Polymerized-type I collagen for the treatment of patients with rheumatoid arthritis. Effect of intramuscular administration in a double blind placebo-controlled clinical trial

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Abstract

Objective

To determine the efficacy, tolerance and safety of intramuscular injections of porcine type I collagen-PVP in patients with RA in a long term-therapy.

Methods

The study was a double blind placebo-controlled and included 30 patients with active RA (ACR). Patients were treated with intramuscular injections of 2 ml of collagen-PVP (3.4 mg of collagen) or 2 ml of placebo during 6 months. The follow up was done during the next 6 months. 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 analogue scale (VAS), and Spanish-health assessment questionnaire (HAQ-DI). Improvement was determined using American College of Rheumatology response criteria (ACR20, 50 and 70).

Results

Collagen-PVP was safe and well tolerated. There were no adverse events. Patients had a statistically significant improvement ($p < 0.05$) in collagen-PVP-treated vs. placebo at 6 months of treatment in: swollen joint count (7.1 ± 0.8 vs. 16.0 ± 1.6), RI (8.1 ± 0.8 vs. 15.2 ± 1.5), morning stiffness (9.2 ± 3.1 vs. 29.1 ± 5.9 min), HAQ-DI (50.0 ± 10.8 vs. 22.9 ± 10.3), DAS (3.0 ± 0.2 vs. 4.9 ± 0.3), ACR20 (78.6 vs. 71.4%), ACR50 (57.1 vs. 0%) and ACR70 (7.1 vs. 0%) and CRP (1.1 ± 0.4 vs. 2.5 ± 0.7). Patients treated with collagen-PVP required lower doses of methotrexate vs. placebo (12.6 ± 0.6 vs. 14.2 ± 0.7 at 6 months and 12.3 ± 0.8 vs. 15.4 ± 0.6 at 12 months; $p < 0.05$). Serological or haematological parameters remained unchanged.

Conclusion

Collagen-PVP has been shown to be a safe and well-tolerated drug for the long-term treatment of RA. Combination of collagen-PVP plus methotrexate was more efficacious than methotrexate alone. This biodrug can be useful in the treatment of RA.

Key words

Rheumatoid arthritis, collagen-PVP, biological therapy.

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease of unknown aetiology, in which affected joints exhibit inflammation, synovial hyperplasia, fibrosis and eventually the degradation of articular cartilage and erosion of subchondral bone. RA affects almost 1% of the population (1) and is associated with rapid functional loss and reduced life expectancy (2). Guidelines delineate goals for treatment, including preservation of function, prevention or control of joint damage and remission of disease activity. Between the novel biological therapeutic agents, oral or nasal administration of type II collagen is included. It induces antigen-specific peripheral immune tolerance. The studies show a trend towards clinical improvement with the use of this treatment strategy (3, 4). Kalden (5) and Choy *et al.,* (6) have suggested that the type and source of the administered collagen might be important as well as administration way and formulation of collagen.

We have evaluated the γ-irradiated mixture of atelopeptidic porcine type I collagen and polyvinylpyrrolidone (collagen-PVP), which has anti-inflammatory properties. Intralesional injection of biodrug once per week during 3 months in scleroderma skin lesions diminishes inflammatory infiltrates. Collagen-PVP modulates types I and III collagen turnover, and downregulates the expression level of IL-1β, TGF-β, ELAM-1 and VCAM-1 (7). Besides, one percent collagen-PVP addition to synovial tissue cultures from non-RA and RA cultures does not induce any change in DNA concentration or metabolism. However, the addition of the biodrug to RA synovial tissue cultures modifies the histological and biochemical pattern of fibrosis, without changing the total collagen content. Collagen-PVP induces the recovery of type III collagen at similar levels to those detected in normal synovial tissue. Collagen-PVP diminishes the accumulation of dense and tightly packed type I collagen fibres and contributes to establish similar tissue architecture to that observed in normal synovium. The last effect is due to the biodrug decreases collagenolytic activity, mainly calcium-independent collagenase activity (cathepsins) and increases the amount of type III collagen, as well as TIMP-1 production. The chronic inflammatory process is altered by collagen-PVP action, presumably due to the down-regulation of IL-1β and TNF-α, since both cytokines are capable of inducing the expression and activation of collagenolytic enzymes, principally MMP-13 and MMP-1, who plays a significant role in the progression of erosions through the proteoglycan-rich cartilage matrix (8) and cathepsins, (9) as well as inducing proliferation and migration of synovial cells via cell adhesion molecules (ELAM-1, VCAM-1, ICAM-1) and inducing Cox-1 activation (9). Also, down production of TNF-α and IL-1β seems to stimulate only activated synovial cell death via apoptosis in synovium cultures, the last may contributed to inhibit the outgrowth of synovial cells that eventually leads to hyperplasia or pannus formation and the destruction of RA joints (10, 11). We infer that collagen-PVP mechanism of action might be mediated through regulation of certain transcription factors such as NF-*k*B and AP-1. Particularly, NF-κB regulates the expression of proinflammatory enzymes, cytokines, chemokines, immunoreceptors, and cell adhesion molecules as well as apoptosis, it has been often termed a "central mediator of the immune response" (12, 13). Because of this key role, we suggest that collagen-PVP could be contribute considerably to the anti-inflammatory effects observed through the down-regulation of NF-κB. Finally, collagen-PVP subcutaneous administration to RA patients has been safe and well-tolerated drug for the short term-treatment. The biodrug has induced a statistically significant improvement in basal *versus* 3 month's treatment in morning stiffness, Ritchie Index (RI), swollen joint count, Disease Activity Score (DAS), Visual Analogue Scale (VAS), Health Assessment Questionnaire-Disability Index (HAQ-DI), and ACR 20, 50 and 70% (14). Due to, we decided to evaluate the intramuscular administration of collagen-PVP in patients with RA in a long-term therapy

