EXPERT REVIEWS

Treatment of knee osteoarthritis with Orthokine®-derived autologous conditioned serum

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Osteoarthritis (OA) is the most prevalent arthritis in the world with increasing numbers of people expected to acquire the disease as the population ages. Therapies commonly used to manage the disease have limited efficacy and some carry significant risks. Current data suggest that the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1Ra) can alter the inflammatory response and cartilage erosion present in OA. Intra-articular gene expression of IL-1Ra has shown promising results in animal models to provide symptomatic improvement and minimize osteoarthritic changes. Orthogen AG (Dusseldorf, Germany) has developed a method to produce an autologous conditioned serum (ACS) rich in IL-1Ra marketed as Orthokine®. Study participants treated with ACS have improved pain and function; however, these results are preliminary and need confirmation. If ongoing trials prove that ACS can retard cartilage degeneration and reduce inflammation, the management of OA would be dramatically altered, perhaps providing a mechanism to prevent the disease or at least its progression.

Keywords: autologous conditioned serum • IL-1 receptor antagonist • Orthokine® • osteoarthritis

Osteoarthritis (OA) is the most common arthritis worldwide, affecting millions of people and causing the WHO to designate 2000–2010 the Bone and Joint Decade because of its effect on global wellness [1]. This disease is characterized by joint cartilage degradation, bone remodeling and synovitis, with the hip and knee being the most commonly involved joints. Afflicted individuals suffer joint pain, loss of function and inability to perform routine daily activities. OA has been linked with poor quality of life outcomes due to its effect on daily activities [2]. Indeed, musculoskeletal conditions have been associated with poorer quality of life parameters than other chronic medical conditions, primarily as a result of physical impairment, disability and lower life satisfaction [3]. Due to the significant and widespread impact of OA on individual pain and functioning, as well as on the global healthcare system in terms of costs and services, additional therapies to control, abate or halt disease progression are ongoing. A therapy that can accomplish these goals with few adverse effects and contraindications or interactions, which is readily available and economically priced, would be a welcome adjunct to the list of therapeutic interventions available to sufferers.

The US 2005 National Health Survey estimated that 47.8 million people were affected by OA and expected an increase to 67 million by 2030, with more than 50% of the OA cases predicted to be among those older than 65 years of age. Approximately 1.3–1.75 million people in England and Wales have OA and in France approximately 6 million new cases are diagnosed every year [4].

The diagnosis of OA is primarily a clinical one based on history and physical examination findings. The need for radiographic findings to confirm the diagnosis is not necessary since the degree of pain does not correlate with the severity of disease radiographically, particularly in the early stages of the disease [5]. There are established radiologic guidelines that can assist with diagnosis if needed. While the hip and knee joints are most commonly involved, the shoulders, hands and back are also frequently affected. Those afflicted with this condition present with pain, morning stiffness of less than

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an hour with progressive improvement during the day, and disability due to loss of function and an inability to perform routine daily activities.

The greatest risk factor for OA is aging. In 2000, an estimated 9.6% of men and 18% of women over 60 years of age had symptomatic OA [1]. With age being the greatest risk factor for acquiring OA, the burden of disease will only increase as the population ages. In the USA, if current trends persist, by 2050 approximately 25 million people will suffer significant daily activity limitations as a result of OA [6]. By 2050, those over 60 years of age are estimated to represent approximately 33% of the population in Europe and approximately 27% in North America. The greatest growth will be seen in those 80 years of age and above, with a greater than 100% increase anticipated by 2050 [7,8].

A substantial part of the workforce will also be affected, with a third of the cases of OA affecting those between 45 and 64 years of age. Approximately 25% of persons over the age of 55 years experience knee pain most days of the month [9]. During a 2-week period, workers in the USA aged 40–65 years experiencing pain related to OA had more lost wages and time off work than workers without exacerbations. These costs were estimated at over US$7 billion per year. Approximately 66% of this amount was attributed to only 38% of the surveyed workforce [10]. Other costs related to the disorder are direct costs attributable to hospital and outpatient visits, medications, home health services and assistive devices [11].

There are some modifiable and nonmodifiable risk factors for OA. Under the age of 45 years, the risk of OA is more common in men. The risk rises and is more common in women after 55 years of age. It is more likely to affect those with obesity, advancing age, sports interests such as running, prior joint injuries, particular employment histories, especially farming, and those with a genetic predisposition [1].

Osteoarthritis patterns vary along ethnic and cultural lines. Black people from Jamaica, South Africa, Nigeria and Liberia, as well as Asians, have a lower hip OA prevalence compared with Europeans. In some Native American populations, particularly the Blackfoot and the Pima nations, there is less OA despite a generally heavier body habitus compared with white people [12]. In the Beijing Osteoarthritis Study, it was postulated that greater knee height as measured at 90° in a sitting position in persons over 60 years of age correlated with more severe knee pain as a result of mechanical forces exerted along the cartilaginous surfaces. In this mixed gender group, more knee pain was observed in persons with higher knee height, especially in women [13].

