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# Autologous Conditioned Serum in the Treatment of Orthopedic Diseases

## The Orthokine® Therapy

*Peter Wehling*,[1,2] *Carsten Moser*,[2,3] *David Frisbie*,[4] *C. Wayne McIlwraith*,[4] *Christopher E. Kawcak*,[4] *Ruediger Krauspe*[3] and *Julio A. Reinecke*[5]

1 Centre for Molecular Orthopaedics, Düsseldorf, Germany
2 Orthogen AG, Düsseldorf, Germany
3 Department of Orthopaedics, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany
4 Department of Clinical Sciences, Equine Orthopedic Research Center, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA
5 Orthogen Veterinary GmbH, Düsseldorf, Germany

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## Abstract

The common strategies for the treatment of patients with orthopedic diseases do not address the underlying pathogenesis. Several biologically based, local therapies aiming to influence the cytokine imbalance are either in development or in the initial stages of clinical use. A method based on exposure of blood leukocytes to pyrogen-free surfaces (e.g. glass spheres) elicits an accumulation of anti-inflammatory cytokines, including interleukin-1 receptor antagonist, and several growth factors, including insulin-like growth factor-1, platelet-derived growth factor, and transforming growth factor-$\beta_1$, in the liquid blood phase. Based on these observations, a new therapy using cell-free, autologous conditioned serum (ACS) from the incubation of whole blood with glass spheres was developed. The injection of ACS into affected tissue(s) has shown clinical effectiveness and safety in animal models and studies, as well as in human clinical studies, for the treatment of osteoarthritis, lumbar stenosis, disc prolapse, and muscle injuries.

A number of the commonly practiced orthopedic treatment options lack adequate documentation of efficacy.[1] Surgical methods of preserving and restoring articular cartilage are practiced, but their effectiveness has not been clearly proven in randomized, controlled trials.[2,3] Joint arthroplasty is an excellent treatment for advanced osteoarthritis, but is limited to the end stages of osteoarthritis. Although analgesics and NSAIDs are used widely, their efficacy has been questioned.[4-7] The frequent and serious adverse effects of NSAIDs and corticosteroids have recently been highlighted.[8-12] In addition, randomized, controlled clinical studies of

intra-articular injections of hyaluronan against osteoarthritis have shown variable results, some trials suggesting long lasting pain relief, and others failing to show a difference between hyaluronan and placebo.[13-17]

The need for additional evidence-based treatments stimulates the continuing search for alternative, symptom-modifying and possibly disease-modifying drugs for use in orthopedic diseases. The ultimate aim is to address the underlying pathologic mechanisms.

## 1. Biologic Mechanisms and Targets in Orthopedic Diseases

In addition to biomechanical factors, which play a role in the onset of orthopedic diseases, the concomitant activation of a whole cascade of biologic factors within the cytokine network is important in disease pathogenesis.[18] The therapeutic use of cytokine inhibitors and growth factors was first proposed in the late 1970s and early 1980s. Growth factors, which target the revitalization of damaged tissue function by modulating the underlying pathologies, are playing an increasing role in the local treatment of orthopedic diseases. Within this context, osteoarthritis is characterized by a number of uniform molecular and biologic processes. One of the primary molecular pathologic features is the local release of cytokines that trigger the destruction of hyaline cartilage and its matrix. This leads to additional production of intracellular pro-inflammatory and destructive factors that promote the further destruction of hyalin cartilage by causing an imbalance in matrix turnover.[19]

Of the cytokines identified in orthopedic diseases, interleukin-1 (IL-1) appears to be of special importance.[20] Interleukin-1 receptor antagonist (IL-1ra) is a competitive receptor antagonist of IL-1, with affinity to type I and II IL-1 receptors.[21-23] One hypothesis is that the local IL-1ra concentration is too low in degenerative diseases to inhibit the destruction of cartilage, muscles, spine tissue, and other joint structures. Although differing in detail, published studies generally agree that a 10- to 1000-fold excess of IL-1ra over IL-1 is required to effectively block all of the available IL-1 receptors or the IL-1 triggered effects.[24-27] These data result from a number of different receptor binding experiments usually performed with recombinant forms of IL-1ra, and thus may not fully reflect the biologic function of the native *in vivo* polyglycosylated IL-1ra protein. At present, it is not clear that a complete blockage of all biologically active IL-1 receptors is necessary to significantly impact such pathologic conditions such as osteoarthritis or nerve inflammation. It is now evident that several natural and recombinant additional anti-inflammatory cytokines and soluble receptors which display differential dissociation rates for IL-1α, IL-1β, and IL-1ra,[28] can affect IL-1 receptor signaling and inflammatory conditions.

