Subcutaneous Administration of Polymerized-type I Collagen for the Treatment of Patients with Rheumatoid Arthritis. An Open-Label Pilot Trial

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ABSTRACT. ***Objective.*** To determine the efficacy, tolerance and safety of subcutaneous injections of porcine type I collagen-polyvinylpyrrolidone (PVP) in patients with rheumatoid arthritis (RA).

Methods. Eleven patients with active RA on stable therapy with methotrexate (MTX) were enrolled in a 3 month prospective and longitudinal study. Patients were treated weekly with subcutaneous injections of 0.2 ml of collagen-PVP (1.7 mg of collagen) in the 8 most painful joints. The primary endpoints included the Ritchie index (RI), swollen joint count, disease activity score (DAS), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). The secondary endpoints included morning stiffness, pain intensity on a visual analog scale (VAS), and the Spanish-Health Assessment Questionnaire Disability Index (HAQ-DI). Improvement was determined using American College of Rheumatology (ACR) response criteria.

Results. Collagen-PVP was safe and well-tolerated and there were no adverse events. Patients had a statistically significant improvement ($p < 0.05$) in basal versus 3 month's treatment in morning stiffness (Δ -32.3, -68.6%), RI (Δ -10.2, -46.4%), swollen joint count (Δ -10.7, -71.8%), VAS (Δ -39.9, -63.8%), HAQ-DI (Δ-0.5, -48.5%), DAS (Δ -1.35, -70.5%) and ACR20, 50, and 70 (80.0%; 60.0% and 20.0% respectively). We found no differences in serologic or hematologic variables.

Conclusion. Collagen-PVP was a safe and well-tolerated drug for the short term treatment of RA. The combination of collagen-PVP plus MTX was more efficacious than MTX alone. However, double-blind placebo-controlled phase II and III clinical trials are necessary to determine whether this drug could be useful in the longterm treatment of RA. (J Rheumatol 2003;30:256–9)

Key Indexing Terms:
RHEUMATOID ARTHRITIS COLLAGEN-PVP BIOLOGICAL THERAPY

Rheumatoid arthritis (RA) is characterized by progressive joint destruction, deformity, and loss of function. Among the novel biological therapeutic agents, oral or nasal administration of type II collagen is included. It induces antigen-specific peripheral immune tolerance. Studies have shown a trend towards clinical improvement with the use of this treatment strategy[1,2]. Kalden[3] and Choy, *et al*[4] have suggested that the source of the collagen might be important as well as its route of administration and formulation.

Porcine type I collagen-polyvinylpyrrolidone (PVP) has antiinflammatory properties. Intralesional injection once per week over 1 to 3 months in human hypertrophic scars diminishes inflammatory infiltrates. It modulates type I and III collagen turnover, and downregulates the expression level of interleukin-1ß (IL-1ß), tumor necrosis factor-α (TNF-α), platelet derived growth factor (PDGF), and vascular cellular adhesion molecule-1 (VCAM-1)[5]. In addition, a 1% preparation of biodrug modified collagen turnover in synovium cultures from RA patients[6]. Proinflammatory cytokines, adhesion molecule expression, as well as cyclooxygenase-1 expression was downregulated in RA synovium treated with collagen-PVP. In supernatants, the collagenolytic activity has been shown to be downregulated[7].

We evaluated the subcutaneous administration of γ-irradiated, pepsin-digested collagen-PVP in patients with RA.

MATERIALS AND METHODS

Patients. Only patients who gave informed consent to participate were recruited. We included patients who fulfilled the 1987 American Rheumatism Association (American College of Rheumatology, ACR) criteria for RA[8].