Single joint OA, although unusual, does exist; however, when multiple joints are affected, everyday activities such as ambulation become more difficult to perform as a result of the pain, swelling and stiffness associated with the disease, resulting in poorer quality of life outcomes [2]. Persons with single disease of the knee or foot were 14-times more likely to experience functional disability than those without knee disease. Notably, those who had multiple joint disease (hip, knee, feet and back) were 60-times more likely to experience difficulties with standing and walking [2]. Chronic diseases in general result in impaired functioning across various physical, mental and psychosocial parameters. Psychological and behavioral coping methods impact pain perception in knee OA and significantly impact disability [14]. Musculoskeletal conditions have been linked to poorer quality of life parameters than cardiovascular, neurologic, endocrine and renal diseases, primarily as a result of physical impairment, disability and poorer life satisfaction [3].

Osteoarthritis not only affects the sufferer but also has a significant impact on healthcare costs, employer costs and job performance. These are divided into direct and indirect costs. Direct costs attributable to arthritis include hospital and outpatient visits, pharmaceuticals, home health services and assistive devices. In 75–79-year-olds with OA, prescription drug costs were 102% higher than a similar cohort without the disease and their outpatient visits were more than doubled [11]. Similar high costs were noted in this earlier trial for persons with a diagnosis of OA with increases seen in the areas of diagnostic testing, physician visits and other direct medical costs. Prescriptions were substantially more significant in those with OA than in those without this diagnosis [15]. Indirect costs include lost wages and work absences. A community-based study in Ontario, Canada found similar indirect costs resulting from lost wages and inability to perform activities of daily living as identified in the USA; however, additional costs identified were for informal caregivers, primarily in those with other comorbidities and more severe OA. Incorporation of the value of these informal caregivers significantly increases the economic burden of the disease [16]. In Ontario, the estimated total annual cost per person living with OA was approximately US$5700 from May 1999 to May 2000. This was divided into US$3952 annually as direct costs, with 39% being for prescription drugs and US$1760 per year as indirect costs with 4% being time lost from work [17]. Similar results were reported by the CDC when they explored both national and state-specific direct and indirect costs of OA in 2003 and discovered that these totaled US$128 billion dollars or US$1752 per person of direct costs and US$1590 per person of lost wages among working adults [18].

Market overview

Osteoarthritis affects all aspects of life for each person with the disease. There is chronic pain with intermittent acute exacerbations, reduced function, disability and reduced quality of life. For those employed, there are lost wages and absenteeism due to the acute exacerbations of the disease. As the population ages globally, healthcare costs will escalate. With pain being the most prevalent and limiting factor, most current therapy choices attempt to reduce pain, improve function and reduce exacerbations. Individual joint OA substantially impacts pain and function but this is magnified when multiple joints are involved; therefore, those therapies that address multiple joints would be expected to have the greatest impact. Current recommendations for management of OA are based on available evidence and expert opinion.

Both the European League against Rheumatism and the Osteoarthritis Research Society International convened an expert review committee and developed guidelines for the management of hip and knee OA. Each recommends a combination of nonpharmacological and pharmacological modalities. Both committees agree that education, exercise and knee bracing are nonpharmacological

options proven to assist with personal management of the disease. Weight loss is recommended for those patients with hip or knee OA who are overweight. In addition, each guideline recommends acetaminophen as the initial drug therapy and the preferred long-term oral analgesic if the medication is tolerated and effectively controls the pain [19–21]. However, concerns regarding acetaminophen and potential hepatotoxicity at current therapeutic dosages seriously impact its chronic use [22].

Nonsteroidal anti-inflammatory drugs and selective cyclooxygenase-2 inhibitors taken orally or applied topically are recommended for those patients who are unresponsive to acetaminophen. Unfortunately, these medications taken orally can precipitate severe adverse events such as gastrointestinal hemorrhage in some patients or can be contraindicated in others, leaving few choices to control pain and suffering. Intra-articular injections of steroids have demonstrated a reduction in pain from 1–4 weeks postinjection, especially if triamcinolone is used; however, there are no head-to-head studies to recommend a particular steroid preparation. It is not considered a long-term treatment option [19–21,23].

Intra-articular injections of hyaluronate (HA) may be considered in patients who have been unresponsive to nonpharmacological or drug therapy. However, recent reviews of the literature reveal contradictory evidence regarding its effectiveness and have questioned the long-term efficacy of this therapy to alter the progression of the disease [19,20]. HA has a similar onset of action for reduction of pain as intra-articular steroids but there is sustained improvement at 14–26 weeks. Neither steroids nor HA demonstrated effectiveness at 1 year [23].

Many studies have examined the effectiveness of glucosamine and/or chondroitin sulfate to improve pain and function in knee OA. In general, glucosamine therapy has failed to demonstrate consistent improvement in the pain, function, and stiffness associated with OA, with many trials demonstrating manufacturer bias [24]. Indeed, there seems to be little evidence to support the use of glucosamine hydrochloride [25], however, there is some evidence supporting the use of glucosamine sulfate in combination with chondroitin sulfate to improve pain and function, with some trials also suggesting a chondroprotective effect. Generally, glucosamine and chondroitin are well tolerated with minimal side effects that primarily consist of gastrointestinal upset [21,26].

Narcotics can help to control pain but should be limited to those with severe pain that cannot be controlled with other therapeutic options. There is insufficient evidence for chronic narcotics in the management of the pain and disability of knee OA; however, they are widely used in clinical practice when either non-steroidal anti-inflammatory drugs (NSAIDs) or acetaminophen are ineffective or contraindicated. There is evidence that the use of opioids can reduce the amount of NSAIDs required. Total knee arthroplasty is available for those with refractory pain and markedly reduced function despite a combination of medications and nonpharmacological therapies. This is especially true for patients with reductions in health-related quality of life parameters [19–21]. As new information regarding the pathophysiology of OA is emerging, biochemical therapies are dominating current research.

