Low-dose intra-articular autologous conditioned serum in treatment of primary knee osteoarthritis

Fatma K. Abd-EL Motaal, Amal M. Elganzoury, Mahmoud M. Fathalla, Ola Abdulkareem

Department of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Correspondence to Mahmoud M. Fathalla, MD, Department of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, Cairo, Egypt
Tel: +20 2 2418 44 00; fax: +20 2 2917855; e-mail: mmfathalla@yahoo.com

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Background
Interleukin-1 (IL-1) plays an important role in the pathogenesis of osteoarthritis. Hence, agents that inhibit such cytokine have a high therapeutic potential. A method of therapy depends on competitive inhibition of IL-1 at the receptor level – that is, IL-1 receptor antagonist; such antagonist is called Orthokin, which is a normal product of monocytes and is prepared within autologous conditioned serum (ACS) from the patient's own blood cells. It is capable of blocking the effects of IL-1, including the induction of matrix metalloproteinases, prostaglandin E_2 synthesis, and expression of other cytokines.

Objective
The aim of the study was to clinically evaluate the effect of intra-articular injection of low-dose ACS enriched with Orthokin on primary knee osteoarthritis to assess its validity in treatment.

Patients and methods
This study included 30 knees with primary osteoarthritis. Baseline clinical evaluation using WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index) score was performed. Then ACS enriched with Orthokin (IL-1 receptor antagonist) was prepared. The knee joint was injected with 1 ml ACS weekly for 3 successive weeks. Patients were assessed using WOMAC questionnaire (1 week after each injection for 3 weeks and monthly after the last injection for 3 months).

Results
On comparing WOMAC score with baseline data, there was a highly significant improvement in all scores, where *P* was less than 0.01 during all assessment periods and improvement persisted until the end of follow-up after 3 months in comparison with baseline data.

Conclusion
The synthesis and introduction of interleukin-1 receptor antagonists derived from own blood cells established a promising strategy in the treatment of osteoarthritis.

Keywords:
autologous conditioned serum, interleukin-1 receptor antagonist, osteoarthritis, WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index)

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Introduction

Osteoarthritis (OA) is the most common, multicomponent joint disease mainly characterized by destruction of articular cartilage, joint pain, tenderness, crepitations, occasional effusion and physical disability [1]. The pathogenesis of the arthritic process depends on inflammatory mediators released from mononuclear cells, synoviocytes, and chondrocytes, such as interleukin-1 (IL-1), which suppress the synthesis of collagen type II, characteristic of hyaline cartilage and reducing aggrecan synthesis [2].

There is no consensus on what is the best treatment to improve OA symptoms and slow disease progression but the goal of treatment is to reduce pain and inflammation and improve joint function. Various conservative methods may be used to achieve this goal, such as weight reduction, physical therapy, systemic and topical NSAIDs, acetaminophen, analgesics, intra-articular steroids, or viscosupplementation to restore the thickness of synovial fluid and improve joint lubrication. Finally, surgery is reserved for patients with severe OA who were unresponsive to the conservative measures [3].

Nowadays, disease-modifying osteoarthritis drugs (DMOADs) would be highly desirable adjuncts to symptomatic relief as they may delay the disease process. They can be divided into three groups on the basis of their predominant mode of action: those targeting cartilage, inflammatory pathways, and subchondral bone. IL-1 inhibitors are considered as DMOADS [4].

IL-1 plays an important role in osteoarthritis. Hence, agents that inhibit the action of such cytokine have a high therapeutic potential in OA. Alternative method of therapy depends on competitive inhibition of IL-1 at the receptor level — that is, IL-1 receptor antagonist

(IL-1Ra); such antagonist is called Orthokin, which is a normal product of monocytes and is prepared within autologous conditioned serum (ACS). It is capable of blocking the effects of IL-1, including the induction of matrix metalloproteinases, nitric oxide, prostaglandin E_2 synthesis as well as expression of other cytokines [2].

Consequently, this study aimed to clinically evaluate the effect of intra-articular injection of low-dose ACS enriched with Orthokin on primary osteoarthritic knee to assess its validity in treatment.

Patients and methods

This was a prospective study that was approved by the ethics committee of the Ain Shams faculty of medicine. Informed consent was obtained from all participating individuals before initiating any study-related activities.

Patient selection

This study included 30 knees with primary OA recruited from physical medicine and rheumatology department of Ain Shams University Hospitals (Cairo, Egypt). Patients were diagnosed according to the ACR classification of OA of the knee [5].