## 2. Production of Autologous Conditioned Serum (ACS) Utilizing Orthokine®

We describe a method that selectively increases the production of anti-inflammatory cytokines and growth factors using human blood. Arend and coworkers[25,29-32] demonstrated that surface-bound immunoglobulin G (IgG), in addition to molecules such as lipopolysaccharide (LPS), phorbol myristate acetate (PMA), IL-1, and tumor necrosis factor-alpha (TNFα), induces IL-1ra production by isolated human monocytes. A new technique has been developed that achieves similar IL-1ra induction without the need to coat surfaces with IgG.[33] Experimental testing of different materials such as plastic polymers, glass, and quartz led to the identification of borosilicate glass spheres as the most suitable surface to induce IL-1ra *de novo* production and accumulation without concurrent IL-1β production in whole blood.

Autologous conditioned serum (ACS) is generated by incubating venous blood in the presence of medical grade glass beads (Orthogen, Düsseldorf, Germany). Peripheral blood leukocytes produce elevated amounts of endogenous anti-inflammatory cytokines, including IL-1ra, which accumulates in the serum (figures 1 and 2; table I). Following centrifugation and extraction, ACS is portioned and either stored until use or injected into the affected region of the human or animal patient. In randomized controlled human clinical trials, injections were given in a series of

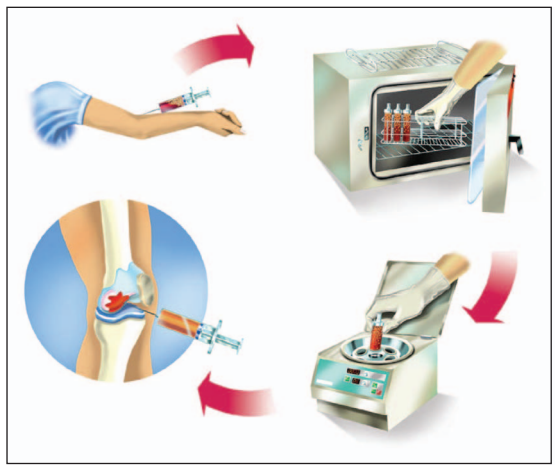

**Fig. 1.** Flowchart showing the processing of patient venous blood; Orthokine® BioLab (in humans) or interleukin-1 receptor antagonist protein (IRAP) VetLab (in horses) [courtesy Orthogen AG, Düsseldorf, Germany]. Cell-free autologous conditioned serum (ACS) is generated by incubation of aseptically aspirated venous blood in a syringe in the presence of medical-grade glass spheres. Peripheral blood leukocytes produce elevated amounts of different endogenous anti-inflammatory cytokines, such as interleukin-1 receptor antagonist, that are recovered with the serum. Following centrifugation and portioning, ACS is stored or aseptically injected into the affected region of the human or animal subject. In randomized controlled human clinical trials, injections were given in a series of 3 injections once weekly for spinal applications, or 6 injections twice weekly for the treatment of osteoarthritis.

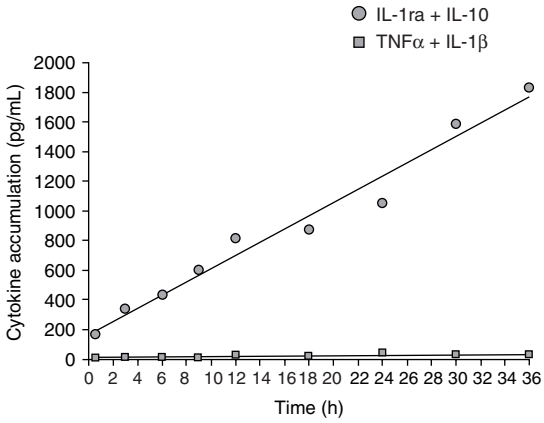


**Fig. 2.** Time course of cytokine induction in the Orthokine® system. During incubation of venous whole blood in the closed container of the 10mL Orthokine® syringe, cytokines accumulate in the serum fraction. The experiment depicted shows the selective and significant increase in the combined concentrations of interleukin (IL)-1 receptor antagonist (IL-1ra) and IL-10 versus the combined total of tumor necrosis factor-alpha (TNFα) and IL-1β. TNFα and IL-1β remain relatively stable. There were 19 independent samples per timepoint. Linear trend lines have been superimposed on the graph.

3 injections once weekly for spinal applications, or 6 injections twice weekly for the treatment of osteoarthritis.

The products released by mononuclear cells and platelets through ACS production are partly derived from intracellular reservoirs and partly synthesized *de novo*. A substantial part of the accumulating IL-1ra derives from *de novo* synthesis, as has been shown by cycloheximide inhibition of IL-1ra accumulation in human whole blood.[28] The list of cytokines present in ACS is probably much more extensive than the number that has been detected (see table I), and the mean concentrations of the cytokines detected showed a high standard deviation (SD), indicating that cytokine concentrations vary greatly between individuals. It is hypothesized that the clinical and preclinical effects detected when using ACS cannot be attributed to single components, but rather that the synergistic action of all factors present in the ACS contribute to the effect. Age stratification of human knee osteoarthritis study patients[34] did not reveal a clear correlation between recorded ACS IL-1ra levels and therapeutic effect. IL-1ra concentration alone may therefore not be significantly linked to clinical outcome. Taking into account that many more cytokines (growth factors) are accumulating in the serum, it stands to reason that clinical effects observed in the studies presented here may partially result from endogenous wound healing mechanisms activated by the exposure of blood cells to 'alien' surfaces, inducing mechanisms such as the coagulation cascade and tissue repair.