Design. The study was an open-label phase I trial that included 11 patients with active RA. They were treated for up to 12 weeks with weekly subcutaneous injections of 0.2 ml of collagen-PVP (1.6 mg of collagen) in the 8 most painful joints. The rationale to inject subcutaneously only 8 joints was based on previous encouraging experience gained from a previous study involving the treatment of hypertrophic scars with collagen-PVP[6]. In that study, the treated group included 3 patients with erosive active RA. Each patient received 6 weekly subcutaneous injections with maximum dose of 1.6 ml of collagen-PVP. In accordance with the administration route established in that study, we decided to inject collagen-PVP subcutaneously into the joint. The injection sites in each patient changed during the study, as each patient point-

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ed out their most painful joints. However, in all patients, at all visits, the same dose of collagen-PVP (1.6 ml) was injected to maintain the quantity of drug used.

Concomitant medication. Patients must have been receiving oral methotrexate (MTX) and a dosage of concomitant nonsteroidal antiinflammatory drugs (NSAID) for at least 3 months with no break in treatment of more than 1 week during this period.

Clinical evaluation. Patients were evalulated at baseline, 7, and 13 weeks. Each patient was assessed for concomitant medication, duration of morning stiffness, Ritchie Index (RI), and number of swollen joints (72-joint count), global pain intensity self-assessment on a 100 mm visual analog scale (VAS), patient's global assessment of disease activity on a 10-point rating scale, physician's global assessment of change in disease activity at the end of the treatment, and disease activity disability as measured by responses on the Spanish Health Assessment Questionnaire Disability Index (HAQ-DI)[9]; disease activity score (DAS) also was determined[10].

Criteria for response. The ACR response criteria (20, 50 and 70) were used. The percentage change for tender and swollen joints, and erythrocyte sedimentation rate (ESR) refers to the difference between the value at the end of study and the value at entry[11].

Laboratory assessment. The evaluation was made pre-treatment and at 13 weeks' post-treatment and included ESR, determined by Westergren method, CRP and rheumatoid factor (RF) (determined by nephelometric methods). Anti-double stranded DNA antibodies were determined by both *Crithidia luciliae* substrate and by ELISA. Anti-RNP antibodies were also quantified.

Statistics. Statistical analysis was performed by the non-parametric 2 tailed Mann-Whitney U test.

RESULTS

Demographic and clinical characteristics of patients at the time of their baseline visit and last dose of collagen-PVP are summarized in Table 1.

Safety. Collagen-PVP was well tolerated during therapy and adverse events were not detected, except pain lasting less than 5 min in the injection site.

Efficacy and clinical benefit. Swollen and tender joint (RI) counts (Figure 1) improved after collagen-PVP treatment (RI: Δ -10.2, -46.4%; swollen joint count: Δ -10.7, -71.8%). This improvement was sustained during longterm therapy. We found similar and highly significant differences in changes in other variables of disease activity such as morning stiffness and pain (morning stiffness: Δ -32.3, -68.6%; VAS: Δ -39.9, -63.8%; Figure 1).

Patients also had a statistically significant improvement in DAS (Δ -1.35, -70.5%, $p < 0.02$). The ACR20 was achieved by 80.0% of patients at 3 months. Similarly, 60.0% of patients achieved the ACR50 and 20.0% the ACR 70 at this time (Figure 1). The physical functional status (Spanish-HAQ-DI) also showed a significant improvement from baseline values after 3 months of collagen-PVP treatment (HAQ-DI: Δ -0.5, -48.5%; $p < 0.05$, baseline vs. 13 weeks; Figure 1).

Concomitant medication. No patients modified their oral MTX or dosage of concomitant NSAID during the 3 months of the study.

Laboratory assessment. There were no changes in ESR, hematological constants, CPR or RF. None of the patients was positive for antibodies to DNA and RNP at baseline or after treatment.

DISCUSSION

We used collagen-PVP derived from porcine type I dermal collagen because pigs are among the primary animal species proposed as sources for xenografts for a variety of practical, ethical, and safety reasons. In the manufacturing process, collagen enzymatic digestion cleaves the telopeptide end of the molecule, which contains the major antigenic determinants, and the new structure is formed by the minimal antigenic central helical structure[12]. The homopolymer of N-vinyl-2-pyrrolidone confers pharmaceutical properties that differ from those observed in collagen or PVP alone. The covalent binding with PVP conveys both increased collagen stability and reduced collagen antigenicity. Because of its unique chemical

Table 1. Demographic and disease history at baseline and 3 months of study.

