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Autologous interleukin-1 receptor antagonist improves function and symptoms in osteoarthritis when compared to placebo in a prospective randomized controlled trial

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Summary

Introduction: Incubation of blood with $CrSO_4$-coated glass beads stimulates the synthesis of anti-inflammatory cytokines, such as interleukin-1 receptor antagonist (IL-1ra), IL-4, IL-10, and IL-13. As IL-1β is thought to play a key role in the development of osteoarthritis (OA), this product, also known as Orthokin, might be a viable treatment for symptomatic knee OA. The aim of the current study was to evaluate the efficacy of Orthokin for treatment of symptomatic knee OA in a randomized, multicentre, double-blind, placebo-controlled trial.

Patients and methods: One hundred and sixty-seven patients received six intra-articular injections either with Orthokin or physiological saline. The primary efficacy objective consisted of 30% superiority on the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) at 3, 6, 9, and 12 months post-treatment. Additionally, the patients completed the visual analogue scale for pain, the Knee injury and Osteoarthritis Outcome Score (KOOS) and Knee Society Clinical Rating System.

Results: Orthokin and placebo treatment resulted in similar improvements on the WOMAC (16.8% vs 16.5%, respectively; n.s.). Orthokin resulted in significantly more improvement for KOOS symptom ($P = 0.002$) and KOOS sport ($P = 0.042$) parameters as compared to placebo treatment. For most other outcome parameters, Orthokin-treated patients consistently showed higher improvement compared to placebo-treated patients, although none of these differences were statistically significant. Two serious adverse events were observed in the Orthokin group: one patient with repeated severe inflammatory reactions of the knee joint within hours after the injection and one patient with septic arthritis which was attributed to the injection procedure rather than the product.

Conclusion: The statistically significant improvement of KOOS symptom and sport parameters together with the consistently higher, though non-statistically significant, improvement of most other parameters demonstrates that Orthokin clearly induces a biological response different from placebo treatment and warrant future investigations into the possible chondroprotective effect of Orthokin. However, in the current study the primary efficacy objective was not met and, therefore, the use of Orthokin currently cannot yet be recommended for the treatment of OA.

Key words: Osteoarthritis, Interleukin-1, Disease modifying osteoarthritic drugs, Placebo, Trial.

Introduction

Biological joint reconstruction is a viable and realistic goal in regenerative medicine. Altering the joint homeostasis, i.e., factors that determine the intra-articular environment, can be an important pathway by which we may improve treatment of OA and cartilage defects.

Osteoarthritis (OA) is a slowly progressive, degenerative, and disabling disease of articulating joints that not only affects the elderly, but also involves younger, more active patients, e.g., post-traumatic or due to prolonged participation in high demanding sports[1–4]. Treatment of this young population is especially troublesome as current treatment options, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2) inhibitors, do not prevent the progression of OA, although they have been proven to effectively reduce symptoms of OA[5,6]. In addition, prolonged use of these drugs is related to important drawbacks, such as increased risk of upper gastrointestinal bleeding and cardiovascular ischaemia through platelet activation[7–10].

Over the last two decades, OA research has increasingly focussed on drugs that not only improve the patients' symptoms, but additionally are capable of altering the course of OA development and consequently postpone or even prevent the need for total joint replacement, the so-called

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disease modifying osteoarthritis drugs (DMOADs). Best known are glucosamine and chondroitin sulphate. When used in combination, these have recently demonstrated to be effective in reducing clinical symptoms in a subgroup of patients with moderate-to-severe knee pain[11]. However, whether glucosamine and chondroitin sulphate are effective modifiers of OA progression is still controversial, although some studies suggest that these substances may inhibit the radiographic progression of OA[12,13]. In addition, the mechanism through which these substances should alter the course of OA development remains unclear, although recent studies have suggested a chondroprotective effect through inhibition of the expression of matrix degrading enzymes such as MMP-13[14].

A viable target for DMOADs are pro- and anti-inflammatory cytokines, as these are known to be involved in OA development[15–20]. Of these, interleukin-1β (IL-1β), a pro-inflammatory cytokine, has been proposed to play a key role[21–23]. It induces the production of collagenase and prostaglandin and results in decreased synthesis of cartilage specific collagens and proteoglycans[19,24–26]. In an experimental equine OA model, *in vivo* delivery of the IL-1Ra gene results in significant improvement in clinical parameters of pain and disease activity, preservation of articular cartilage, and beneficial effects on histological parameters of the synovial membrane and adjacent articular cartilage[27].

Orthokin (Orthogen, Düsseldorf, Germany) is a product in which whole blood is incubated with $CrSO_4$-coated glass beads. This process has been demonstrated to stimulate the synthesis of IL-1 receptor antagonist (IL-1ra) and other anti-inflammatory cytokines, such as IL-4, IL-10, and IL-13[28]. Therefore, Orthokin might have a beneficial effect on the symptoms and disease progression of OA. The aim of this study is to investigate the efficacy of this from of autologous IL-1ra for the relief of symptoms in patients with OA of the knee in a prospective, randomized, double-blind, placebo-controlled study of intra-articular injection therapy.