in order to determine whether other administration way could downmodulate systemic inflammation and was more efficacious than subcutaneous administration.

Methods

Patients

The protocol was approved by the Committee of Medical Ethics of the National Institute of Medical Sciences and Nutrition. Only patients who gave informed consent to participate were recruited. We included 30 patients who fulfilled the 1987 American Rheumatism Association (ACR) criteria for RA (15).

Design

The study was double blind placebo-controlled. Patients on stable therapy with methotrexate (MTX) and/or non-steroidal anti-inflammatory drugs (NSAIDs) were enrolled in a 1 year prospective, comparative and longitudinal study. Patients were randomly allocated and they were treated in accordance to Freyberg scheme with intramuscular injections of 2 ml of collagen-PVP (3.4 mg of collagen) or 2 ml of placebo (citric/citrate buffer) for up to 6 months with every third day intramuscular injections during 2 weeks (6 injections), weekly intramuscular injections for 5 weeks (5 injections), every 2 weeks intramuscular injections for 2 months (4 injections) finally every month during 2 months (2 injections). The rationale to inject intramuscular collagen-PVP was based on the previous encourage experience related to the biodrug systemic effect observed during the course of the treatment of hypertrophic scars and RA patients with collagen-PVP (14, 16). The primary endpoints were done according to Ritchie index (RI, 72-joint count), 72-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 analogue scale (VAS), and Spanish-health assessment questionnaire (HAQ-DI) (17). The improvement was determined using American College of Rheumatology response criteria (ACR20, 50 and 70). The follow up was done during the last 6 months of the study.

Radiographic measurements

Radiographs of the hands were taken at baseline and after one year. Radiographs were evaluated by two independent observers who were unaware of the patient data. These observers used the Larsen's scoring method (18).

Concomitant medication

Patients must also have been receiving oral MTX and a dosage of concomitant NSAIDs for at least 3 months with no break in treatment of more than 1 week during this period.

Clinical evaluation

It was performed in basal, and every month during the year of the study. Each patient was assessed for concomitant medication, duration of morning stiffness, RI and number of swollen joints (72-joint count), global pain intensity self-assessment on 100 mm 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, disease activity disability as measured by responses on the Spanish HAQ-DI (17), DAS was also determined (19).

Criteria for response

The ACR response criteria (20, 50 and 70) were used. The percentage change for the variables tender and swollen joints, ESR or CRP refers to the difference between the value at the end of study and the value at entry (20).

Laboratory assessment

The evaluation was made pre-treatment and at 6 and 12 month's post-treatment and included ESR, determined by Westergren method, CRP and Rheumatoid factor (RF) (determined by nephelometric methods). Soluble CD5 was evaluated by ELISA. Anti-nuclear antibodies were evaluated by indirect immunofluorescence in Hep-2 cells.

Statistics

Statistical analysis was performed by the non-parametric two tailed Mann-Whitney U test. Data were expressed as the mean ± SD. The p values smaller than or equal to 0.05 were considered as significant.

Results

Characteristics of the patients

The majority of patients in placebo treatment were women (14; 93%), with a mean age of 42.2 ± 10.9 years (range 22-63 years) and a mean disease duration of 6.7 ± 3.7 years (range 2-16 years). Meanwhile all patients in collagen-PVP treatment were women with a mean age of 39.1 ± 11.3 years (range 22-57 years) and a mean disease duration of 5.9 ± 3.3 years (range 1-10 years). Clinical characteristics of patients at the time on their baseline visit and last dose of collagen-PVP are summarized in Table I.

Safety

Collagen-PVP was well tolerated during extended 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 (Fig. 1) improved after collagen-PVP or placebo treatment. However collagen-PVP-treated group showed statistically significant difference compared to placebo-treated group (RI: Δ-13.7, ≅-47.4% *vs.* Δ-20.6, ≅-71.8%; swollen joint count: Δ-9.3 ≅-36.8% *vs.* Δ-15.2, ≅-68.2%; placebo *vs.* collagen-PVP). This improvement was sustained during long-term therapy and during follow up. We found similar and highly significant differences in changes in other variables of disease activity such as morning stiffness (Δ-72.3 ≅-71.3% *vs.* Δ-85.4 ≅-90.3%; placebo *vs.* collagen-PVP; Fig. 1).

