precise dose is delivered. This is especially important with native CII, which is an adhesive protein and requires a low pH environment. Therefore, CII was previously administered in orange juice. However, this liquid formulation is unlikely to deliver a precise dose. In this study, we have developed a new formulation in which CII is absorbed into a lactose base, which may deliver a more consistent dose of CII and hence render the result more replicable. Further studies would be required to confirm these hypotheses.

In our study, treatment with bovine CII tablets was well tolerated, especially when compared with standard SAARDs, and 0.5 mg/day produced a statistically significant disease improvement, but the therapeutic window was narrow. In summary, the conflicting results of previous studies with oral CII have led many to abandon mucosal tolerance as a treatment strategy for human disease. We believe our data demonstrate that oral CII can safely suppress inflammation in RA, although, as monotherapy, it cannot achieve sufficient disease control. However, various other methods, such as administering CII protein or peptide nasally (24) or conjugation with cholera toxin (25), have been shown to enhance mucosal tolerance in animal models. This study suggests these approaches will be worth pursuing in the future in RA.

**REFERENCES**

7. Nagler-Anderson C, Bober LA, Robinson ME, Siskind GW, Thorbecke GJ. Suppression of type II collagen-induced arthritis by...