Inclusion criteria were painful primary knee osteoarthritis, radiologically proven (Kellgren and Lawrence grade I–III) [6] and requiring the prescription of a symptomatic treatment of pain.

Exclusion criteria included knee OA grade IV by Kellgren and Lawrence, patients receiving antiosteoarthritic treatments, hyaluronic acid, knee tidal lavage, and secondary causes of knee OA, such as rheumatic, septic, endocrinal, metabolic, and traumatic disorders, osteonecrosis, osteochondritis, Ehler Danlos syndrome, hyperlaxity syndrome, and Paget's disease.

Baseline clinical and radiological evaluation

Baseline clinical evaluation using WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index) score was performed.

ACS preparation and intra-articular injection

Induction of ACS [7]

A volume of 10 ml whole blood was withdrawn from the same patient without anticoagulants using 10 ml plastic syringes containing glass beads to initiate monocyte activation and increase internal surface area. Blood was incubated aseptically at 37°C, 5% CO_2 for 6 h. After incubation, blood was centrifuged at 3500 rpm for 10 min then 3 ml serum was retrieved, aliquoted into three portions, 1 ml each, and stored at −20°C until use.

Now, the ACS enriched with IL-1Ra (Orthokin) is ready for intra-articular injection. The knee joint is injected with 1 ml ACS weekly for 3 successive weeks.

Intra-articular knee injection

Patient is placed in the supine position with knee flexed 80°. After sterilization, local anesthesia is injected 1 inch below and lateral to the lower pole of the patella then ACS is injected intra-articularly using filter.

Outcome assessment

Patients were assessed using Likert version of WOMAC Questionnaire for seven times (preinjection, 1 week after each injection for 3 weeks, and monthly after the last injection for 3 months). Such version of WOMAC Questionnaire contains 24 items with five subscales for pain, two for stiffness, and 17 for physical function [8].

Three dimensions were assessed: pain, stiffness, and physical function. All questions reflect the patients experience over the past 48 h.

The Likert version uses descriptive adjectives (none, mild, moderate, severe, and extreme) translated to numerical ordinal scale (0–4); lower scores indicate lower levels of dysfunction. Score range for pain, stiffness, and physical function subscales is summed to a maximum score of 20, 8, and 68, respectively [8].

Statistical analysis

Statistical analysis was performed using statistical package for social science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, Illinois, USA). Before statistical analysis, the Kolmogorov–Smirnov test was performed to assess the normality of the continuous data. The main comparative analysis, within the treatment group, was performed using the Wilcoxon signed-rank test to assess the statistical significance of the difference of nonparametric quantitative variables, where P value less than 0.05 was the level of significance.

Results

Patients age ranged from 42 to 67 years with a mean of 54.21 (5.95) years. The percentage of male and female patients was 42 and 58, respectively. Baseline WOMAC scores (total and subscores) are shown in Table 1 and Fig. 1. The Kolmogorov–Smirnov test

revealed that continuous outcome data were not normally distributed; hence, nonparametric analysis was performed. Comparison between baseline WOMAC score (total and subscores) with scores recorded during different assessment periods (1 week after first, second, and third injections and 1, 2, and 3 months after last injection) is shown in Tables 2–5 and Fig. 1.

Discussion

A randomized controlled trial (the German Osteoarthritis Trial) was conducted by Baltzer *et al.* to study the clinical efficacy of high-dose ACS enriched with IL-1Ra in OA patients. A total of 126 osteoarthritic patients received intra-articular knee injection with 2 ml ACS twice weekly for 3 successive weeks, and the outcome was assessed using WOMAC score at 7, 13, and 26 weeks postinjections [9,10]. In addition, Yang *et al.* [11] investigated 167 patients with osteoarthritis knee after receiving the same high dose of ACS intra-articularly (twice weekly for 3 successive weeks), and the outcome was assessed by the knee injury and osteoarthritis outcome score 3, 6, 9, and 12 months postinjection.

In our study, although low-dose ACS was used (only 1 ml ACS injected intra-articularly once weekly for 3 successive weeks), the outcomes showed similar results to that of Baltzer and Yang, where there was significant reduction in WOMAC score (total and subscores) after 13 weeks follow-up ($P < 0.01$) in both studies. Our results suggest that low-dose ACS gives a similar result to high dose of ACS; in addition, reducing the dose and number of injection times in our study may help to reduce the side effects of intra-articular injection in comparison with Yang study where serious adverse events were observed in the high-dose ACS group: one patient with severe inflammatory reaction 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.