## 3. ACS in Animals

### 3.1 Controlled Equine Osteoarthritis Model

Joint disease, specifically osteoarthritis, is one of the most prevalent and debilitating diseases affecting horses. An experimental model of osteoarthritis has been used in horses for >10 years for assessment of the pathophysiologic processes and biologic markers of the disease, as well as evaluation of the efficacy of therapeutic substances in a controlled fashion.[34-37] A study undertaken at Colorado State University assessed the clinical, biochemical, and histologic effects in intra-articular administration of ACS in the treatment of experimentally-induced osteoarthritis in 16 horses.[34]

Osteoarthritis was induced arthroscopically in one middle carpal joint of each of the 16 horses. In 8 placebo- and 8 ACS-treated horses, 6mL of phosphate-buffered saline (PBS) solution or 6mL of ACS was injected into the osteoarthritis-affected joint on days 14, 21, 28, and 35, respectively; PBS solution was administered in the other sham-operated joint. Evaluations included clinical assessment of lameness and synovial fluid analysis (performed biweekly); gross pathologic and histologic examinations of cartilage, and synovial membrane samples were performed at necropsy. No adverse treatment-related events were detected. Horses that were treated with ACS had significant clinical improvement in lameness, unlike the placebo-treated horses (figure 3).

Synovial fluid examination showed an increase in the percentage of neutrophils and the total white blood cell count in joints with and without osteoarthritis that were treated with ACS. For instance, in osteoarthritic-affected joints treated with ACS, the neutrophil percentage was 11.4%, compared with 6.8% in the sham-operated joints. This change was not considered to be clinically significant.

The synovial fluid concentration of IL-1ra (as assessed by the use of human anti-IL-1ra antibody) was unchanged by induction of osteoarthritis or treatment with ACS throughout the study. When the IL-1ra concentration in synovial fluid was estimated by use of mouse anti-Il-1ra antibody, mean values calculated from both joints of the ACS-treated horses at days 35 and 70 were significantly increased ($p = 0.005$), compared with mean values calculated from both joints of the placebo-treated horses (figure 4). In a previous report[33] of ACS preparation from human blood, using the same method as our study, significantly increased the concentration of IL-1ra and presumably other anti-inflammatory factors in the serum samples. Our results suggest that that mouse anti-IL-1ra antibody is more appropriate than the human anti-IL-1ra antibody for estimating IL-1ra concentrations in those samples. In an earlier study, Frisbie et al.[38] assessed the administration of equine IL-1ra by gene therapy using enzyme-linked immunosorbent assay (involving human anti-IL-1ra antibody),

**Table I.** Content of cytokines and growth factors in human autologous conditioned serum (ACS) using 10mL whole blood in the Orthokine® system. The system is optimized to limit the accumulation of pro-inflammatory mediators. The list of cytokines present in the ACS is probably much more extensive than the number measured in this experiment. Note the high standard deviation (SD) of the individual mean concentrations detected indicating that cytokine concentrations vary greatly between individuals. Unless indicated, the values are from measurements of serum and/or unspecified plasma samples[a]

| | Cytokine | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | IL-1ra | IL-1β | IL-6 | TNFα | IL-10 | FGFb | VEGF | HGF | IGF1 | PDGF AB | TGFβ |
| No. of patients | 224 | 224 | 200 | 92 | 92 | 92 | 92 | 92 | 92 | 92 | 80 |
| **Concentration (pg/mL) prior to incubation (0 hours)** | | | | | | | | | | | |
| Basal[b] | 236 | <3.9 | <12.5 | <15.6 | <7.8 | 14.6 | 61 | 431 | 86 000 | 205 | 1 165 |
| **Concentration (pg/mL) after incubation (6 hours)** | | | | | | | | | | | |
| Mean | 2 014.8 | 7.9 | 28.7 | 10.1 | 33.4 | 26.6 | 508.6 | 1 339.3 | 117 208.8 | 39 025.6 | 97 939.0 |
| SD | 4 381.1 | 8.7 | 48.1 | 9.6 | 18.9 | 20.8 | 307.7 | 928.7 | 51 644.4 | 10 515.8 | 113 418.3 |
| Minimum | 390.3 | 1.4 | 0.9 | 3.0 | 4.1 | 2.8 | 114.1 | 691.4 | 37 430.0 | 19 601.0 | 13 067.0 |
| Maximum | 31 057.0 | 48.9 | 250.2 | 69.7 | 105.0 | 104.5 | 1 694.0 | 6 473.0 | 440 000.0 | 66 208.0 | 823 000.0 |

a All measurements were performed using enzyme-linked immunoabsorbant assay kits (ELISA; R&D Systems, Minneapolis, MN, USA). Basal values are normal values of healthy donor as measured by the kit manufacturer. Serum retrieved from 10mL of whole blood.

b All basal levels given with a '<' are lower limits of the kit's sensitivity. The accuracy of readings lower than these values are suboptimal.