Pathomechanisms

Knee OA is a degenerative disease that ultimately leads to joint destruction. The pathophysiology of the disease is probably multifactorial, encompassing mechanical stresses, genetics and an imbalance between anabolic and catabolic mechanisms and inflammatory mediators. As the disease progresses, there is cartilage erosion and synovial inflammation [27]. Chondrocytes and synovial cells produce increased levels of proinflammatory cytokines, particularly IL-1 and TNF-α, which increase catabolic activity and reduce collagen synthesis, ultimately resulting in the destruction of articular cartilage. IL-1β is the predominant cytokine produced and with TNF-α stimulates the production of additional cytokines, namely IL-6 and IL-8 [28]. IL-1β stimulates chondrocytes to produce inducible nitric oxide synthase, cyclooxygenase type 2 and type 2 phospholipase A, resulting in the production of nitric oxide, prostaglandin-E2 and platelet-activating factor. These mediators promote articular cartilage degradation as seen in OA by inhibiting proteoglycan and collagen synthesis and activating matrix metalloproteinases (MMPs). Nitric oxide has been implicated in chondrocyte apoptosis and MMPs cleave collagen [29–31]. MMP-13 is abundant in OA and, therefore, may play a more important role in OA due to its preferential degradation of Type II collagen. A disintegrin and metalloproteinase with thrombospondin motifs are responsible for the degradation of cartilage proteoglycan, aggrecan, which is a major component of cartilage, giving elasticity and compressibility to the matrix. Loss of aggrecan impairs the weight-bearing function of the cartilage. Two forms of degraded aggrecan have been discovered in the synovial fluid of patients with OA [30,32]. Anti-inflammatory or inhibitory cytokines normally produced include IL-4, IL-10, IL-1 receptor antagonist (IL-1Ra) and interferon [33].

As previously mentioned, IL-1β is the predominant cytokine produced and is synthesized as a 31-kDa precursor and converted by IL-1β-converting enzyme (also known as caspase-1) to the mature 17.7-kDa active form. IL-1 mediates its activity by affinity to two receptors, type I and type II. The type I receptor has a higher affinity to IL-1α while the type II receptor has a higher attraction for IL-1β. The IL-1 receptor type II lacks a cytoplasmic domain and binds IL-1 but no signal is transmitted. This is termed a decoy receptor. In addition, IL-1 receptor type II preferentially binds IL-1β and prevents it from binding to the functional type I receptor and the accessory protein [31]. Interestingly, there are a larger number of type I IL-1 receptors in the chondrocytes and synovial cells in OA, thereby explaining the increased sensitivity of these cells to IL-1. TNF-α, although less potent and present in smaller quantities, is believed to play a pivotal role in matrix degradation and synovitis. The precursor protein has 76 amino acids with cleavage by a TNF-α-converting enzyme, resulting in the 17-kDa secreted protein. IL-6 and IL-8 are believed to be involved in the pathological process of OA. The function of IL-6 is not fully known. It may increase inflammatory cells in the synovium, stimulate the propagation of chondrocyte tissue and increase the production of MMPs. IL-6 may also stimulate the production of IL-1Ra and activate NF-κβ [34]. IL-8 exerts its effects primarily

on neutrophils, resulting in their movement and production of oxygen free radicals like nitric oxide. IL-8 may also play an important role in acute inflammation [27,35].

Anti-inflammatory cytokines are also present in abundance in the synovial membrane, synovial fluid and cartilage in patients with OA. Examples include IL-4, IL-10 and IL-13. Important properties of these cytokines are the reduction of IL-1β, TNF-α and MMPs, and the increased production of IL-1Ra [27]. IL-1Ra was first reported in 1985 and was discovered in human macrophages cultured on IgG and eventually in macrophages in the synovium in OA [24]. The human *IL-1Ra* gene was mapped to band q14-q21 in the long arm of chromosome 2. Three structural variants have been identified: a 17-kDa form secreted from monocytes (sIL-1Ra); an 18-kDa form that remains in the cytoplasm of keratinocytes (icIL-1Ra); and a 16-kDa form discovered intracellularly in neutrophils, monocytes and hepatocytes [36]. Later, the IL-1Ra genome was cloned and expressed in *Escherichia coli* producing the 18-kDa molecule. An identical IL-1Ra protein was made by stimulation of human monocytes with granulocyte colony-stimulating factor [35,37,38]. As the factors responsible for the development of OA become well understood, therapeutic agents targeting aspects of the pathophysiological pathways have attracted particular interest. Such areas of interest include the inhibition of cytokines, particularly IL-1β, which seems to be the principal cytokine responsible for the inflammatory changes found in OA, and the MMPs, which play a role in the degradation of the cartilage matrix.