Materials and methods

A prospective double-blind placebo-controlled randomized multi-centre trial was conducted to evaluate the efficacy of intra-articularly injected Orthokin vs placebo (physiological saline) in reducing symptoms of OA in the knee. This trial was conducted over a period of 30 months; the first patient was included in February 2004 and the last patient completed follow-up in August 2006. The trial was performed at seven centres in the Netherlands and was approved by medical ethics comity of the University Medical Center in Utrecht and the local ethics committee at each participating study site.

PATIENTS

Eligible patients were aged >18 years and had clinical evidence of OA as judged by the orthopaedic surgeon, defined by the presence of typical knee symptoms (pain, stiffness, disability) and radiographic evidence of OA (grades I–III on the Kellgren–Lawrence index). Other inclusion criteria were knee complaints surpassing the threshold indicated by at least two of the following questionnaires: maximal 60 points out of 100 points on the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), the pain sub-item of the Knee injury and Osteoarthritis Outcome Score (KOOS), or the Knee Society clinical rating scale (KSCRS), and minimal 40 mm for the 100-mm Visual Analogue Scale (VAS) for pain.

Exclusion criteria were poor general health as judged by the orthopaedic surgeon; concomitant painful or disabling disease of the spine, hips, or lower limbs that would interfere with evaluation of the afflicted knee; suspicion of ipsilateral coxarthrosis and hip prosthesis loosening; any clinically significant or symptomatic vascular or neurological disorder of the lower extremities; crystalline, inflammatory and infectious arthropathies, known human immunodeficiency virus (HIV), hepatitis, cytomegalovirus or syphilis infections, current diagnosis of osteomyelitis, alcohol/drug abuse, OA grade IV; known immunodeficiency; participation in other trials within 3 months of inclusion, surgical and intra-articular pharmacological treatment within 6 months of inclusion, known coagulopathy; corticosteroid and anti-coagulant usage or morbid obesity.

RANDOMIZATION

All participants were enrolled between February 2004 and August 2005. They were first seen in the practice of their orthopaedic surgeon, who evaluated eligibility for enrolment based on in- and exclusion criteria. Furthermore, the patients received a written information brochure and were informed verbally after which the patients were given at least 24 h to consider participation in the trial. Subsequently, all patients were seen by a specially trained local study physician, who provided information concerning the trial, the products, and the alternatives, and obtained written informed consent. Next, in order to assess whether the participants met all inclusion criteria, the patients completed all questionnaires and x-rays (weight-bearing antero-posterior, so-called notch view, weight-bearing lateral, and patellar skyline) were obtained. If the participants still met all in- and exclusion criteria, they were randomized. Therefore, a computer-generated randomization code was produced according to the "random-permuted-block within strata principle" by a researcher not affiliated with the trial using SYSTAT for windows (SYSTAT Inc., Evanston, IL, USA). The randomization was stratified for gender, age (<45 and >45 years of age) and NSAID usage, i.e., a separate randomization scheme was generated for each stratum separately. In cases of severe subjective symptoms, patients were able to indicate whether NSAID usage would be continued during the trial. The randomization code was managed by a trial coordinator, who allotted the participants either to the Orthokin of the placebo group in a sequential manner according to the randomization code. The participants and their orthopaedic surgeon were blinded for the treatment to which the patients were assigned throughout the study.

One knee per patient was analysed. Patients who needed bilateral treatment were randomized as described above and were treated with the same product in both knees. However, since two knees in the same patient cannot be analysed statistically as independent specimens, an additional randomization step was performed to determine which knee would be analysed by using a randomization scheme that was also generated using a random permuted block design.

Patients were informed that all placebo group patients would be given the opportunity to have the Orthokin treatment if effectiveness was determined at completion of the trial.

INTERVENTION

After randomization, all participants returned to the local study physician, where 50 ml of venous blood was obtained using the Orthokin syringe, which contains the $CrSO_4$-coated glass beads. The syringe was gently rotated to ensure complete mixing and maximal contact of beads and blood, immediately stored at 37°C and shipped to the Orthogen laboratory within 24 h in a designated transport incubator. At the laboratory, the blood samples were tested for Hepatitis A and B, and HIV because of uncertainty of virus titre response to such incubation. If blood samples were found positive for one of these diseases, the patients were retested using new blood samples. In case of repeated positive test-outcome, the patients were excluded from the study. When the patient was tested negative, the Orthokin product was prepared by the Orthogen laboratory and was returned to the hospital after 14–21 days in 2-ml vials at −20°C. Subsequently, an injection regime of six injections was started in a rigid scheme comprising 3 weeks: injections were given on day 0, 3, 7, 10, 14 and 21. The participant was placed in a supine position, the knee was disinfected with alcohol draped in a sterile fashion. A sterile 21-gauge needle was placed supero-laterally into the supra-patellar pouch. The synovial fluid present was aspirated to minimize drug dilution. The needle was left in place and 2 ml of Orthokin or 2 ml of placebo (physiological saline) was injected through a 0.22-μm pore size anti-bacterial sterile filter. All procedures were identical for both the Orthokin and the placebo injections.