Variable	Baseline	3 Months
Demographics		
Sex (F/M)	11/0	10/0*
Age, yrs, mean ± SD (range)	45.7 ± 12.8 (27–68)	45.7 ± 12.8 (27–68)
Disease duration, yrs, mean ± SD (range)	12.1 ± 9.6 (6–33)	12.1 ± 9.6 (6–33)
Clinical variables		
DAS, mean ± SD (range)	4.6 ± 1.1 (2.55–6.16)	3.2 ± 0.83** (1.9–4.6)
No. of swollen joints, mean ± SD (range)	14.9 ± 8.1 (7–35)	4.2 ± 4.2** (1–15)
Ritchie index, mean ± SD (range)	22.0 ± 12.2 (11–45)	11.8 ± 6.5** (5–26)
Duration of morning stiffness, min, mean ± SD (range)	47.1 ± 28.1 (5–75)	14.8 ± 9.3** (0–90)
Visual analog scale, mm, mean ± SD (range)	62.5 ± 12.9 (40–75)	22.6 ± 19.4** (0–56)
HAQ, mean ± SD (range)	1.1 ± 0.3 (0.65–1.6)	0.6 ± 0.6** (0.05–1.7)
Laboratory variables		
ESR, mm/h, mean ± SD (range)	31.1 ± 23.1 (1–59)	30.2 ± 26.0 (2–81)
RF positive (%)	90.0	90.0
CRP, mg/l, mean ± SD (range)	2.3 ± 3.5 (0.1–11.9)	1.93 ± 2.3 (0.1–7.8)

* Premature discontinuation on 7th week. One patient had a final clinical and laboratory examination and a set of questionnaires to answer. ** $p < 0.05$.