Introduction to the drug

Since intravenous, intramuscular and oral routes of medication delivery offer poor access to joints and risk systemic effects, intra-articular delivery has become a targeted method of medication delivery in therapeutic research for knee OA. Gene expression of IL-1Ra has been an area of interest in treating OA as a means to circumvent the short half-life of recombinant IL-1Ra directly injected into affected joints [39,40]. Intra-articular gene expression of IL-1Ra was first successfully transferred via synovial cells into normal rabbit joints [41]. Subsequently, additional studies have focused on the efficacy of IL-1Ra on structural changes and production of IL-1Ra at selected joints in animal models. One study used a plasmid vector to introduce canine IL-1Ra into surgically induced menisectomies in rabbit knees and another used an adenoviral vector to transfer equine IL-1Ra into horses with osteochondral defects representative of human OA. In both studies arthritic changes were markedly minimized, horse lameness was improved and IL-1Ra levels were augmented for approximately 4 weeks after the intra-articular injections [42,43]. Two other animal OA model experiments utilized *ex vivo* delivery of human IL-1Ra via autologous synovial cells. Cells were cultured and genetically modified by a retrovirus and reintroduced into the animal OA models, one in rabbits and the other in dogs. Gene expression of IL-1Ra was present 14 days after implantation in the rabbits along with reduced cartilage severity and degradation, with similar results in the surgically induced canine OA model [44,45].

Human recombinant interleukin receptor antagonist (IL-1ra) is structurally related to IL-1β and IL-1α and weighs 18 kDa [28]. It is currently approved for the treatment of rheumatoid arthritis. Chevalier *et al.* performed two studies utilizing IL-1ra [46,47]. The first was to determine its safety and the second sought to determine its efficacy in symptomatic knee OA. There were no adverse events in either study; however, there was also no significant clinical improvement in OA symptoms. Pharmacokinetic data revealed that IL-1ra has a half-life of approximately 4 h after intra-articular injection [46,47]. Previous methods for induction of IL-1Ra are considered too laborious and time intensive to be used therapeutically; however, the potential benefits of IL-1Ra to alter progression of OA by neutralizing proinflammatoy cytokines remains under investigation. This theoretical benefit led to the development of a novel method of producing an endogenous source of IL-1Ra, autologous conditioned serum (ACS), marketed as Orthokine® (Orthogen AG, Dusseldorf, Germany). It is also referred to as IL-1Ra protein, especially when used in the management of equine OA. Orthokine is the medical device (syringe) used to produce the ACS containing anti-inflammatory cytokines, particularly IL-1Ra.

Chemistry

The method for production of ACS by Orthokine is the same for horses and humans. The following is the method as described by Meijer *et al.* [48]. First, 50–60 ml of blood is collected into special syringes containing 200 medical-grade glass beads. The glass beads are washed with distilled water and the surface of the beads incubated with chromium sulfate for 5 min. The beads are washed again with distilled water until the pH is equal to that of distilled water. Sterilization occurs by autoclaving or γ-radiation. The whole-blood syringes are incubated aseptically at 37°C with 5% CO_2 for 24 h and then centrifuged for 10 min. From the syringes, 10 ml of serum is removed and stored at -20°C. After this process the serum is injected into the affected joint of the patient. A cell-surface interaction stimulates monocytes to generate cytokines. Meijer *et al.* measured the cytokine content of the serum and screened it for contaminants and infectious diseases such as hepatitis and syphilis [48]. Via this method, they found no significant increase in the proinflammatory cytokines IL-1β or TNF-α; however, anti-inflammatory cytokines IL-4, IL-10, IL-13 and IL-1Ra were recovered starting at 30 min. Multiple growth factors such as TGF-β, insulin-like growth factor 1 and PDGF were also recovered [49,50]. At 24 h, Meijer *et al.* demonstrated a significant increase in levels of IL-4 from 8.1 ± 2.1 pg/ml (time 0 h) to 17.2 ± 2.8 pg/ml, while IL-10 increased from 4.1 ± 1.1 pg/ml to 8.9 ± 1.2 pg/ml; thus, both increased by a factor of 2. However, IL-1Ra increased by a factor of 140, as indicated by levels of 73 ± 8 pg/ml at 0 h which increased to levels of 10254 ± 165 pg/ml at 24 h. Only serum proteins, glucose and potassium levels were measured before and after incubation as a means of evaluating blood cell integrity. Serum protein levels were unchanged. Glucose was reduced by a third from 94 ± 6 to 35 ± 3.9 mg/dl. This was believed to indicate ongoing cellular metabolic activity and cell survival. Potassium levels increased from 4.4 ± 0.094 mmol/l to 8.6 ± 0.62 mmol/l. This indicated a moderate degree of hemolysis and was felt to be a typical finding after

incubation at 37°C [48]. ACS may not only act via the induction of anti-inflammatory cytokines, IL-1Ra, but also via the induction of growth factors. ACS has been shown to accelerate the healing of muscle tissue after trauma in animal models and in professional sports athletes [51,52].

Pharmacodynamics & pharmacokinetics/metabolism

There is no information available specifically for Orthokine-produced ACS in the areas of pharmacodynamics or pharmacokinetics of the product since its foundation is venous blood taken from the future recipient of the prepared serum and contains normally occurring biological products. This was confirmed via communication with Orthogen AG. No studies could be located that determined the elimination of ACS after intra-articular injection, only that of human recombinant IL-1ra, as noted previously.