Patients also had a statistically significant improvement in DAS (DAS: Δ-0.9 ≅-15.5% *vs.* Δ-2.6, ≅-46.4; placebo *vs.* collagen-PVP). Collagen-PVP-treated group improvement was important meanwhile placebo-treated group improvement was scarce. At 6 months the ACR20 was achieved by 71.4% of patients from placebo-treated group and 78.6% of patients from collagen-PVP-treated group. Conversely, none

Table I. Clinical and laboratory data at baseline, 6 and 12 months of study.

Variable	Baseline		6 months		12 months	
Clinical	Placebo	Collagen-PVP	Placebo	Collagen-PVP	Placebo	Collagen-PVP
DAS, mean±SD Change from baseline, (Δ)	5.8 ± 1.2	5.6 ± 1.0	4.9 ± 0.3 Δ=-0.9, -15.5%	3.0 ± 0.6* Δ=-2.6, -46.4%	3.5 ± 0.7** Δ=-2.3, -39.7%	3.3 ± 0.9** Δ=-2.3, -41.1%
Swollen joints, mean±SD Change from baseline, (Δ)	25.3 ± 8.5	22.3 ± 9.7	16.0 ± 1.6 Δ=-9.3, -36.8%	7.1 ± 0.8* Δ=-15.2, -68.2%	7.6 ± 3.12** Δ=-17.7, -69.9%	7.7 ± 5.3** Δ=-14.6, -65.5%
Ritchie index, mean±SD Change from baseline, (Δ)	28.9 ± 10.3	28.7 ± 10.2	15.2 ± 1.5 Δ=-13.7, -47.4%	8.1 ± 0.8* Δ=-20.6, -71.8%	9.6 ± 2.8** Δ=-19.3, -66.8%	9.3 ± 3.7** Δ=-19.4, -67.6%
Morning stiffness, min, mean±SD Change from baseline, (Δ)	101.4 ± 24.3	94.6 ± 14.4	29.1 ± 5.9 Δ=-72.3, -71.3%	9.2 ± 3.1* Δ=-85.4, -90.3%	8.7 ± 2.5** Δ=-92.7, -91.4%	16.3 ± 5.5** Δ=-78.3, -82.8%
Patient VAS, mm, mean±SD Change from baseline, (Δ)	6.8 ± 1.8	6.6 ± 2.3	4.1 ± 2.4 Δ=-2.7, -39.7%	3.2 ± 2.5 Δ=-3.4, -51.5%	3.4 ± 2.5** Δ=-3.4, -50.0%	3.9 ± 2.7** Δ=-2.7, -40.9%
Physician VAS, mm, mean±SD Change from baseline, (Δ)	6.6 ± 1.3	7.3 ± 0.8	4.6 ± 1.1 Δ=-2.0, -30.3%	4.3 ± 1.5 Δ=-3.0, -41.1%	3.9 ± 1.1** Δ=-2.7, -40.9%	4.4 ± 1.7** Δ=-2.9, -39.7%
HAQ, mean±SD Change from baseline, (Δ)	1.05 ± 0.53	1.38 ± 0.15	0.81 ± 0.15 Δ=-0.24, -22.9%	0.69 ± 0.16* Δ=-0.69, -50.0%	0.60 ± 0.10 Δ=-0.45, -42.9%	0.71 ± 0.15** Δ=-0.67, -48.6%
MTX, mg/week mean±SD (range)	13.4 ± 1.9 (10 – 15)	12.9 ± 2.6 (7.5 – 15)	14.2 ± 2.5 (10 – 20)	11.6 ± 2.3 (7.5 – 15)	15.2 ± 2.3 (10 – 20)	12.3 ± 3.0* (7.5 – 15)
Laboratory						
ESR, mm/h, mean±SD Change from baseline, (Δ)	28.6 ± 2.6	32.8 ± 4.5	28.9 ± 4.7 Δ=0.3, 1.0%	32.0 ± 7.6 Δ=-0.8, -2.43%	24.9 ± 3.7 Δ=-3.7, -12.9%	30.5 ± 7.2 Δ=-2.3, -7.0%
RF, UI/ml, mean+SD Change from baseline, (Δ)	470.6 ± 112.8	371.8 ± 86.8	490.4 ± 674.9 Δ=19.8, 4.2%	244.0 ± 57.3* Δ=-127.8, -34.4%	465.9 ± 84.6 Δ=-4.7, -1.0%	204.3 ± 58.4*/** Δ=-167.5, -45.1%
CRP, mg/l, mean±SD Change from baseline, (Δ)	1.9 ± 0.5	1.9 ± 0.5	2.5 ± 0.6 Δ=0.6, 31.6%	1.1 ± 0.5* Δ=-0.8, -42.1%	2.0 ± 0.5 Δ=0.1, 5.3%	1.1 ± 0.6/** Δ=-0.8, -42.1%