FOLLOW-UP

At 3, 6, 9 and 12 months after the first injection, the patients completed the same questionnaires as at baseline, namely the VAS for pain, the KOOS and the KSCRS. The WOMAC scores were deducted from the separate KOOS items. All questionnaires were sent by mail and were completed prior to the follow-up visits with the treating orthopaedic surgeon. At these follow-up visits, the treating orthopaedic surgeon performed a physical examination of the knee, completed the surgeon part of the KSCRS, checked for adverse events and changes in NSAID and other analgesic use.

During the treatment and follow-up period, the patients were allowed to use only Acetaminophen (Paracetamol; maximum of 4 g/day). For patients stratified into the NSAID group, additional NSAID use was permitted. However, all patients were asked to stop all analgesics at least 1 week before completing the questionnaires and visiting their treating orthopaedic surgeon.

STUDY OUTCOMES

For the WOMAC, the KOOS sub-domains [pain, stiffness, function, sport and Quality of Life (QoL)] and the KSCRS, a higher score represented

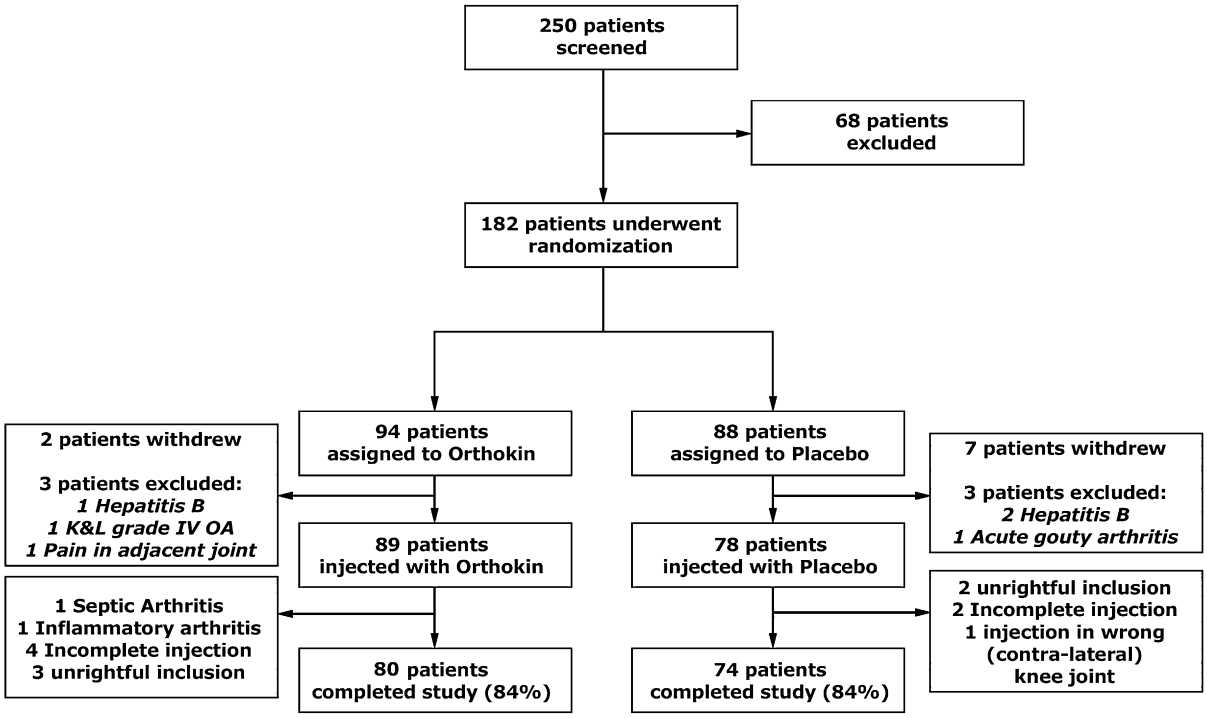

Fig. 1. An overview of enrolment of the patients.

a better outcome; the possible values ranged from 0 (worst score) to 100 (best score). In contrast, for the VAS for pain, 0 indicated no pain and 100 most severe pain. An absolute increase in the response rate of 30% on the WOMAC scale, as compared to the rate in the placebo treatment was considered a clinically relevant effect. With an expected standard deviation of 40%, as frequently found in other OA treatment studies, 100 patients were required to obtained a power of 90%.

Treatment failures were defined as follows: patients who underwent a different treatment of the afflicted knee during the 12 months follow-up period; patients randomized in the non-NSAID group who started NSAIDs during the follow-up period; patients randomized in the NSAID group who increased use of NSAIDs.