**FGFb** = fibroblast growth factor-b; **HGF** = hepatocyte growth factor; **IGF1** = insulin-like growth factor-1; **IL** = interleukin; **PDGF** = platelet-derived growth factor; **TGF**β = transforming growth factor-β; **TNF**α = tumor necrosis factor-α; **VEGF** = vascular endothelial growth factor.

and found that synovial fluid concentrations of IL-1ra increased by an order of magnitude greater than that detected in the present study. Although it is clear that the gene therapy protocol was more effective, it would seem that, based on the mouse antibody, the ACS treatment in the horse certainly does increase the amount of equine IL-1ra protein. We are currently doing further work to confirm this with mass spectroscopy.

Another interesting finding in this study was that the mean synovial fluid IL-1ra concentration of all joints of horses in the ACS-treated group significantly increased with time, compared with the mean value in all the joints in horses in the placebo-treated group. This difference became apparent at day 35 and was still evident at day 70. Although the IL-1ra concentration was numerically highest in the osteoarthritis-effected joints in which ACS was directly administered, it appears that the increased synovial fluid IL-1ra concentration was a systemic event. A similar finding was also apparent after gene transfer of equine IL-1ra.[38] These data may suggest the administration of IL-1ra stimulates endogenous production of IL-1ra.

At necropsy examination, the total score for articular cartilage erosion and synovial membrane hemorrhage in osteoarthritis-affected joints treated with ACS was improved (i.e. decreased from $4.2 \pm 0.6$ to $2.9 \pm 0.6$), but this difference was not significant ($p = 0.093$). The histologic evaluation of the synovial membrane revealed a significant decrease in the degree of intimal hyperplasia in osteoarthritis-affected joints treated with ACS (score, $0.4 \pm 0.3$), compared with osteoarthritis-effected joints treated with placebo (score, $1.3 \pm 0.3$).

Results of this study (the first controlled study in the horse) indicated that there were significant clinical and histologic improvements in the osteoarthritis-affected joints of horses following treatment with ACS compared with placebo. On the basis of these findings, further controlled clinical trials to assess this treatment are warranted, and investigation of the mechanisms of action of ACS should be pursued concurrently.[39]

### 3.2 Treatment of Muscle Injuries by Local Administration of Conditioned Serum in Mice

Experimental and clinical results using local administration of conditioned serum in animals and humans in the treatment of muscle injuries were reported by Wright-Carpenter et al.[40,41]

In short, muscle injuries represent a major part of sports injuries and are a challenging problem in traumatology. Strain injuries and contusions are common muscle injuries; they can lead to significant pain and (sometimes lasting) disability. Despite the frequency of strain injuries, traditional treatment is limited and generally not sufficient to accelerate muscle regeneration efficiently when fast resumption of sports activity is a primary target (e.g. professional athletes). Several growth factors play a specific role in tissue regeneration, but if available through pharmacy for systematic

application often bear the problem of being 'doping' relevant. Additionally, recombinant growth factors need to be applied in rather high dosages, with the concomitant risk of adverse effects. ACS prepared using the Orthokine® [1] technique contains increased concentrations not only of cytokines but also of growth factors, compared with baseline values in plasma. Therapeutic levels are still in the physiologic range and, locally applied, with this low local concentration (in comparison with systemic recombinant growth factors) there is no systemic load for the patient. Therefore the health risk is expected to be low and not doping relevant in respect to performance-enhancing substances. The factors measured in mouse serum by Wright-Carpenter and colleagues[40,41] showed an increase after 24 hours of incubation.

In both a controlled animal study with mice and a patient observation with human professional athletes, the subjects were treated with ACS versus placebo (mouse) or ACS versus the commonly used Actovegin® (a deproteinized hemoderivative of calf blood [Nycomed; Roskilde, Denmark]) and Traumeel® (a homeopathic preparation [Biologische Heilmittel Heel GmbH; Baden, Germany]).[2] The objective was to evaluate whether ACS accelerates the regeneration of injured muscle tissue compared with saline (mouse) or standard of care (human athletes). The study designs are described in detail by Wright-Carpenter et al.[40,41]