[1]Premature discontinuation of one patient for lack of efficacy of the treatment. [2]Elimination of one patient due to develop psoriatic arthritis. *$p < 0.05$ (placebo *vs.* collagen-PVP). **$p < 0.005$ (baseline *vs.* 12 months).

of placebo-treated patients and 57.1% of collagen-PVP-treated patients achieved the ACR50. Similarly, none of placebo-treated patients and 7.1% of collagen-treated patients were ACR 70 at this time (Fig. 1). At 12 months the ACR20 was achieved by 85.7% of patients from placebo-treated group and 78.6% of patients from collagen-PVP-treated group. The 21.4% of placebo-treated patients and 57.1% of collagen-PVP-treated patients achieved the ACR50. Similarly, none of placebo-treated patients and 14.3% of collagen-PVP-treated patients were ACR 70 at this time (Fig. 1). The physical functional status (Spanish-HAQ-DI) also showed a significant improvement from baseline values after 6 months of collagen-PVP treatment (HAQ-DI: Δ-0.24, ≅-22.9% *vs.* Δ-0.69, ≅-50.0%, placebo *vs.* collagen-PVP; Fig. 1).

Radiographic measurements

Values for radiographic damage, erosion, and joint narrowing remained unchanged before and after treatment in placebo and collagen-PVP- treated group (data not shown).

Concomitant medication

All patients have not modified their oral MTX and dosage of concomitant NSAIDs during the first 6 months of the study (Table I, Fig. 2). However, the last 6 months placebo-treated group increased MTX dose at statistically significant levels, and required more DMARDs compared to collagen-PVP-treated group (Table II).

Laboratory assessment

There was no change in ESR neither haematological nor liver constants. However CRP (CRP: Δ 0.6, ≅ 31.6% *vs.* Δ-0.8, ≅-42.1%, placebo *vs.* collagen-PVP at 6 months) and RF (RF: Δ 19.8, ≅4.2% *vs.* Δ-127.8, ≅-34.4%, at 6 months and Δ-4.7, ≅-1.0% *vs.* Δ-167.5, ≅-45.1%, at 12 months, placebo *vs.* collagen-PVP) decreased at statistically significant levels in collagen-PVP-treated group compared to placebo-treated group (Fig. 2). Two collagen-PVP-treated patients and 3 placebo-treated patients increased two-fold titles (1:160 to 1:320) for antibodies to nuclei at 6 and 12 months compared to baseline. Meanwhile, 5 placebo-treated patients had positive titles for antibodies to nuclei and remain at the same title at 6 and 12 months (data not shown).

CD5 assessment

Detectable levels of sCD5 were found in all patients. Negative values were related to sCD5 levels of 100 healthy