Table I
Baseline demographic and clinical parameters. Baseline values of both treatment groups were comparable.

	Orthokin, $n = 80$		Placebo, $n = 73$	
	n	%	*n*	%
Male	49	61	43	59
Analgesic medication use	22	28	24	33
PCM	12	15	17	23
NSAID	7	9	6	8
Other analgesics	3	4	1	1
	Mean	SD	Mean	SD
Age (years)	54	11	53	11
Weight (kg)	83	16	87	14
BMI (kg/m^2)	27	5	28	14
WOMAC	54	18	50	16
KOOS pain	47	16	45	15
KOOS activity daily life	55	18	51	17
KOOS symptoms	55	18	48	17
KOOS sport	25	19	21	15
KOOS QoL	29	14	26	14
VAS for pain	60	20	63	18
KSCRS, patient part	69	23	68	20
KSCRS, Surgeon part	47	16	47	13

STATISTICAL ANALYSIS

FileMaker Pro 6.0 for Windows (Filemaker Inc, Santa Clara, CA, USA) and SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA) were used for data management and statistical analysis.

For comparison of the efficacy of Orthokin vs placebo treatment, a repeated measure analysis was performed. When multivariate analysis showed an interaction between the treatment effect and time, an additional repeated measure analysis was performed in order to study the early (0–3 months) and late (6, 9, and 12 months) treatment effects. Although, randomization was stratified for NSAID usage, gender and age (<45 and >45 years old), sub-analysis was performed for these groups. Finally, correlation analysis has been performed to study whether baseline characteristics, such as age, gender, and degree of symptoms, could predict the outcome of the treatment. Patients who were considered treatment failures, were excluded for further follow-up. The data sets of these participants were completed using the last-observation-carried-forward method. Statistical analysis of treatment failure frequency between groups was separately done using the Chi-square test. *P*-values less than 0.05 were considered statistically significant. All graphs show mean values with standard error of mean (S.E.M.).

Results

DEMOGRAPHICS AND BASELINE CHARACTERISTICS

A total of 250 patients were screened for possible inclusion into the study. Sixty-eight patients were excluded because they did not meet the inclusion/exclusion criteria or voluntarily withdrew after receiving written and oral information. One hundred and eighty-two met the inclusion and exclusion criteria and were randomized to receive Orthokin ($n = 94$) or placebo ($n = 88$). Before injection, six patients were excluded and nine patients withdrew informed consent (Fig. 1). Of the remaining 167 patients, 89 patients were randomized to the Orthokin group and 78 were randomized to the placebo group. Subsequently, 14 patients were excluded from further analysis, because of serious adverse events and major protocol violations (Fig. 1), i.e., 153 patients were analysed: 80 patients received Orthokin treatment and 73 patients received placebo treatment.

Table II

Outcome scores per treatment over time. Values are given for the complete data set (all patients) and for the data set after stratification to NSAID use during the trial. Note the fact that Orthokin-treated patients score consistently better at most data points suggesting a beneficial biological effect of Orthokin

	WOMAC		KSCRS patient part		KSCRS Surgeon part		VAS	
All patients (n = 153)								
Orthokin	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	54.49	17.6	47.43	16.4	68.98	22.8	59.68	20.2
3 months	63.37	20.6	58.87	19.8	76.53	22.9	43.63	26.5
6 months	62.90	23.7	58.29	21.2	74.81	21.2	48.59	28.5
9 months	61.78	23.4	58.46	21.1	75.52	21.9	48.91	27.7
12 months	65.02	24.1	58.96	20.2	77.17	21.6	47.32	28.0
Placebo	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	50.47	15.6	47.20	12.7	67.66	20.4	63.44	18.2
3 months	59.51	19.6	54.84	19.3	74.13	19.2	47.51	26.5
6 months	60.00	21.6	55.03	19.3	72.86	20.4	49.06	27.4
9 months	59.08	22.2	53.04	20.0	72.27	21.4	50.79	25.4
12 months	57.26	22.3	55.20	18.8	70.76	23.5	49.76	26.7
Non-NSAID using patients (n = 140)								
Orthokin	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	55.14	17.8	69.03	23.2	47.84	16.4	59.88	20.7
3 months	65.32	20.5	76.86	23.0	58.65	19.6	47.23	26.7
6 months	62.77	23.6	75.05	21.4	58.05	21.4	49.21	28.5
9 months	61.53	23.3	75.99	21.7	58.28	20.9	50.03	27.2
12 months	65.57	23.7	77.85	21.1	59.12	20.1	47.79	27.8
Placebo	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	50.75	15.9	67.66	20.9	47.93	12.6	62.61	18.7
3 months	59.90	19.2	75.03	19.1	55.57	19.1	46.14	26.5
6 months	61.05	21.4	74.44	20.3	56.12	19.3	47.66	27.7
9 months	60.31	22.5	73.91	21.4	54.19	20.2	49.38	25.9
12 months	58.49	22.6	72.47	23.4	56.79	18.3	48.45	27.0
NSAID using patients (n = 13)								
Orthokin	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	47.77	14.6	68.43	19.6	43.21	16.6	59.71	15.3
3 months	65.92	22.7	73.14	23.0	61.16	23.0	40.57	25.6
6 months	64.29	26.3	72.29	20.8	60.90	21.5	42.29	29.8
9 months	64.29	26.2	70.71	24.4	60.09	24.8	37.43	32.4
12 months	59.52	29.1	70.29	27.3	57.10	24.0	42.57	31.0
Placebo	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	47.40	12.4	67.67	13.7	36.92	9.5	72.67	7.3
3 months	55.21	24.9	64.33	18.1	47.38	21.3	62.33	24.1
6 months	48.78	21.8	56.17	12.5	44.48	16.2	64.00	20.9
9 months	45.49	13.7	54.50	12.7	41.38	13.3	66.33	12.5
12 months	43.92	14.5	52.50	15.8	36.15	15.4	64.00	18.7