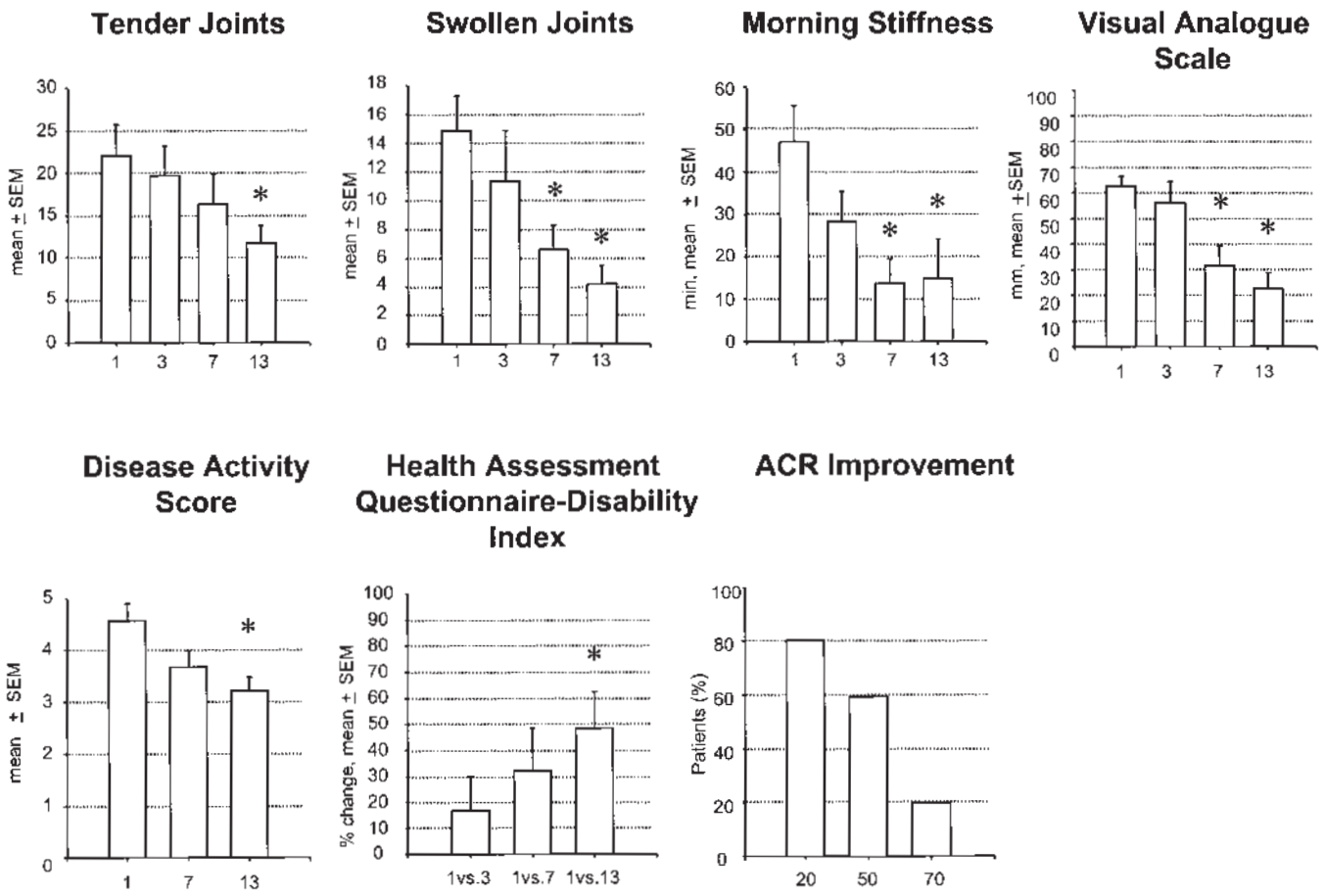

Figure 1. Changes in clinical manifestations and response to therapy evaluated by disease activity score (DAS), Health Assessment Questionnaire-Disability index (HAQ-DI) and American College of Rheumatology (ACR) criteria, during the treatment course with collagen-PVP. A collagen-PVP dosage of 1.6 ml produced a sustained reduction of active joints. Tender joint count: *p = 0.02 (1 vs 13 weeks); swollen joint count: *p = 0.007 (1 vs 7 weeks); *p = 0.001 (1 vs 13 weeks); morning stiffness: * p < 0.009 (1 vs 7 weeks) and *p < 0.02 (1 vs 13 weeks); pain: *p = 0.007 (1 vs 7 weeks); *p = 0.001 (1 vs 13 weeks). DAS: *p = 0.015 (1 vs 13 weeks) and HAQ-DI: *p = 0.045 (1 vs 13 weeks). 1: First visit or baseline; 3: Third visit and 2nd subcutaneous injection of collagen-PVP; 7: Seventh visit and 6th subcutaneous injection of collagen-PVP; 13: 13th Visit and 12th subcutaneous injection.

nature, its low molecular weight makes PVP biologically inert and safe[13].

In contrast with other studies using collagen administration, we did not find any adverse effects[14]. We suggest that subcutaneous collagen-PVP might also act like a tolerance inducing molecule more effectively than the other routes of administration and with fewer toxic effects.

Using ACR criteria, the 20% response rate was 80.0% at month 3. Good results were seen with more demanding criteria (60.0% response at ACR50 and a 20.0% response at ACR70). These values were similar to those obtained by etanercept in a phase II trial[15].

Although we observed a trend toward decreasing C-reactive protein, it was not statistically significant. We would like to evaluate other routes of administration, such as intramuscular injection, in order to determine if it is possible to increase the systemic antiinflammatory effect of the collagen-PVP.

In most cases, there was a continuous improvement over the 12 weeks, and this could be taken as evidence that a longer treatment period might be more favorable.

Given these encouraging results and the fact that this therapy has no known side effects further studies evaluating its effectiveness, optimal doses, mechanism of action, and other pathways of administration are warranted. We are also planning a placebo controlled study in order to evaluate the clinical relevance of these findings.

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