Clinical efficacy

Animal studies

Studies have been conducted in equine and human OA models. Two small studies in equine OA models using IL-1Ra revealed clinical improvement as evidenced by improved lameness when compared with placebo and increased levels of IL-1Ra as assessed by mouse IL-1Ra antibody but not when assessed by human IL-1Ra antibody [53,54]. One study of 16 horses with surgically induced unilateral mid-carpal joint OA demonstrated improvement in synovial hyperplasia at 70 days in the ACS treatment group compared with the saline group. Either 6 ml of ACS or saline was injected into the affected joint on days 14, 21, 28 and 35. On day 14, an exercise program of 5 days per week for the remaining 8 weeks of the study was initiated. There were no observed adverse events. Interestingly, in this study, no measurable levels of IL-1Ra were found in the prepared ACS assessed by human IL-1Ra antibody as has been reported in other studies [53]. In a similar study involving 16 horses divided equally into ACS and saline groups, OA was unilaterally surgically induced in a middle carpal joint. On day 7, blood was obtained from each horse and ACS prepared according to the manufacturer's instructions. On days 14, 21, 28 and 35, 6 ml of the prepared serum or saline was injected into the affected joint and all were exercised on treadmills 5 days per week for 8 weeks starting on day 15. There was a significant reduction in lameness and reduced osteochondral erosion and synovial hyperplasia in the ACS-treated group. In this study, there was a significant increase in IL-1Ra in the ACS-treated joints when assayed by mouse IL-1Ra antibody, but not human IL-1Ra antibody. No adverse events were reported [54].

A larger equine study enrolled 262 horses with resistant OA that had previously been treated with HA or glucocorticoids in the affected joints. The horses were given two or three injections of 2 ml of ACS separated by 8–12 days. Different joints were treated in this study. Treatment joints and numbers were as follows: 110 coffin joints, 87 fetlock joints, 26 carpal joints, 33 hock joints and six hip joints. Either elimination of or improvement in lameness was seen in 221 of the 262 horses in the study 6 weeks after treatment. At 12 weeks, 178 horses still had no evidence of lameness. No adverse events or reactions were recorded after any of the injections [55]. A smaller study of 20 horses unresponsive to HA or cortisone therapy produced similar results, with 17 out of 20 returning to full activity 3 months after ACS treatment [56]. These favorable results in the management of equine OA led to trials in humans.

Human studies

Owing to the autologous nature of the procedure, no specific Phase trials have been completed. This was confirmed by communication with the manufacturer. However, two human trials were located that evaluated the efficacy of ACS in symptomatic OA of the knee utilizing Orthokine. The method for administration of ACS was the same in each trial and as recommended by the manufacturer. The study knee was cleansed with alcohol and draped in a sterile fashion. A 21-gauge needle was placed supero-laterally and the synovial fluid removed. The needle was left in place and 2 ml of Orthokine-derived ACS was injected through a 22-µm pore sterile filter [57,58].

The first prospective study to evaluate the efficacy of ACS produced by this syringe was placebo-controlled and patient-and observer-blinded with an intention-to-treat analysis. The study is known as the German Orthokine Osteoarthritis Trial (GOAT). Outcomes were measured using the Western Ontario and McMaster Universities (WOMAC) Index, the visual analogue scale (VAS) self-assessment for pain, global patient assessment and the SF-8 Health Survey Questionnaire. The WOMAC is a 24-question validated survey assessing pain, function and mobility. The global patient assessment incorporates pain, function, emotional effects of disease and physician assessment sections. The study was designed to detect a 20% change in the WOMAC between ACS and HA and between HA and saline. Participants were recruited from five orthopedic clinics between October 2003 and July 2004. Key inclusion criteria were as follows: at least 30 years of age, willing to discontinue all analgesics for 6 months, a clinical diagnosis of OA for at least 3 months along with Grade 2–3 radiographic OA as determined by Kellgren–Lawrence criteria and a VAS score of at least 50 mm. Participants could have had previous surgical intervention on the affected knee if at least 3 months had elapsed prior to injections starting. Those with Grade IV OA were excluded, along with women who were pregnant or lactating and those with other arthropathies, cancer, abnormal laboratory values and a history of substance abuse. There were 464 patients screened for inclusion with 65 excluded for not meeting one of the inclusion criteria of at least 50 on a 100-mm VAS. Only 376 patients were eligible for randomization into one of three intention-to-treat groups: an ACS treatment group, a HA treatment group or a saline group [57].

A 3-week washout period for all analgesics started on the day of inclusion. Only acetaminophen could be used as rescue medication. No NSAIDs were allowed. The observer was blinded to the treatment group, although the physician administering the intra-articular injections was not. All participants had 50 ml of venous blood withdrawn. The blood of those in the HA or saline group was discarded. The blood of those in the ACS group was prepared for injection by Orthogen as described previously and screened for hepatitis B and C, HIV and syphilis. All participants saw a physician twice per week for 3 weeks. The saline and HA groups received one intra-articular injection in the affected knee each week

and topical heparin-natrium cream was applied on the second visit of the week, while the ACS group received two injections per week for 3 consecutive weeks. Outcome assessments were performed at baseline and then at weeks 7, 13 and 26. Those in the ACS group performed better in all WOMAC subscales ($p < 0.001$) than those in the HA or saline groups, with no differences between the saline and HA groups. The VAS scores were lowest in the ACS group with at least a 50% improvement at week 26 ($p < 0.001$) but were also significantly reduced in the HA and saline groups, although by lesser amounts, at 32 and 33%, respectively. In the SF-8 health survey, the ACS group had demonstrated the greatest improvement compared with either control group ($p < 0.001$). There were no differences in use of rescue medications among the study groups and adverse events were limited to local reactions in all groups. None of the reactions in the ACS group required intervention. Of note for this study, 60% of the participants had undergone previous knee surgery. This 6-month study concluded that ACS was an effective alternative therapy for the improvement of pain and function in symptomatic knee OA; however, the results are preliminary and require confirmation. The results should be considered in light of the differences in the treatment arms. The ACS group received two injections per week while the HA and saline groups received only one. This trial was not designed to evaluate any disease- or structure-modifying effect of ACS [57].