The baseline characteristics of the Orthokin and placebo group were comparable (Table I), no significant differences between the treatment groups were found with respect to age, gender, weight, body mass index (BMI) or use of NSAIDs before and during the study period.

TREATMENT EFFECT

Both Orthokin and placebo-treated patients showed a significant improvement on all outcome measures ($P < 0.001$), as compared to baseline values. Comparable improvements were found for the Orthokin and placebo treatment on the WOMAC [28% vs 23% at 3 months, 15% vs 18% at 6 months, 14% vs 17% at 9 months, and 19% vs 13% after 12 months; n.s.; Table II, Figs. 2(A) and 3(A)]. On all outcome parameters, Orthokin-treated patients scored consistently better as compared to placebo-treated patients. However, the differences between the two treatment groups were small. With respect to improvement over time, Orthokin resulted in significantly more improvement for KOOS symptomatology ($P = 0.002$) and KOOS sport ($P = 0.042$), as compared to placebo treatment [Fig. 2(C) and (D)]. This coincides with the clinical observation that patients describe an initial effect on pain and subsequently choose for an increase in activities in daily life (ADL), in sports and in hobby. Furthermore, Orthokin-treated patients consistently showed higher relative improvements compared to placebo-treated patients for all other outcome parameters, except for the VAS at 3 months (21% vs 25%), the KOOS sport at 3 and 9 months (41% vs 43%, and 42% vs 43%, respectively), and the KOOS ADL at 6 and 9 months (15% vs 17%, and 13% vs 16%, respectively),