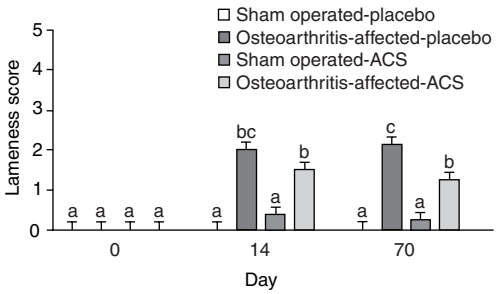


**Fig. 3.** Lameness scores (0–5, with 0 representing normal gait and 5 representing severe lameness) assigned to the middle carpal joints of 16 horses involved in a study of the clinical and histologic effects of intra-articular administration of cell-free autologous conditioned serum (ACS) in the treatment of experimentally induced osteoarthritis. In all horses, osteoarthritis was induced in one middle carpal joint (osteoarthritis-affected joints) and a sham operation was performed on the other (sham-operated joints); horses received either ACS treatment of the osteoarthritis-affected joint and placebo (phosphate-buffered saline solution) treatment of the sham-operated joint (n = 8), or placebo treatment of both joints (n = 8). Scores were assigned at baseline (day 0; prior to induction of osteoarthritis), after osteochondral fragment creation and osteoarthritis induction but prior to treatment (day 14), and at termination of the study 35 days after the last treatment (day 70). **a–c** Different letters indicate a significant ($p < 0.05$) difference between bars (i.e. **a** is significantly different from **b** and **c**; **b** and **c** are significantly different from each other, and from **a**, when shown on separate bars) [reprinted from Frisbie et al.,[34] with permission].

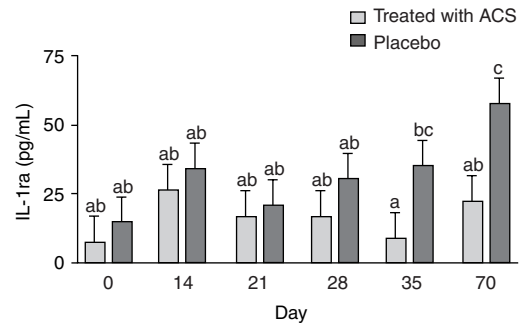


**Fig. 4.** Synovial fluid concentrations of horse interleukin-1 receptor antagonist (IL-1ra) [estimated by use of mouse anti-IL-1ra antibody] in the same horses described in figure 3 at the indicated intervals during a 70-day study of the clinical and histologic effects of intra-articular administration of cell-free autologous conditioned serum (ACS) in the treatment of experimentally induced osteoarthritis. Values represent the mean IL-1ra concentration of both osteoarthritis-affected and sham-operated joints in horses treated with ACS or those treated with placebo. **a** is significantly different ($p < 0.05$) from **bc** and **c**, but not from **ab**; **c** is significantly different from **ab** and **a** but not from **bc**. **bc** is not significantly different from **ab** (reprinted from Frisbie et al.,[34] with permission).

### 3.3 Experimental Mouse Muscle Contusion Model

The model described here has been published by Lefaucheur and Sebille.[42] The experimental mouse muscle-injury systembasically consists of a 16.3g steel ball falling from an height of 1m onto the gastrocnemius muscle of a test mouse. Mice were treated with an intralesional 10μL injection of either conditioned serum or saline 2, 24, and 48 hours post-injury, and sacrificed at relevant timepoints for muscle histology.

The histologic evaluation of the injured muscle treated with either conditioned serum or saline showed a marked difference between the two groups at day 7 post-injury, clearly demonstrating the strongly accelerated healing of tissue (figure 5) in the conditioned serum group. At day 21 the contused muscle looked very much the same in both the conditioned serum and the saline group. These experiments demonstrated that ACS treatment is a powerful tool for the acceleration of muscle contusion healing (figure 5). No unwanted effects were recorded. Of the growth factors measured, fibroblast growth factor (FGF)-2 and transforming growth factor-β1 were especially elevated in the conditioned serum group compared with fresh serum.

**1** The use of trade names is for product identification purposes only and does not imply endorsement.
**2** Actovegin® and Traumeel® are complex biologic preparations and there is no generic name for the active ingredients.

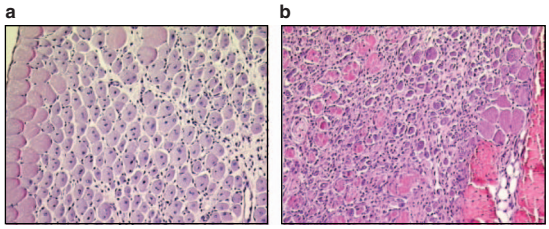


**Fig. 5.** Histologic representation of the regeneration process in the mouse muscle contusion model. Images are hematoxylin + eosin + saffranin stained gastrocnemius muscle sections (magnification: 100×) taken at day 7 post-injury: (**a**) autologous conditioned cell free serum (ACS)-treated; (**b**) saline-treated. The percentage of small diameter (<7.84μm) regenerating myofibers was greater in the control (b) than in the ACS-treated (a) cases (42.92 vs 12.58%; $p < 0.001$). In (a), more cells have a large diameter (>7.84μm) as regenerating myofibers. The normal healthy muscle fiber, the endomysium and the granulomatous tissue can also be seen here. Moreover, it was possible to detect a larger percentage of necrotic muscular tissue in the saline than in the ACS cases. Both these parameters were also true for days 6 and 8 after injury. The tissues exhibited similar appearance at 21 days. The ACS group had a much increased regeneration speed.