At 2 years, 310 of the 345 patients in this trial were traced and re-evaluated by a blinded observer. A total of 122 of these patients had sought other treatments such as surgery, medications or injections. Statistically significant differences for the ACS group over the HA and saline groups persisted past the initial 6-month study in all outcome parameters. For instance, WOMAC declined from 124 at baseline in the ACS group to 58 at 2 years. The WOMAC was 88 and 84, respectively, for HA and saline. The VAS for ACS was 30 out of a scale of 100 from an average of 70 in the initial study. Pain scores were 39 and 37 for the HA and saline groups, respectively. Notably, the effects seen in the HA and saline groups also persisted for the additional 18 months [59]. Another randomized controlled trial (RCT) compared Orthokine-produced ACS with a saline control [58]. A total of 182 patients were enrolled from February 2004 to August 2005, however, 16 were excluded prior to injection. An additional 14 patients were excluded from analysis due to adverse events or protocol violations; therefore, an intention-to-treat analysis was not undertaken in this trial. The primary end point of this trial was a 30% improvement in the WOMAC Index. In addition, patients were asked to complete the VAS for pain, the Knee Injury and Osteoarthritis Outcome Score (KOOS) and the Knee Society Clinical Rating System (KSCRS) at baseline and at 3, 6 and 12 months after injections. The validated KOOS and KSCRS cover pain, other disease symptoms, function, sport and recreation, and a range of motion parameters. This trial was conducted over 30 months at seven sites in The Netherlands. Participants were enrolled from orthopedic offices, and all participants were older than 18 years with clinical evidence of OA and radiographic evidence of OA by the Kellgren–Lawrence index. Some exclusion criteria included other arthropathies, history of or current infectious diseases, vascular or neurologic disorders, surgical or intra-articular therapies within 6 months, coagulopathies and severe (Grade IV) OA. All participants were subjected to the withdrawal of 50 ml of venous blood and screened for hepatitis A and B and HIV. After testing negative, the blood was prepared by the Orthogen laboratory via the previously described Orthokine method and returned for use after 14–21 days in 2 ml vials at -20°C. A series of six injections were given over 3 weeks on days 0, 3, 7, 10, 14 and 21. This same schedule was used for those in the control arm. The procedure for intra-articular injection of saline and ACS were identical. Rescue medications were limited to acetaminophen up to 4 mg/day; however, 13 patients were placed into an NSAID group in which these additional rescue medications were permitted. The type and amount of NSAIDs was not specified. All rescue medications were to be discontinued 1 week before the scheduled follow-up evaluation. The primary outcome measure of 30% reduction in the WOMAC Index was not reached. Interestingly, both the ACS and saline arms had significant improvements in all outcome measures ($p < 0.001$); however, the ACS group scores were consistently higher, although they did not reach significance. There was statistically significant improvement identified in the KOOS symptomatology ($p = 0.002$) and the KOOS sport measured over time ($p = 0.042$) compared with the saline treatment group. There were statistically significant differences identified in a secondary data analysis of those allowed NSAIDS while receiving ACS treatment in the KOOS sport parameters ($p = 0.011$) and the physician section of the KSCRS ($p = 0.005$). Adverse events were relatively equal between both groups. Although clinical improvement in OA symptoms with Orthokine-derived ACS was suggested in this trial, the primary outcome measure was not met. The clinical relevance of these results is uncertain. Of particular concern is the lack of intention-to-treat analysis, the unexplained use of NSAIDs in a small group of participants, and the influence of NSAIDs on the results noted in the Orthokine plus NSAID group. The chondroprotective effect of ACS was not evaluated in this RCT [58].

Safety & tolerability

The available data regarding the safety and tolerability of ACS produced by the Orthokine method was acquired from the two studies by Baltzer *et al.* and Yang *et al.* [57,58]. During these trials, reactions were localized to the injection joint and were primarily mild-to-moderate in nature. No infections occurred in any of the three groups in the GOAT trial (the ACS, HA or saline group). The only adverse reactions in the ACS group were pain and pressure associated with the injection and all improved within 24 h and required no further intervention [57]. There were 219 adverse events reported in the RCT by Yang *et al.* [58]. These were primarily local reactions and most were associated with an increase in joint pain during the procedure. A total of 162 events were knee related. These were described as pain, swelling and local irritation. There were two serious adverse drug events that occurred in the ACS study participants. One patient developed septic arthritis; this event was attributed to the injection procedure rather than the study sample because the ACS was administered via a 0.22-µm sterile filter and there was no microbiological evidence of bacterial contamination of the serum prior to administration. Another patient in the ACS

study group developed a recurrent severe inflammatory reaction demonstrated by pain, swelling and increased warmth within hours after three injections, which necessitated withdrawal. Other musculoskeletal adverse events included other joint pain such as hip or shoulder pain, back pain, and systemic events such as influenza, headache and pneumonia. No details were provided regarding the duration of symptoms. Notably, there were no statistically significant differences between the ACS and saline groups except that there was more back pain in the saline group ($p = 0.009$) [58].