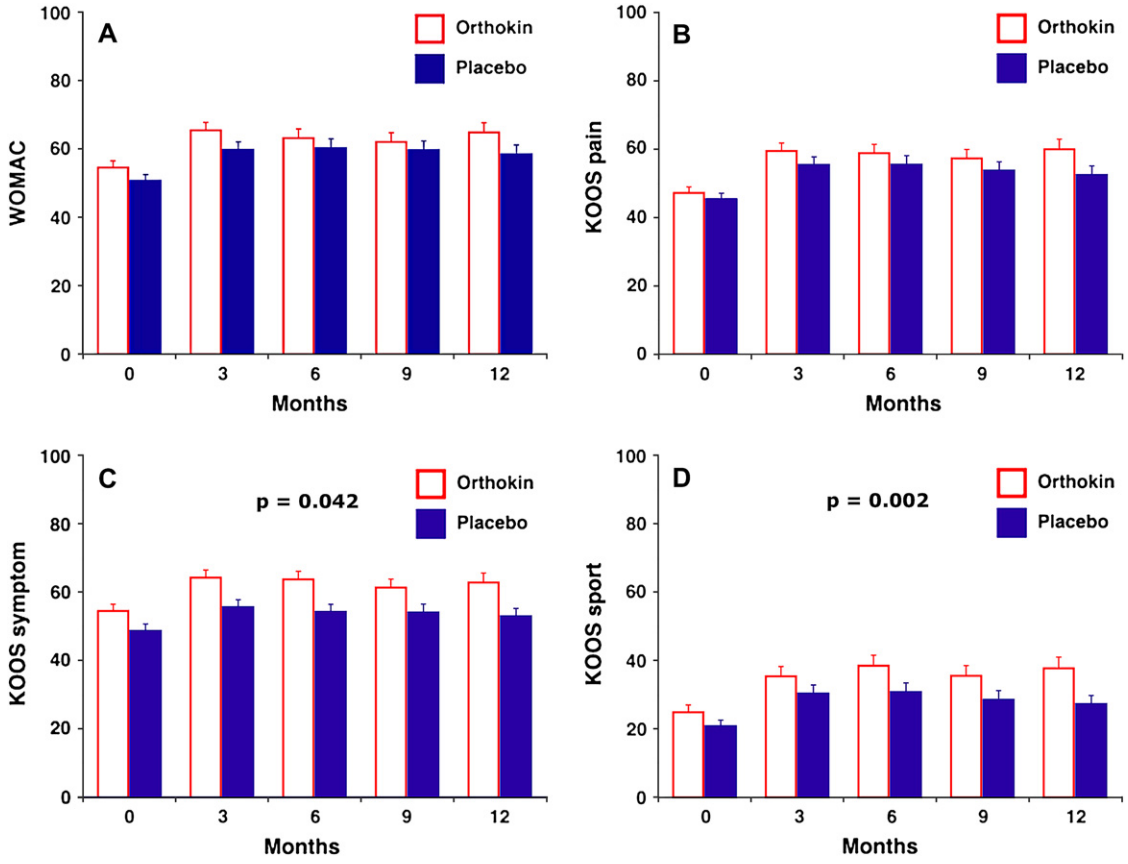

Fig. 2. These graphs show the effect of Orthokin and placebo treatment over time on separate KOOS items. (A) WOMAC, (B) KOOS pain, (C) KOOS symptomatology, and (D) KOOS sport. Bars represent mean ± S.E.M.

although none of these differences were statistically significant. Treatment failures were equally distributed over both treatment groups (Orthokin 8, placebo 7; chi-square: $P = 0.954$), and were mainly due to worsening of symptoms for which these patients were treated either with NSAIDs or by surgical intervention.

Sub-analysis for age (<45 and >45 years old) and gender did not show statistically significant differences in responsiveness between these subgroups. In addition, correlation analysis did not show significant relations between baseline patient characteristics (age, gender, symptom severity) and treatment outcome. Interestingly, the superior improvement resulting from Orthokin treatment, as compared to placebo treatment, appeared even more pronounced upon sub-analysis for the patients who continued using NSAIDs during the trial (Fig. 3 and Table II). For these patients ($n = 15$), repeated measure analysis showed that Orthokin treatment resulted in statistically significant more improvement of the KOOS sport parameters as compared to placebo treatment ($P = 0.011$; Fig. 3C). Furthermore, Orthokin resulted in significantly more improvement of the KSCRS, surgeons part: as compared to placebo treatment ($P = 0.005$; Table II).

ADVERSE EVENTS

During the trial, 219 adverse events were reported (Table III). One hundred and fifty-nine adverse events were knee related, of which the majority was attributable to (subjective) increase of knee pain (Orthokin: 44 vs Placebo: 50; n.s.). The involved surgeons graded two adverse events as serious; both cases were Orthokin-treated patients. The first serious adverse event was due to a patient with a septic arthritis of the knee joint. Because the product was injected through a 0.22-μm sterile filter and because no bacterial contamination was demonstrated by microbiological testing of the sample, it was concluded that this event was caused by the injection procedure and not by contamination of the product. The second adverse event was related to a patient with repeated severe inflammatory reactions of the knee joint within hours after the injection, as reflected by severe pain, swelling, and warmth of his knee joint. As a result, this patient discontinued the treatment after three injections.

Discussion

Due to its high prevalence (6−12% of the adult population), OA is associated with high cost for society induced by healthcare consumption and lost of productivity at work[29,30]. These costs are expected to increase even more due to the ageing of the population. Therefore, over the last decades, an increasing interest for drugs that may alter the course of OA development (DMOADs) and thereby possibly delaying or even prevent the need for surgical interventions, such as total joint replacement has developed. Orthokin is an autologous blood product in which the production of various anti-inflammatory cytokines, such as IL-1ra, have been