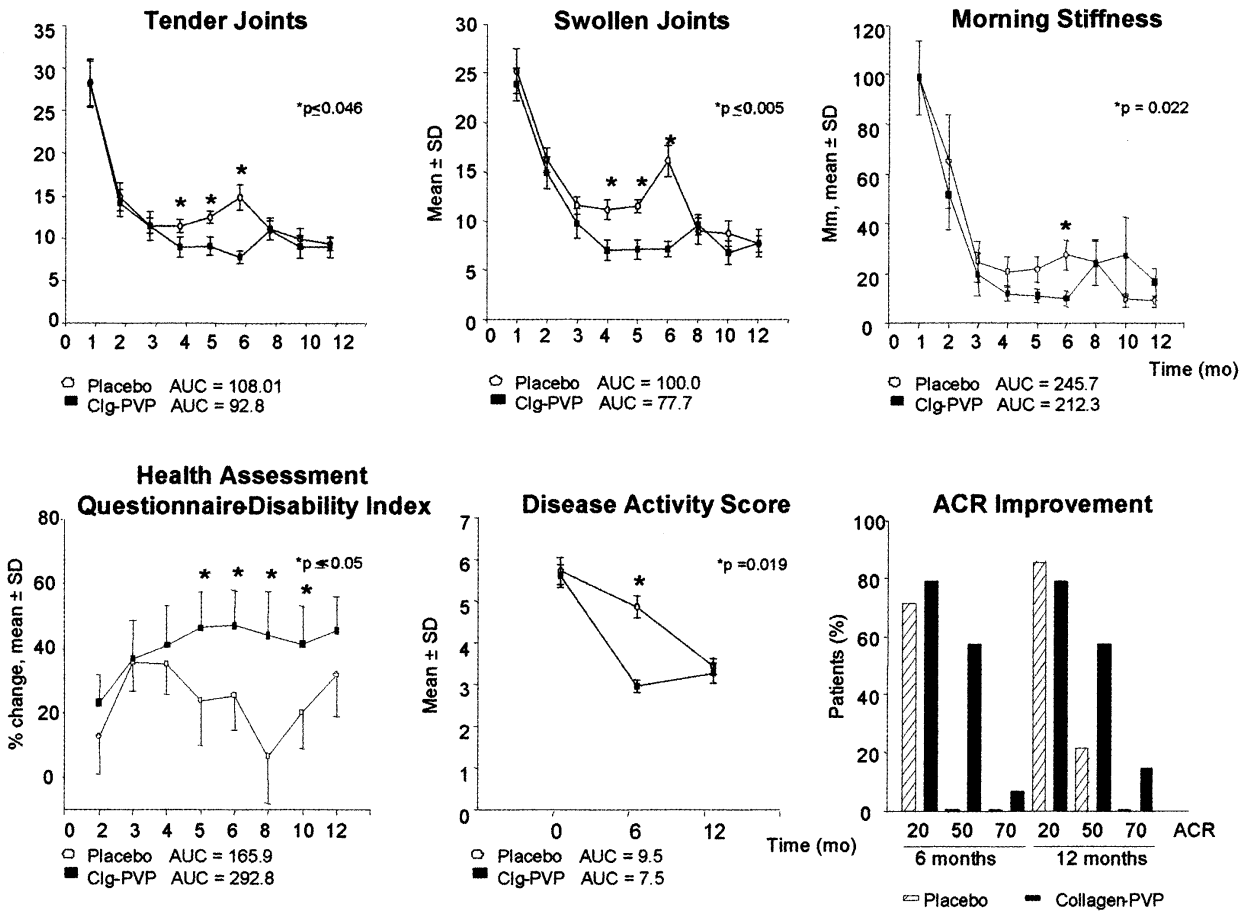

Fig. 1. Changes in clinical manifestations and response to therapy evaluated by disease activity score (DAS), health assessment questionnaire-disability index (HAQ-DI) and American College of Rheumatology (ACR) criteria, during the treatment course with collagen-PVP. A collagen-PVP dosage of 2 ml produced a sustained reduction of active joints.

pool sera. We found lower levels of sCD5 in some patients from collagen-PVP-treated group at 12 months compared to baseline. Meanwhile sCD5 levels were increased in placebo-treated patients at 12 months compared to baseline (Table III).

Discussion

In this study we used collagen-PVP which is made of porcine type I dermal collagen, due to pigs are among the primary animal species proposed as sources for xenografts in a variety of practical, ethical, and safety reasons. Porcine derivates do not produce zoonosis. In the manufacturing process, collagen enzymatic digestion cleaves telopeptide end of the molecule, which contain the major antigenic determinants, and the new structure is now formed by the minimal antigenic central helical structure (21). Besides, the homopolymer of N-vinyl-2-pyrrolidone or PVP confers on the biodrug pharmaceutical properties different than 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 nature, low molecular weight makes PVP biologically inert and safe (22).

Differently from the previous study with subcutaneous collagen-PVP administration (9), we found that intramuscular injection was more effective to down regulate systemic inflammation determined by decreasing of CRP, RF (Fig. 2) and sCD5 (Table III). However, clinical improvement was observed 2 month later when compared

Table II. Concomitant DMARD therapy administration according to study drug assignment.

DMARD Therapy	Baseline		12 Months	
	Placebo (n = 14)	Collagen-PVP (n = 14)	Placebo (n = 14)	Collagen-PVP (n = 14)
Methotrexate	14 (100%)	14 (100%)	14 (100%)	14 (100%)
Sulfasalazine	6 (42.9%)	6 (42.9%)	9 (64.3%)	4 (28.6%)*
Chloroquine	9 (64.3%)	6 (42.9%)	10 (71.4%)	7 (50.0%)*
Penicillamine	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Minocycline	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)
Leflunomide	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)