Cost

The cost of the procedure depends upon the number of syringes used during the procedure and the number of injections. Usually three to six injections are used. There is no fixed reimbursement for this procedure, therefore charges may vary by physician. The cost is approximately US$675–1350 for three and six injections, respectively, at the current exchange rate from Euros to Dollars. These pricing schemes were supplied by Orthogen AG.

Regulatory status

The biologic preparation of ACS produced via the Orthokine method has been available for clinical use since 1998 and marketed by Orthogen AG, a biopharmaceutical group of companies. The European Commission approved the medical device in February 2006, and it later became available to clinicians in Australia and some Asian countries. Application was also apparently made to the US FDA in 2006 but remains under investigation and to this date has not received approval in the USA. Because the use of ACS falls within the physician scope of practice, it does not require drug approval, but rather the syringe requires approval for distribution [101,102]. Orthogen AG has recently received clearance concerning athletic doping. Reportedly, athletes must declare use of the Orhtokine method but no prior approval is required.

Conclusion

Osteoarthritis is a common problem that is increasing in prevalence. Current therapies are costly and many are only marginally effective. Inadequately treated disease leads to poor quality of life and adversely impacts the work force. This new therapy seems generally safe and may be an effective alternative for clinicians to consider depending on cost and availability of processing; however, the results and effectiveness of this product remain unconfirmed. More data are required before it can be recommended. The chondroprotective effect of this product needs to be studied.

Expert commentary

Patients with OA suffer pain, reduced quality of life and functional impairment. The disease has a significant economic impact. Past research and recommended treatment options have focused primarily on pain relief based on the premise that OA was a 'wear and tear' degenerative process, with a perceived inability to really alter the course of the disease. In fact, most of the therapies currently recommended for management of the symptoms lack supporting evidence and much is based on expert opinion. As more and improved therapies have been investigated, the theories regarding the pathophysiology of OA have evolved. It likely has a multifactorial etiology; therefore, combination therapy targeting different mechanisms is most advantageous. This includes pharmaceutical medications, assistive devices and bracing, exercise and conditioning, weight control, patient education and behavioral modification.

Current research suggests that OA has many similarities to an inflammatory arthritis, along with the degenerative changes to the synovium and cartilage erosion. Inflammatory mediators produced by chondrocytes and synovial cells reduce cartilage synthesis and increase its degradation. As the disease advances, proinflammatory cytokine production exceeds the production of anti-inflammatory cytokines and results in an imbalance of the catabolic and anabolic steady state. Therapies aimed at reducing the effects or production of the proinflammatory cytokines could greatly alter the course of the disease. One proposed therapy is ACS produced by the Orthokine method. An advantage of this method is that it is convenient and produced from the blood of the recipient. This potentially limits adverse events. It is delivered intra-articularly, which places the therapy at an affected joint and has shown efficacy in managing the clinical signs and symptoms of single-joint OA, particularly pain and function. There appear to be additional benefits from ACS besides the increase in anti-inflammatory cytokines, although these are yet to be determined. Despite promising early results in humans and successful utilization in veterinary medicine to treat equine OA in Europe, these results are preliminary and require confirmation. Because of this, it is unlikely that ACS will soon enjoy widespread acceptance and utilization in the USA. It will most likely be relegated to those patients that have failed more conventional therapies and are not candidates for surgical intervention. The imbalance in cytokine production, the cartilage destruction and the accompanying signs of inflammation have caused the emergence of a new area of research in OA, namely gene therapy. Therapies and interventions that retard cartilage erosion or replace damage cartilage will be the focus of future research. Orthokine-derived ACS could provide an immediately available method of providing cost-effective IL-1Ra with low risk for those with knee OA, providing pain control, improved function and the potential to retard the cartilage destruction. More studies are needed utilizing this method for producing ACS to evaluate its effectiveness in OA. Ideally, strategies aimed at prevention would be superior.

Five-year view

There currently is no cure for OA but great strides have been made in attaining a greater understanding of the pathophysiology of the disease. There has been extensive work performed evaluating the biochemical mechanisms of the chondrocyte, its differentiation and apoptosis [60]. This has led to extensive research targeting the inflammatory response in OA and its structural modifications. There are ongoing trials evaluating therapies that attack inflammation. One such medication under investigation is diacerein, whose active metabolite rhein is an IL-1 inhibitor. Diacerein in doses of 50 mg twice daily has been shown to produce a significant reduction in joint space width (0.5 mm) over a 3-year period in patients with hip OA. Lengthier studies will be needed to determine if

this result is reproducible in knee OA. In another study utilizing diacerein, a dose of 100 mg per day significantly improved pain with motion when compared with placebo [61]. However, a Cochrane systematic review found that diacerein use resulted in small improvements in pain and slowed the progression of hip OA but not knee OA [62]. This could become a helpful add-on therapy for those requiring incremental interventions to control symptoms but more studies are required to determine its effectiveness.

Another area of interest is antibiotics with anti-inflammatory action. Laboratory studies suggest that doxycycline may inhibit MMPs and reduce the production of IL-1 and IL-6 in chondrocytes harvested from human articular cartilage [61]. A large RCT recently completed and sponsored by the NIH found that doxycycline significantly slowed the rate of radiographically-determined joint space narrowing in established knee OA at 30 months but had no effect on the contralateral knee, suggesting that some other pathomechanism is at work there [63]. A Cochrane systematic review found no evidence to support its use as a disease-modifying agent in hip or knee OA [64]. Additional research into this and other medications that possess anti-inflammatory activity and potentially have fewer serious adverse events is expected.