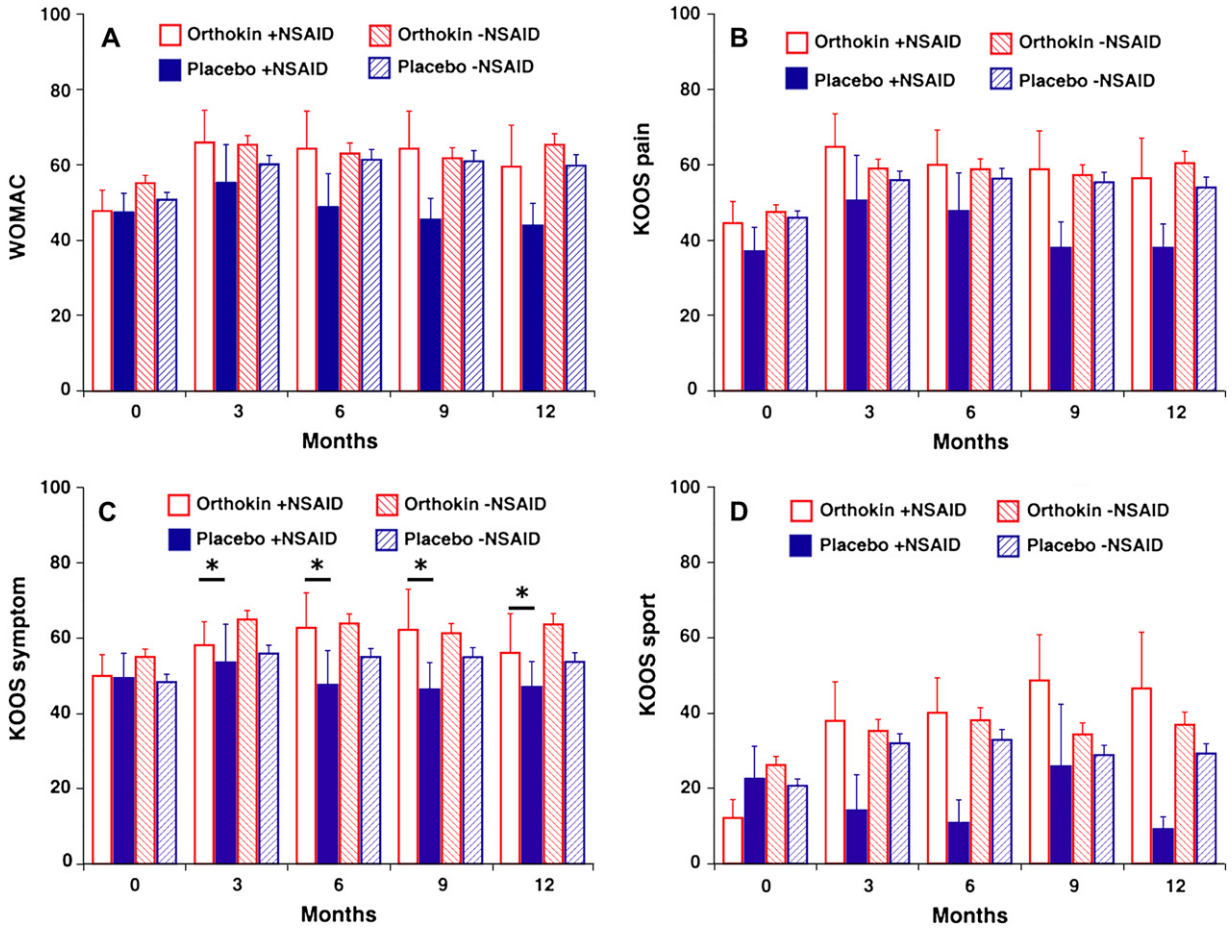

Fig. 3. These graphs show the effect of Orthokin and placebo treatment over time on separate KOOS items subdivided for NSAIDs (+NSAID) and the non-NSAID (−NSAID) strata. (A) WOMAC, (B) KOOS pain, (C) KOOS symptomatology, and (D) KOOS sport. Bars represent mean ± S.E.M., * indicated statistically significant difference between groups.

demonstrated to be upregulated[28]. By competitive inhibition of IL-1β in OA knee joints, this product may have a beneficial effect on the development of degenerative articular changes.

The aim of the current study was to evaluate the efficacy of Orthokin for treatment of knee OA in a randomized, multicentre, double-blind, placebo-controlled trial with a primary efficacy objective to demonstrate 30% superiority of the Orthokin treatment on the WOMAC OA index at 3, 6, 9, and 12 months post-treatment, as compared to placebo treatment. In the current trial this objective was not met, as the WOMAC showed comparable improvement ratios for Orthokin and placebo treatment. However, absolute values of the WOMAC and most other outcome measures showed that Orthokin-treated patients scored consistently better, compared to placebo-treated patients. In addition, Orthokin treatment was found to result in statistically significant more improvement on the KOOS symptomatology scores and the KOOS sport parameters. Altogether, these findings suggest a beneficial biological effect of Orthokin on clinical symptoms arising from knee OA.

This hypothesis is further supported by the data set of patients who continued NSAID use during the trial (NSAID group), in which Orthokin treatment resulted in even more apparent improvement of the KOOS sport parameter and, in addition, induced an improved knee function as measured by the surgeon on the KSCRS. Furthermore, when comparing the absolute values of the NSAID and the non-NSAID group, a striking trend was observed, namely that patients who were treated with Orthokin in combination with NSAIDs showed improvement similar to or more than both Orthokin and placebo-treated patients of the non-NSAID group. In contrast, for the placebo-treated patients of the NSAID group, improvements were much smaller, or not observed at all. This may be explained by the fact that all patients were required to stop all analgesics, including NSAIDs, 1 week before completing the questionnaires and visiting their treating surgeon. Patients using NSAIDs experienced more pain during this week than those not requiring NSAIDs, an effect that was not seen in the NSAID group treated with Orthokin. The absence of this trend for Orthokin-treated patients is at least interesting, as this finding supports the hypothesis that Orthokin induces a beneficial biological effect.

Despite these findings, incorporation of Orthokin to the standard spectrum of treatment modalities for symptomatic knee OA needs careful consideration. The clinical benefit found in the current study was small and did not meet the initial trial objective of 30% superiority on the WOMAC. This may be due to several causes.