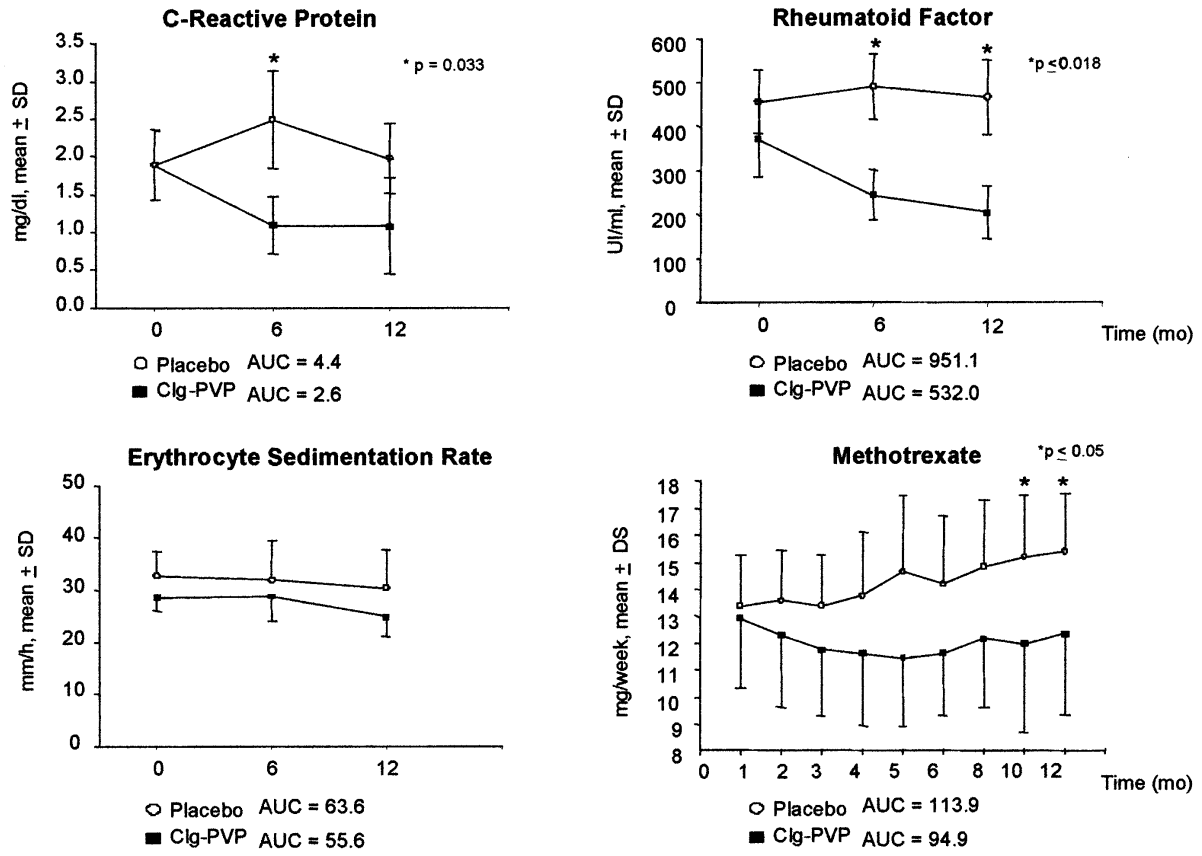

Fig. 2. Changes in laboratory assessment.

with subcutaneous administration (Fig. 1) (14). RF decreased in collagen-PVP-treated group (Δ=-127.8, ≅-34.4% *vs.* Δ=-19.8, ≅4.2% collagen-PVP *vs.* placebo). At the end of the study, 4 patients from the collagen-PVP-treated group (28.6%) were transformed in negative RF. The last results are remarkable due to previous works and have reported that patients with a persistently positive RF test, have more radiological abnormalities, more disease activity, worse functional ability, more extra-articular manifestations, and need more treatment with second line drugs than patients with persistently negative or variably positive and negative test results during the follow up (23). CRP decreased in collagen-PVP-treated group during the first 6 months (Δ=0.6, 31.6% vs. Δ=-0.8, -42.1%; placebo *vs.* collagen-PVP) and low levels remained during the follow up compared to placebo-treated group (Δ=0.1, 5.3% *vs.* Δ=-0.8, -42.1%; placebo *vs.* collagen-PVP).

Table III. Soluble CD5 (sCD5) serum levels according to study drug assignment at baseline and 12 months of the study.

Patient	Placebo		Clg-PVP	
	Baseline[1]	12 Months	Baseline	12 Months
1	7.20	11.95 ↑	-3.58	-3.53
2	-.82	-0.1 ↑	-0.89	-0.31
3	-3.50	-2.76 ↑	32.33	4.29 ↓
4	-4.26	74.28 ↑	-0.32	-1.79 ↓
5	4.67	27.20 ↑	-2.99	-3.07 ↓
6	2.42	-0.64	-0.80	-0.01
7	-3.42	-3.37	-3.66	-3.56
8	-3.58	-3.53	1.18	0.39 ↓
9	55.45	58.70 ↑	-3.40	-3.95
10	-0.89	-0.31 ↑	159.60	53.33 ↓
11	-1.94	-1.57	-1.45	-2.81 ↓
12	-1.45	-2.82	-1.37	0.99
13	4.00	38.82 ↑	9.04	5.62 ↓
14	-3.77	-3.79	12.29	9.84 ↓

[1]ng/μl.

The last results are remarkable due to previous works and have reported that patients with a persistently positive RF test, have more radiological abnormalities, more disease activity, worse functional ability, more extra-articular manifestations, and need more treatment with second line drugs than patients with persistently negative or variably positive and negative test results during the follow up (23). CRP decreased in collagen-PVP-treated group during the first 6 months (Δ=0.6, 31.6% vs. Δ=-0.8, -42.1%; placebo *vs.* collagen-PVP) and low levels remained during the follow up compared to placebo-treated group (Δ=0.1, 5.3% *vs.* Δ=-0.8, -42.1%; placebo *vs.* collagen-PVP).