In addition, our results are supported by that of Baltzer *et al.* [12]who studied intra-articular injection of hip with ACS, where they reported that treatment resulted in a statistically significant improvement for patients and that neither cortisone nor cortisone with recombinant IL-1 receptor antagonist increased the beneficial treatment effect over and above that of ACS alone. In such study, a high dose of ACS was injected (5.94 ± 0.03 injections of 2 ml each) and patient assessment was performed using visual analogue scale (VAS) for pain only, but, in our study, a low-dose ACS was used and evaluations were performed using WOMAC (total and subscores).

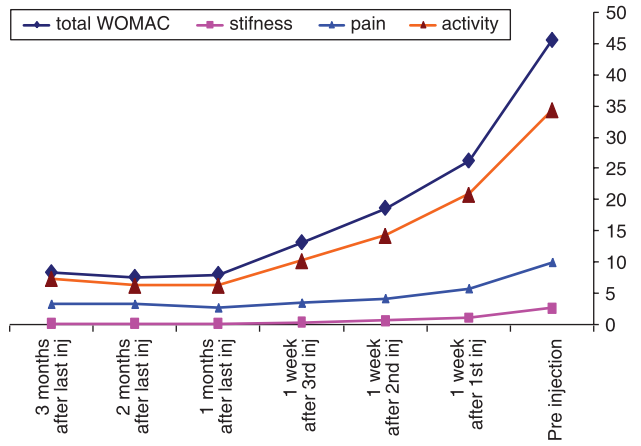
In another study conducted by Chevalier *et al.* [13], 13 osteoarthritic patients received 150 mg recombinant human IL-1Ra (Anakinra) intra-articularly twice weekly for 3 successive weeks and follow-up was

Table 1 Preinjection WOMAC score (total and subscores)

Scores		Preinjection		
	N	Mean (SD)	Minimum	Maximum
Total WOMAC score	30	45.63 (9.99)	29.00	63.00
Stiffness score	30	2.61 (1.04)	0.00	4.00
Pain score	30	9.87 (2.11)	6.00	14.00
Daily physical function score	30	34.40 (7.72)	21.00	48.00

WOMAC, western ontario and McMaster universities osteoarthritis index.

Figure 1



WOMAC score (total and subscores) at different intervals of assessment.

Table 2 Comparison of total WOMAC score with preceding and preinjection scores

	Total WOMAC score				Vs. preceding score		Vs. preinjection	
	Mean	SD	Range		Z	*P*	Z	*P*
Preinjection	45.63	9.99	29.0	63.00				
One week after first injection	26.23	11.50	6.00	53.00	4.78	0.000*	4.78	0.000*
One week after second injection	18.63	10.77	1.00	47.00	4.31	0.000*	4.78	0.000*
One week after third injection	13.10	9.81	2.00	41.00	4.23	0.000*	4.71	0.000*
One month after last injection	8.00	7.28	2.00	26.00	4.49	0.000*	4.62	0.000*
Two months after last injection	7.52	6.91	2.00	26.00	0.77	>0.05	4.71	0.000*
Three months after last injection	8.27	5.45	3.0	24.0	0.86	>0.05	4.79	0.000*

Wilcoxon signed-rank test. WOMAC, western ontario and McMaster universities osteoarthritis index, *$P < 0.05$ is significant.

performed until 12 weeks by VAS and WOMAC scores. Such study showed a significant improvement of VAS and WOMAC scores after 12 weeks ($P = 0.008$ and 0.005, respectively). Later, in 2009, another study was performed by the same author where they reported that single intra-articular injection of 50 or 150 mg Anakinra was not associated with improvements in OA symptoms compared with placebo [14]. Our study shows that intra-articular injection of three doses of autologous IL-1Ra provides same results as six doses of recombinant human IL-1Ra.

Oral IL-1 inhibitors are available in the form of the semisynthetic oral anthraquinone diacerein, which is a novel chondroprotective agent intended for the treatment of osteoarthritis where rhein (an active metabolite of diacerein) interferes with IL-1 (an inflammatory mediator), downregulates the gene expression and production of promatrix metalloproteinases, and upregulates the tissue inhibitors of metalloproteinase-1 production [1,15,16].

A study conducted by Brahmachari *et al.* [17] included 64 patients suffering from OA knee who received diacerein 50 mg twice daily for 8 weeks followed by VAS and WOMAC physical function score assessment where VAS showed highly significant changes ($P < 0.01$) and physical function showed a significant change ($P < 0.05$). In our study, the pain and physical function scores showed a highly significant change in both ($P = 0.007$ and $P < 0.0001$, respectively) after 2 months. It is to note that diacerein was classified as a treatment of uncertain appropriateness in the OA Research Society International 2014 guidelines for the nonsurgical management of knee osteoarthritis where 29 treatment modalities were considered for recommendation [18]. In addition, diacerein causes side effects in the form of diarrhea, loose stool, and colic and urine discoloration, which was absent with low-dose ACS intra-articular injection.