## 4. ACS in Clinical Human Studies

### 4.1 Pilot Observation in Muscle Regeneration for Human Athletes

In a study by Wright-Carpenter et al.,[41] two groups of professional athletes (football, basketball, hockey players) with a variety of muscle strains were treated in a nonblinded, nonrandomized fashion with either standard of care (n = 11) Actovegin® + Traumeel® injections (5mL) into the lesion, or with ACS (n = 18) injections (2.5mL + 2.5mL saline). In addition, both groups received the standard program of physiotherapy, lymph drainage, and massage. Bromelian/trypsin/rutoside (Phlogenzym®; Mucos Pharma (Berlin, Germany) was also given as an oral antiphlogistic drug. Success was defined by ability to perform 100% under competition conditions as judged by both a physiotherapist and the professional athlete. Treatment progress was documented by MRI between days 14 and 16 after the start of treatment. No local or systemic unwanted effects were detected in either group. The results show a substantial tendency towards shorter recovery time in the ACS group compared with standard of care (table II). Recovery time (in days) for athletes treated with ACS was reduced by almost one-third. This is an important finding, as training pauses can lead to athletes experiencing a massive loss in muscle mass and fitness. The shortened recovery time strongly reduces the build-up training phase that is necessary in order to regain full performance, which is of paramount importance in professional athletes.

The ACS treatment offers an effective way to reduce injury 'down' times in professional athletes, and given the autologous nature of the treatment and the physiologic levels of cytokines and growth factors, the risk/benefit ratio is excellent.

Clinical studies are planned to clarify whether the results from these initial observations can be confirmed in a larger cohort. In summary, injecting ACS into injured muscle is effective and well tolerated, and accelerates muscle regeneration in laboratory mice and human athletes. Optimized treatment frequencies in clinical muscle injuries should be determined in further randomized studies.

### 4.2 Randomized Controlled Trials

#### *4.2.1 Knee Osteoarthritis*

The first data regarding the clinical efficacy and safety of ACS from a randomized controlled trial (the German Osteoarthritis Trial [GOAT]; University of Düsseldorf, Germany) were presented recently at the 2007 American Academy of Orthopedic Surgeons (AAOS) meeting in San Diego.[43] Patients with confirmed knee osteoarthritis (n = 376) were included in an intention-to-treat (ITT) analysis in a prospective, randomized, patient- and observer-blind trial with three parallel groups, in which intra-

**Table II.** Recovery time (return to full training) for individual athletes treated with autologous conditioned serum (ACS) or Actovegin®/Traumeel® (A/T)[a] after muscle fiber tear. All treatment modalities, including ACS Injections, were performed by placing 5 needles along the injury site and injecting 1mL of medication per needle. This procedure was generally repeated every second day for a total of 5–6 times. The mean number of treatments in the A/T group was 8–9

| Muscle | Recovery time (days) | |
|---|---|---|
| | ACS group (n = 18)[b] | A/T group (n = 11)[b] |
| Hamstring | 12, 14, 16, 17, 18, 21 | 16, 18, 23, 24, 28 |
| Adductor | 10, 15, 17, 18, 21, 23 | 19, 24, 25, 26 |
| Iliopsoas | 17, 21 | 24 |
| Gluteus | 20 | |
| Abdominal oblique | 8 | |
| Gastrocnemius | 14 | |
| Rectus femoris | 16 | 18 |
| **Mean** | **16.6** | **22.3** |
| **Standard error** | **0.9** | **1.2** |

a Actovegin® (Nycomed) is a deproteinized hemoderivative of calf blood that is obtained by ultra-filtration for activation of metabolism; Traumeel® (Biologische Heilmittel Heel GmbH) is a homeopathic combination product. Both substances are widely used in German sports medicine for orthopedic problems such as muscle injuries and were, therefore, regarded as 'standard of care.'

b Recovery time is shown for each individual athlete with the designated injury (i.e. 6 ACS-treated athletes and 5 A/T-treated athletes had hamstring tears, etc.).

articular injections of ACS were compared with intra-articular hyaluronan (Hya-ject®; Ormed, Freiburg, Germany) and placebo (saline). Efficacy was assessed by validated patient-administered outcome instruments (Western Ontario and McMaster Universities Arthritis Index [WOMAC] and visual analogue pain scale [VAS]) after 7 weeks, 3 months, and 6 months. The frequency and severity of adverse events were used as safety parameters. Here we report on the secondary analysis of the gathered clinical data examining the efficacy of the therapy (WOMAC responder criteria according to a method described by Bellamy et al.[44]).

Patients enrolled in the study were over 30 years old and agreed to discontinue all analgesics and NSAIDs for at least 6 months. All patients had idiopathic osteoarthritis for at least 3 months according to the American College of Rheumatology criteria,[45] Kellgren-Lawrence grade 2 or 3 radiographic evidence of knee osteoarthritis,[46] and a weight-bearing pain score of at least 50mm on a 100mm VAS at the time of inclusion. To evaluate pain severity, analgesic and anti-inflammatory medications were discontinued prior to the start of treatment with the test drug. The washout period was 2 weeks from inclusion into the trial to the first injection. During the washout period, the patients were allowed to use acetaminophen (paracetamol; up to $8 \times 500$mg = 4 g/day) if necessary as rescue medication during the trial and could be used for pain in other locations.