On the other hand, CD5 is a highly homologous representative of the scavenger receptor cistein-rich (SRCR) superfamily. CD5 is expressed on thymocytes, mature T cells, B1a cells, and B chronic lymphocytic leukaemia cells. CD5 and CD6 act co-ordinately during activation and/or differentiation of T lymphocytes (24). Previous studies determined the presence of high "cell-free" soluble scavenger receptor CD5 in the sera of Sjögren's syndrome patients *vs.* healthy individuals. It is possible that the CD5 molecules are shed in the serum by activated lymphocytes that subsequently infiltrate the synovium (25). The raised levels determined in placebo-treated group might represent an unsuccessful mechanism of counter-regulation of an enhanced lymphocytic activation, considering that soluble molecules are released when these lymphocytes are activated. Data obtained from placebo correlates with previous studies suggesting that an enhanced activity of $CD5^+$ B cells is related to the production of RF (26).

Notably, during the first six months of the study, one placebo-treated patient achieved remission, meanwhile another patient achieved remission in the last six months. Total number of patients in remission in this group at the final of the study was of one. Conversely, dur-

ing the first six months, two collagen-PVP treated patients (14.3%) were in remission, meanwhile two more patients achieved remission in the last six months. Total number of patients in remission at the final of the study in collagen-PVP-treated group was of 4 (28.6%). Interestingly, MTX dose (Table I) and number of DMARDs (Table II) in placebo-treated group were increased at the end of the study, due to lack of therapeutic efficacy.

The health-assessment questionnaire disability index is most usually clinimetric tool used to assess functional status. Similar to the disease activity results, combination treatment with collagen-PVP was more efficient in reduction of disability as measured by the HAQ-DI questionnaire than MTX alone.

Using ACR criteria, the 20% response at 6 months rate was similar between groups. Good results with collagen-PVP were seen with more demanding criteria (57.1% *vs.* 0.0% response at ACR50 and a 7.1% *vs.* 0.0% response at ACR70 in collagen-PVP *vs.* placebo-treated patients). At 12 months the ACR50 response rate was 57.1% *vs.* 21.4% and a 14.3% *vs.* 0.0% response at ACR 70 in collagen-PVP *vs.* placebo-treated group. These values were similar to those obtained by etanercept and MTX in a phase II trial (27).

We suggest that collagen-PVP could act also, like a tolerance inducing molecule more effective than the other ways of administration and without toxic effects.

In conclusion, Collagen-PVP has been shown to be a safe and well-tolerated drug for the long-term treatment of RA. The combination of collagen-PVP plus MTX was more efficacious than MTX alone in the reduction of disease activity. This biodrug could be useful in the treatment of RA.