Gene therapy remains an area of interest with its success in other inflammatory arthropathies and the finding that osteogenesis is amenable to gene therapy. Additional trials are ongoing evaluating the delivery and effectiveness of anti-inflammatory cytokines and determining which genes are most appropriate, how to deliver them and by what method. The use of growth factors, specifically TGF-β, in OA is currently undergoing clinical trials [65]. TGF-β has been shown to be crucial to cartilage repair and its reduction or absence results in changes consistent with OA; therefore, it is being recognized as a potential therapy for repair or prevention of cartilage damage [66,67]. There is a Phase I trial currently underway in the USA using a TGF-β product developed by TissueGene, Inc. (MD, USA). A similar trial has been completed by Kolon Life Sciences, a subsidiary of Kolon, a biochemical company in Korea, in cooperation with TissueGene, Inc. A total of 12 patients with Grade IV knee OA in Korea and four in the USA have been treated with TGF-β inserted into human articular chondrocytes via a retroviral vector. TGF-β is transferred into the affected joint by direct intra-articular injection. The effect of using allogenic cells for gene transfer on the trial outcome has yet to be determined. Reportedly, there have been no adverse events in these initial trials and a Phase II trial has begun in Korea. The primary outcome measured in this study will be symptomatic improvement in OA symptoms, with a secondary outcome of cartilage regeneration evaluated by MRI [65,103,104]. The results of the initial Phase I trials should be published soon.

Matrix metalloproteinases have frequently been considered for the treatment of OA. Severe dose-dependent musculoskeletal side effects (MSS) have limited their utility in humans. These side effects were thought to be the result of nonselective inhibition. MMP-13 was targeted since it is specifically expressed in the cartilage of human OA and readily cleaves type II collagen. An MMP-13 inhibitor has shown promise as a disease-modifying agent in the treatment of OA in one animal model of OA [68]. Baragi *et al.* performed selective assays utilizing bovine and human articular cartilage incubated with IL-1α and oncostatin M for 11 and 21 days, respectively. Type I and II collagen degradation markers, the carboxyl-terminus cleaved by collagenase, were measured in these cultures by the C1,2C assay. A rat model of monoiodoacetate-induced OA was used to evaluate the effects of MMP-13 inhibition on pain reduction and inhibition of cartilage degradation. In addition, surgically induced OA (full thickness cut of medial meniscus) in rats was used to evaluate the chondroprotective effects of a MMP-13 inhibitor. This same rat model was used to assess the MSS of MMP inhibitors. A selective MMP-13 inhibitor was identified termed ALS 1-0635 and evaluated with ilomastat, a nonspecific MMP inhibitor, as a positive control and a saline control. The results from this study are promising. ALS 1-0635 inhibited bovine cartilage degradation in a dose-dependent manner at 48.7% at 500 nM and 87.1% at 5000 nM, compared with 96.8% at 50 nM ilomastat. Similar results were obtained in human cartilage cultures: 62.8% at 500 nM and 78.3% at 5000 nM compared with 88.6% at 50 nM ilomastat. Scores from two blinded observers found that ALS 1-0635 significantly reduced cartilage damage in monoiodoacetate-induced OA at 41% ($p < 0.05$) in rats given 30 mg/kg administered twice daily. The actual cartilage damage area was also significantly reduced at 48% ($p < 0.05$). ALS 1-0635 also appeared to reduce joint pain as evidenced by improvement in ability to bear weight on the affected knee joint ($p \leq 0.05$). Doses of ALS 1-0635 of 1, 10, 30 and 60 mg/kg twice daily resulted in a dose-dependent reduction in cartilage degradation scores, with consistent beneficial effects noted at the 60 mg/kg dose ($p < 0.05$). Of special note, none of the rats evaluated for MSS given 100 mg/kg/day of ALS 1-0635 exhibited any clinical signs or microscopic tissue changes characteristic of MSS. This study describes a novel MMP inhibitor that demonstrates selectivity and appears to be chondroprotective, improves joint pain and reduces cartilage degradation [68]. Ongoing research is expected with this MMP-13 inhibitor and in the area of MMP inhibitors in general.

Information resources

- National Institute of Arthritis and Musculoskeletal and Skin Diseases: www.niams.nih.gov
- Arthritis Foundation: www.arthritis.org
- Orthogen AG: www.orthogen.com
- TissueGene Inc.: www.tissuegene.com/tg.htm

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Key issues

- Osteoarthritis (OA) affects millions of people worldwide, and many current therapies are costly and only marginally effective.
- The pathomechanism of OA is multifactorial, involving inflammatory mediators and resulting in cartilage erosion and synovial inflammation.
- Some current research is focused on the use of anti-inflammatory cytokines, particularly IL-1 receptor antagonist (IL-1Ra), delivered intra-articularly via gene therapy and autologous methods.
- Currently, Orthokine® (Orthogen AG, Dusseldorf, Germany) is the only method currently available to produce autologous conditioned serum that is rich in the anti-inflammatory cytokine IL-1Ra.
- Current clinical data demonstrate that Orthokine-derived autologous conditioned serum improves pain and function and could be an effective adjunct for those unresponsive to traditional therapies; however, results are only preliminary and need confirmation.
- If chondroprotection and cartilage regeneration can be confirmed in human trials, Orthokine could become an effective alternative in the prevention and management of OA. To date, there are no studies investigating these effects.

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