ITT analysis was performed for the outcome of primary and secondary variables and safety analysis, including all patients who received at least one intra-articular injection using the last-observation-carried-forward method of imputation. At each study visit, data on the tolerability of the three administered drugs was evaluated with regard to any adverse event experienced since the previous visit. Adverse events localized to the injected knee were defined as pain, swelling, or effusion and were reported as such, even though these can also be symptoms of osteoarthritis. The study was conducted in accordance with the principles of good clinical practice and in accordance with the Declaration of Helsinki, and was approved by the regional ethics committee. The investigators obtained signed informed consent from all patients before enrolment.

Between 8 October 2003 and 30 July 2004, 376 patients were randomized and treated with at least one intra-articular injection. Participants in the ACS treatment regimen received 6 injections of 2mL twice a week for the 3 weeks; those in the Hyaluronan and placebo arms received 3 injections of 2mL once a week plus 3 appointments for ointment (spaced at once a week) in a total treatment period of 3 consecutive weeks. The test medications were identifiable during handling for the treating physician (not the patient) because of their differing viscosity. The blind observer technique was therefore used in order to maintain double-blind conditions. The investigator administered the injections and an independent examiner, not involved in the therapy (masked observer), assessed the efficacy and safety for the same patient. In this way, neither the patient nor the examiner was aware of the nature of the treatment. Patient and examiner remained blinded throughout the entire trial.

The 26-week trial was completed per protocol in 345 patients (345 knees). In all treatment groups, intra-articular injections produced a significant reduction in WOMAC scores. However, responses to ACS were significantly superior. The power of ACS responses compared with both hyaluronan and saline was statistically significant with regard to the global WOMAC score at all timepoints. Descriptively, the number and percentage of patients at week 26 with an improvement of symptoms of at least 50% (according to the global WOMAC Score), compared with baseline values, was found to be markedly highest in the ACS group (table III). The mean improvement for patients treated with hyaluronan and saline was less compared with the ACS group ($p < 0.001$). No significant differences were found between hyaluronan treatment and saline injections ($p > 0.05$).

Frequency of adverse advents was comparable between the ACS and placebo groups ($p > 0.05$), whereas there were significantly more adverse events in the hyaluronan group ($p < 0.05$ for the comparison between ACS and saline). Only local adverse events occurred. There was no infection in this series.

The study shows that ACS has a marked clinical and significant therapeutic effect on the major symptoms of knee osteoarthritis after 6 months. The low number of reported adverse events shows the tolerability of this treatment form. Because of its autologous origin, the potential risk of developing adverse effects after treatment with conditioned serum is minimal. The results demonstrate that ACS is highly effective and well tolerated in the management

**Table III.** Number and percentage of patients (n = 376 total)[a] with more than 20, 36, 50, and 70% improvement compared with baseline values at 26 weeks in a study of intra-articular administration of cell-free autologous conditioned serum (ACS) compared with hyaluronan and saline in the treatment of osteoarthritis of the knee (German Osteoarthritis Trial [GOAT]).[43] Note that 57% in the ACS group experienced an improvement of ≥50%, whereas hyaluronan showed 29% and saline 28%, respectively. The difference between ACS and the two control groups is statistically significant ($p > 0.01$) for all comparisons

| Improvement (%)[b] | No. of patients (%) | | |
|---|---|---|---|
| | ACS | Hyaluronan | saline |
| 20 | 105 (78) | 66 (49) | 46 (43) |
| 36 | 91 (68) | 45 (33) | 39 (36) |
| 50 | 76 (57) | 39 (29) | 30 (28) |
| 70 | 51 (38) | 26 (19) | 15 (14) |

a Intention-to-treat population.

b Category of improvement measured by global Western Ontario and McMaster Universities Arthritis Index (WOMAC) [modified method from Bellamy et al.[44]] as a function of treatment group.

of chronic osteoarthritis of the knee. It is significantly superior to hyaluronan and placebo.

#### 4.2.2 Sciatica and Lumbar Back Pain

During the last decade it has become increasingly apparent that low back pain and sciatica are not solely related to biomechanical mechanisms. Biochemical factors are also of major importance in the development of pain and radiculopathy and, thus, ACS may be useful in its treatment.

The potential therapeutic efficacy and safety of ACS in patients with lumbar radicular compression were examined in a prospective, randomized, patient- and observer-blind, reference-controlled, single-center study (n = 84).[47,48] The clinical diagnosis of unilateral nerve root compression was verified by MRI or CT. In eligible patients, pain duration was at least 6 weeks and pain intensity was moderate to severe. Patients in need of surgery were excluded prior to study entry. The observation period was 6 months. Triamcinolone 5 and 10mg were used as comparators, as this modality is widely used as the standard in spine injection therapy.