Based on the WOMAC outcome scores, the placebo effect found in the current study was high, but comparable to other studies studying the efficacy of various oral and intra-articular

Table III
An overview of all adverse events registered during the trial. Prevalences of adverse events were compared using the chi-square test. No statistically significant differences were found, except for back pain

	Orthokin, $n = 80$	Placebo, $n = 73$
Knee related adverse events		
Pain during injection	10	8
Irritation after injection	70	67
Increase knee pain	44	50
Septic arthritis	1	0
Severe inflammatory arthritis (non-septic)	1	0
Foreign body sensation	0	2
Crepitations	1	1
Locking	1	3
Irritation/pain	3	3
Swelling/synovitis	10	3
Redness/warmth	3	1
Stiffness	2	1
Muscle cramps	2	3
Heavy feeling	1	0
Injection in wrong (contra-lateral) knee joint	1	0
Baker's cyst	1	0
Meniscus degeneration	1	0
Ligament ruptures (ACL/MCL)	1	1
Other musculoskeletal adverse events		
Foot pain	1	1
Back pain ($P = 0.009$)	0	6
Hip pain	2	1
Shoulder pain/cuff tendonitis	2	1
Achilles tendon swelling	0	1
Stiffness of other joints	0	1
Hotspot at cuboid − metatarsal 5 joint	0	1
Heel pain	0	1
Fall on knee/knee distortion	9	5
Contra-lateral knee injury	4	1
General adverse events		
Flu	4	1
Pneumonia	1	0
Jaw/molar inflammation	1	1
Infection	1	1
Headache/migraine	2	1
Nephrolithiasis	2	0
Groin pain	1	0
Hysteroscopy	1	0
Endometrium polyp	1	0
Hypertension	0	1
Admission to rehabilitation clinic	1	0
Hospital admission due	1	0
Weight reduction	0	1
Weight increase	0	1

treatment modalities. The latter observation supports the validity of the current data set[11,31–33]. However, demonstrating a beneficial effect of new treatment modalities is hampered by such large placebo effects. Furthermore, it was recently demonstrated that the patients' perception of ones health status and their symptoms of OA changes over time[34].

This phenomenon can have a significant impact on evaluating the effectiveness of interventions, which is known as response shift. This aspect is not taken into account in the current study, which has been demonstrated to increase the risk of a type-2 error[34,35]. On the other hand, it may be argued that the treatment dose was to low, i.e., the small Orthokin volume. However, the treatment was performed strictly according to the manufacturer's instructions. Furthermore, assuming that IL-1ra is the main effective mediator of the Orthokin treatment, 2 ml of Orthokin provides sufficient IL-1ra intra-articularly in order to provide complete inhibition of IL-1β[28,36–39]. Finally, as OA is a slowly progressive degenerative disease with gradually evolving symptomatology, most OA patients are used to a certain degree of pain. As a result, these patients may increase their activity rate until an acceptable degree of complaints is reached, thereby demonstrating improvement on activity scales (KOOS sport), but suppressing improvements on all other scales.

Altogether, autologous induced synthesis of anti-inflammatory cytokines seems an interesting and possibly effective approach in the treatment of symptomatic knee OA. However, Orthokin is based on human serum and therefore, the quality of the product may be variable among different patients. Also, apart from the upregulation of IL-1ra, IL-4, IL-10, and IL-13, the modulation of numerous other proteins by the incubation of blood with $CrSO_4$-coated glass beads has not been clarified. Furthermore, the clinical relevance of the demonstrated differences is disputable. Other, cheaper, less invasive, and effective treatments, such as NSAIDs and COX-2 inhibitors, are available for reducing symptoms from knee OA[5,6,40]. In addition, results from recent *in vitro* studies suggest that selective COX-2 inhibitors, such as Celecoxib, may be an effective DMOAD[41,42]. Nevertheless, specifically the chondroprotective effect of Orthokin on articular cartilage was not studied in the current trial, while potentially being the most important aspect of this treatment. However, the follow-up period of the current trial was to short to reasonably expect a detectable protective effect on knee radiographs. Therefore, we are in the process of to determining the chondroprotective effect of Orthokin by analysis of knee radiographs after an extended follow-up period, and, in addition, by determining cartilage breakdown products, and pro- and anti-inflammatory cytokines in synovial fluid samples from this patient cohort.

Furthermore, cartilage defects have been demonstrated to result in a disturbed joint homeostasis, which negatively affects the outcome of cartilage tissue engineering techniques[43,44]. As cartilage defects can lead to the development of OA[45,46], the disturbed joint homeostasis induced by cartilage defects may very well occur through similar pathways as those responsible for OA development. Therefore, Orthokin may provide a feasible approach for further optimization of cartilage tissue engineering techniques, such as autologous chondrocyte implantation, although this hypothesis remains to be studied in future clinical trials.

In conclusion, Orthokin appears to have a beneficial biological effect on patient documented symptoms arising from knee OA and warrant future investigations into the possible chondroprotective effect, although the improvement in symptomatology seems not clinically relevant.

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