Another inhibitor of IL-1 is botulinum toxin, as it inhibits Rho GTPase, which is necessary for activation of the IL-1 inflammatory pathway [19]. In 2010, a study was performed on 14 cases with grade III Kellgren and Lawrence OA knee; each knee was intra-articularly injected twice with 100 U botulinum toxin for two

Table 3 Comparison of stiffness subscore with preceding and preinjection scores

	Stiffness score	Vs. preceding score		Vs. preinjection	
	Mean (SD)	Z	*P*	Z	*P*
Preinjection	2.61 (1.04)				
One week after first injection	1.06 (0.80)	3.50	0.006*	3.50	0.006*
One week after second injection	0.61 (0.61)	2.31	0.01*	3.77	0.005*
One week after third injection	0.28 (0.57)	1.91	0.03*	3.76	0.005*
One month after last injection	0.11 (0.32)	2.12	0.01*	3.76	0.005*
Two months after last injection	0.11 (0.32)	0.00	1	3.76	0.005*
Three months after last injection	0.11 (0.32)	0.00	1	3.76	0.005*

Wilcoxon signed-rank test, *$P < 0.05$ is significant.

Table 4 Comparison of pain subscore with preceding and preinjection scores

	Pain score			Vs. preceding score		Vs. preinjection	
	Mean (SD)	Minimum	Maximum	Z	*P*	Z	*P*
Preinjection	9.87 (2.11)	6.00	14.00				
One week after first injection	5.69 (2.38)	1.00	10.00	4.73	0.000*	4.73	0.000*
One week after second injection	4.12 (2.16)	1.00	10.00	3.97	0.002*	4.47	0.000*
One week after third injection	3.40 (2.44)	1.00	8.00	2.28	0.01*	3.93	0.002*
One month after last injection	2.64 (2.10)	1.00	6.00	3.26	0.007*	3.52	0.006*
Two months after last injection	3.22 (2.11)	1.00	6.00	0.74	>0.05	3.33	0.007*
Three months after last injection	3.22 (2.11)	1.00	6.00	0	1	3.33	0.007*

Wilcoxon signed-rank test, *$P < 0.05$ is significant.

Table 5 Comparison of the daily physical function subscore with preceding and preinjection scores

	Daily physical function score			Vs. preceding score		Vs. preinjection	
	Mean (SD)	Minimum	Maximum	Z	*P*	Z	*P*
Preinjection	34.40 (7.72)	21.00	48.00				
One week after first injection	20.83 (8.65)	6.00	42.00	4.79	0.000*	4.79	0.000*
One week after second injection	14.23 (8.01)	1.00	34.00	4.49	0.000*	4.79	0.000*
One week after third injection	10.17 (7.29)	0	30.00	4.33	0.000*	4.71	0.000*
One month after last injection	6.23 (5.03)	0	19.00	4.48	0.000*	4.63	0.000*
Two months after last injection	6.23 (5.03)	0	19.00	0	1	4.63	0.000*
Three months after last injection	7.33 (3.85)	1	19.00	0.84	>0.05	4.52	0.000*

Wilcoxon signed-rank test, *$P < 0.05$ is significant.

doses (3 months interval between both injections) and WOMAC assessment was performed monthly for 6 months initiating from the first injection. There was a highly significant change in the WOMAC pain and stiffness scores ($P = 0.001$ and 0.009, respectively) after 1 month from injection, with no significant changes for total WOMAC and physical function scores ($P = 0.245$ and 0.821, respectively), whereas after 3 months there were no significant changes in WOMAC scores (total and subscores) ($P > 0.05$) [20]. In our study, after 1 month, significant improvement in pain, stiffness, physical function, and total WOMAC scores was present, where P was less than 0.001, less than 0.001, less than 0.0001, and less than 0.0001, respectively, and improvement persisted until the end of assessment after 3 months in comparison with baseline data.

Conclusion

It can be concluded that ACS enriched with Orthokin is easily prepared and has good functional and psychological satisfaction in knee OA grade I to III, with no risk for disease transmission because of autologous serum intra-articular injection. In addition, the synthesis and introduction of autologous IL-1 receptor antagonists derived from own blood cells established a promising strategy in the treatment of osteoarthritis.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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