Following a 2-week period for the ACS production, 2mL of the assigned study medication (ACS, triamcinolone 5 or 10mg) was injected once a week for 3 consecutive weeks in a technique described by Theodoridis et al.[48] and Kraemer et al.[49] Neither the administering physician nor the patient were told which injected substance they were receiving. Injection was performed under x-ray control in the close vicinity of the nerve root involved, to verify correct needle placement. A total of 90 patients were recruited, and 84 were evaluated: 32 were in the ACS group, 27 received triamcinolone 5mg, and 25 received triamcinolone 10mg. Follow-up examinations were scheduled at 6 weeks, 12 weeks, and 24 weeks following the first injection using objective and subjective assessment scores (VAS, standardized clinical parameters).

At baseline, the groups were comparable with respect to age, sex, duration of symptoms, and causes of compression signs (e.g. herniated disc, protrusion, spinal stenosis, scars); mean age was 54 years. The VAS pain intensity over time is shown in figure 6, with mean values and standard deviation (SD) depicted. VAS already showed a statistically significant reduction in all treatment groups starting between timepoint 1 and 2. In the first 6–8 weeks there was no evidence of a difference in the reduction of pain between the three therapy modalities. However, 6 months after the first injection, patients who received the ACS treatment reported further pain reduction, whereas patients of both steroid groups (5 and 10mg) showed a tendency to experience increased pain. There were no serious adverse events, and no adverse effects occurred in the three treatment groups. There were no infections in this series. The authors concluded that ACS is effective and safe in treating sciatic pain.

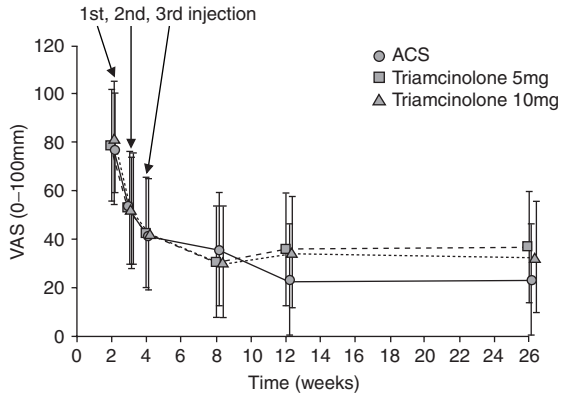


**Fig. 6.** Decrease of pain intensity (visual analog score [VAS]) as a function of time after injection using three different therapeutic modalities in 84 patients with sciatica.[47] Mean scores and standard deviation are shown along the VAS time curves. All three injection therapies (triamcinolone 5mg in 2mL [n = 27], triamcinolone 10mg in 2mL [n = 25], and cell-free autologous conditioned serum [ACS] 2mL [n = 32]) resulted in highly significant clinical improvement. Long term improvement (26 weeks) was significantly superior in the ACS group compared with triamcinolone 5mg.[47]

## 5. Conclusion

In summary, ACS has been shown to be an effective and well-tolerated treatment option in human osteoarthritis of the knee based on the described prospective randomized controlled trials. The same is described for sciatic pain with unilateral lumbar radicular compression.

The autologous production of ACS using the Orthokine® (human) or IRAP (equine) system reproducibly leads to elevated amounts of anti-inflammatory cytokines and growth factors. A clear and significant correlation in humans between IL-1ra levels and clinical improvement was not clearly apparent. This highlights that while IL-1ra may play a role in the composition of the clinically active serum, it is not the only active factor. The relation of IL-1ra versus IL-1 was chosen as a quality parameter during the development process of this novel therapeutic concept in order to ensure an overall anti-inflammatory quality of the conditioned serum.

A number of other factors present in ACS are listed in table I. Preliminary results from mass spectroscopy (unpublished data) indicate the enhancement of certain peaks, suggesting greater differences in the serum composition than mere fluctuations in the nanogram range of many different cytokines. Further research should be directed into this area.

Further application of ACS in the veterinary field (e.g. for dogs) will require confirmatory studies of effectiveness. Regarding muscle regeneration, further randomized controlled trials should be performed to confirm the results of the clinical pilot study and the positive mouse experiments. Investigation of the mechanisms of action of ACS should be pursued concurrently.

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Prof. Dr Wehling is one of the inventors of the Orthokine® procedure. He works as an orthopedic surgeon at the Centre for Molecular Orthopaedics in Duesseldorf, Germany. He is CEO of Orthogen AG, Duesseldorf, which holds patents for the described technology. He holds stock in Orthogen AG. Prof. Dr Wehling was not involved in the reported randomized clinical trials.

Dr Moser carried out the GOAT study (knee ACS study) as an employee of the Heinrich-Heine University, Department of Orthopaedics in Düsseldorf, Germany in collaboration with Dr Axel Baltzer, S. Jansen, and Prof. Dr Krauspe. Currently he works at Orthogen AG, Duesseldorf, Germany.

Dr Reinecke is one of the inventors of the Orthokine® procedure. He works as a molecular biologist and managing director at Orthogen Veterinary. He holds stock in Orthogen AG, Duesseldorf.

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Correspondence: Dr *Peter Wehling*, Centre for Molecular Orthopaedics, Koenigsallee 53-55, D-40212 Düsseldorf, Germany.
E-mail: p.wehling@gmx.de