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

References

1. CARDIEL MH, ROJAS-SERRANO J: Community based study to estimate prevalence, burden of illness and help seeking behaviour in rheumatic diseases in Mexico City. A COPCORD study. *Clin Exp Rheumatol* 2002; 20: 617-24.
2. DROSSAERS-BAKKER KW, DE BUCK M, VAN ZEBEN D, ZWINDERMAN AH, BREEDVELD FC, HAZES JM: Long-term course and outcome of functional capacity in rheumatoid arthritis: the effect of disease activity and radiologic damage over time. *Arthritis Rheum* 1999; 42: 1854-60.
3. BAYRAK S, MITCHISON NA: Bystander suppression of murine collagen-induced arthritis by long-term nasal administration of a self type II collagen peptide. *Clin Exp Immunol* 1998; 113: 92-5.
4. BAGCHI D, MISNER B, BAGCHI M, KOTHARI SC, DOWNS BW, FAFARD RD, PREUSS HG: Effects of orally administered undenatured type II collagen against arthritic inflammatory diseases: a mechanistic exploration. *Int J Clin Pharmacol Res* 2002; 22: 101-10.
5. KALDEN JR, SIEPER J: Oral collagen in the treatment of rheumatoid arthritis. *Arthritis Rheum* 1998; 41: 191-4.
6. CHOY EHS, SCOTT DL, KINGSLEY GH, THOMAS S, MURPHY AGV, STAINES N, PANAYI GS: Control of rheumatoid arthritis by oral tolerance. *Arthritis Rheum* 2001; 44: 1993-7.
7. FURUZAWA-CARBALLEDA J, KRÖTZSCH E, ESPINOSA-MORALES R, ALCALÁ M, BARILE-FABRIS L: Subcutaneous administration of collagen-polyvinylpyrrolidone down-regulates IL-1β, TGF-β1, ELAM-1 and VCAM-1 expression in scleroderma skin lesions. *Clin Exp Dermatol* 2005; 30: 83-6.
8. AINOLA MM, MANDELIN JA, LILJESTRÖM MP, LI T-F, HUKKANEN MVJ, KONTTINEN YT: Pannus invasion and cartilage degradation in rheumatoid arthritis: Involvement of MMP-3 and interleukin-1β. *Clin Exp Rheumatol* 2005; 23: 644-50.
9. HITCHON CA, EL-GABALAWY HS: The histopathology of early synovitis. *Clin Exp Rheumatol* 2003; 21 (Suppl. 31): S28-S36.
10. FURUZAWA-CARBALLEDA J, ALCOCER-VARELA J, DÍAZ DE LEÓN L: Collagen-PVP decreases collagen turnover in synovial tissue cultures from rheumatoid arthritis patients. *Ann NY Acad Sci* 1999; 878: 598-603.
11. FURUZAWA-CARBALLEDA J, ALCOCER-VARELA J, DÍAZ DE LEÓN L: Mediators of inflammation are down-regulated meanwhile apoptosis is up-regulated in rheumatoid arthritis synovial tissue by polymerized-collagen. *Clin Exp Immunol* 2002; 130: 140-9.
12. PAUL HL: Activators and target genes of Rel/NF-kappa B transcription factors. *Oncogene* 1999; 18: 6853-66.
13. ZHANG H-G, HUANG N, LIU D *et al.*: Gene therapy that inhibits nuclear translocation of nuclear factor κB results in tumour necrosis factor α-induced apoptosis of human synovial fibroblasts. *Arthritis Rheum* 2000; 43: 1094-1105.
14. FURUZAWA CARBALLEDA J, CABRAL AR, ZAPATA-ZÚÑIGA M, ALCOCER-VARELA J: Subcutaneous administration of polymerized-type I collagen for the treatment of patients with rheumatoid arthritis. An open-label pilot trial. *J Rheumatol* 2003; 30: 140-9.
15. ARNETT FC, EDWORTHY SM, BLOCH DA *et al.*: The american rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1998; 31: 315-24.
16. KRÖTZSCH-GÓMEZ FE, FURUZAWA-CARBALLEDA J, REYES MÁRQUEZ R *et al.*: Cytokine expression is downregulated by collagen-polyvinilpyrrolidone in hypertrophic scars. *J Invest Dermatol* 1998; 111: 828-34.
17. CARDIEL MH, ABELLO-BANFI M, RUÍZ-MERCADO R, ALARCÓN-SEGOVIA D: How to measure health status in rheumatoid arthritis in non-English speaking patients: validation of a spanish version of the health assessment questionnaire disability index (Spanish HAQ-DI). *Clin Exp Rheumatol* 1993; 11: 117-21.
18. LARSEN A: How to apply Larsen score in evaluating radiographs of rheumatoid arthritis in long-term studies?. *J Rheumatol* 1995; 22: 1974-5.
19. VILLAVERDE V, BALSA A, CANTALEJO M *et al.*: Activity indices in rheumatoid arthritis. *J Rheumatol* 2000; 27: 2756-81.
20. FELSON DT, ANDERSON JJ, LANGE MLM, WELLS G, LAVALLEY MP: Should improvement in rheumatoid arthritis clinical trials be defined as fifty percent or seventy percent improvement in core set measures, rather than twenty percent?. *Arthritis Rheum* 1998; 41: 1564-70.
21. WEISS RA: Xenografts and retroviruses. *Science* 1999; 285: 1221-2.
22. ROBINSON BV, SULLIVAN FM, BORZELLECA JF, SCHWARTZ: PVP. A critical review of the kinetics and toxicology of polyvinylpyrrolidone (Povidone). *Lewis Publishers, Inc.* 1990; 147-9.
23. VAN ZEBEN D, HAZES JM, ZWINDERMAN AH, CATS A, VAN DER VOORT EA, BREEDVELD FC: Clinical significance of rheumatoid factors in early rheumatoid arthritis: results of a follow up study. *Ann Rheum Dis* 1992; 51: 1029-35.
24. GIMFERRER I, FARNÓS M, CALVO M *et al.*: The accessory molecules CD5 and CD6 associate on the membrane of lymphoid T cells. *J Biol Chem* 2003; 278: 8564-71.
25. RAMOS-CASALS M, GARCÍA-CARRASCO FM, CALVO J *et al.*: High circulating levels of soluble scavenger receptors (sCD5 and sCD6) in patients with primary Sjögren's syndrome. *Rheumatol* 2001; 40: 1036-59.
26. ARINBJARNARSON A, JONSSON T, STEINSSON K *et al.*: IgA rheumatoid factor correlates with changes in B and T lymphocyte subsets and disease manifestations in rheumatoid arthritis. *J Rheumatol* 1997; 24: 269-74.
27. KLARESKOG L, VAN DER HEIJDE D, DE JAGER JP *et al.*: Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomized controlled trial. *Lancet* 2004; 363: 675-81.