Injectable orthobiologics and corticosteroids: can they be friends?
Clinical Options

Abstract

Monotherapies for Osteoarthritis and other chronic, degenerative diseases have not been able to "solve the problem". The epidemic causes enormous financial cost and patient suffering. The secondary consequences include obesity and associated diseases. Newer therapy approaches include more advanced glucocorticoids (GC) formulations and hyaluronan (HA) and biological approaches none of which are FDA approved.

Numerous GC and HA products are approved, however are not capable to satisfy the medical need. HA products have to small a clinical effect size vs placebo; GC are effective only for up to six weeks.

Here we suggest an intra-articular adjunct treatment of GC plus an orthobiologic, autologous conditioned serum (ACS).

In vitro data show that ACS ameliorates known side effects of GC. Clinical results demonstrate that GC accelerates the onset of clinical effect and may reduce the number of necessary ACS injections, while ACS extends the duration of the clinical effect. Frequency of intra-articular injections in OA may thus be reduced to a single shot per 6-12 months.

Bullet points Adjunct treatment of OA

Sustainable – ensures long lasting excellent clinical effects.
Protective – enables low dose corticosteroid use.
Patient-friendly – allows single and low frequency injections.

Introduction

Osteoarthritis (OA) constitutes a public health crisis (Osteoarthritis Action Alliance (OAAA) (UNC) supported by CDC). It is a degenerative chronic joint disease characterized by progressive remodeling of joint structures and an inflammatory component.[1,2] OA is a disease with growing prevalence, affecting more than 32.5 million adults in the United States.[3] Central etiologic factors, in addition to a genetic component, include age-related changes in regenerative capacity, (bio)mechanical changes or stresses, metabolic influences, and mechanisms of local inflammation.[1,2,4,5] Osteoarthritic joint fluids are characterized by elevated levels of one or more inflammatory markers.[6,7] The OA joint is subject to oxidative stress, inflammation, and decreased synovial fluid quality.[8,9,10,11] The most common symptoms of osteoarthritis include pain, functional limitations, tenderness, stiffness, inability to bend, grinding, and joint swelling.[1] The irreparable pathophysiological changes and clinical symptoms lead to a stage where OA has serious individual impact with debilitating and critical associations, including premature mortality, comorbidities, serious adverse effects from chronic pain medications, decreased quality of life, and overall health[12]. In short, this epidemic can be defined as a faulty cartilage repair with, ultimately, breakdown of articular cartilage and loss of quality of life.

OA is associated with common work limitations experienced by approximately half of OA patients and denotes a total of $113.2 billion in all-cause earnings losses for all US citizens suffering from

OA. Also, a major socioeconomic and financial burden of $486.4 billion is attributed to OA in the US.[13]
Osteoarthritis is perceived by the public as an unavoidable disease of old people. However, this is not so simple. Metabolic, nutritional, and physical activity aspects play a role in the disease etiology, not only age. Please note, apart from humans, OA also affects 20% of adult dogs and a significant number of other companion animals. OA is regularly treated with systemic anti-inflammatory drugs (NSAIDs), which have severe long-term side effects. Glucocorticoids are also effective against disease symptoms but not disease progression. Other intra-articular treatments include hyaluronic acids (HA), whose effect sizes in studies are close to placebo, and blood preparations such as autologous conditioned serum (ACS) and platelet-rich plasma (PRP). Recently, allogenic intra-articular stem cell products have been introduced, but their use in companion animals is at this point somewhat costly and combined with unwelcome logistics. There is clearly a medical need for local, low frequency, effective, safe and cost-effective injection therapies in OA.
In effort to improve the quality and frequency of patient treatment, a concept of adjunct use of glucocorticoids (GC) and autologous conditioned serum (ACS) was developed and applied in a number of clinical injection studies. This paper aims to provide an overview of the current clinical knowledge regarding this concept.

Glucocorticoids are regularly used as effective anti-inflammatory drugs. Their local and systemic use in orthopedics is widely recognized. In human, equine, and canine OA, GCs are also used intraarticularly, with controversy over their effects. Their mode of action is described in the scientific literature in great depth. McAlindon et al showed in 2017 that “Among patients with symptomatic knee osteoarthritis, 2 years of intra-articular triamcinolone, compared with intra-articular saline, resulted in significantly greater cartilage volume loss and no significant difference in knee pain. These findings do not support this treatment for patients with symptomatic knee osteoarthritis.” [21]
Positive effects have been described in both dogs and horses where low doses of triamcinolone (TA) were administered to treat surgically induced joint damage. The treated groups had significantly better histo-morphologic parameters, including cartilage fibrillation, chondrocyte necrosis, and focal cell loss. No cartilage erosion, cell degradation, or cell death were observed in GC-treated joints.[14,15] Even significant protective effects of low-dose GC on fibrillation, osteophyte formation, and cell viability after chemically induced cartilage injury were demonstrated.[16] Dexamethasone, when used in the short term, can effectively block catabolic effects produced by the combination of proinflammatory cytokines and mechanical injury. Dexamethasone prevented proteoglycan degradation and restored biosynthesis of cartilage matrix[17].
In other studies, adverse effects of corticosteroid application were observed. GCs are described as potent inducers of apoptosis, and they are thought to have an antiproliferative/pro-apoptotic effect on osteoblasts. In turn, increased survival of osteoclasts has been reported.[18] GC treatment has also been reported to inhibit chondrocyte proliferation and hypertrophy, as well as cartilage matrix production.[19,20] In human patients with symptomatic knee osteoarthritis, two years of i.a. TA compared to i.a. saline resulted in significantly greater cartilage volume loss and no significant difference in knee pain[21]. In in vitro studies, TA showed significant

chondrotoxicity as well as a decrease in chondrocyte viability, TA can also induce chondrotoxicity by increasing oxidative stress and altering expression of genes involved in cell death. [22,23,24]. The discrepancy in observed results is possibly due to factors such as the type of organism, dosage, or treatment duration. GC appear to have time- and dose-dependent effects on articular cartilage, with beneficial effects at low doses and problematic effects at high doses and long treatment durations[25].

Autologous Conditioned Serum is a biologic therapeutic (orthobiologic) processed from the patient's own blood. It is an acellular, defibrinated, serum-based, sterile autologous injectate containing inflammation resolving and regenerative factors.
To produce ACS venous blood is collected into a proprietary device (Orthogen, Germany), subjecting it to a defined extracorporeal stimulus. In this environment activated blood cells (platelets and leukocytes) secrete stored as well as *de novo* produced mediators including but not limited to cytokines, growth factors, exosomes, and lipid mediators (Table 1) [26]. The entirety of the secreted factors is defined as a blood cell secretome that accumulates in the liquid phase of the blood after coagulation. Subsequently the serum is isolated from the cellular components by centrifugation and sterile filtration. ACS also contains humoral dissolved proteins present in serum *per se*. The totality of the harvested factors forms the efficacy basis of ACS. ACS is locally injected, to treat disease through regenerative and protective action.
Being autologous and sterile ACS's safety profile is very high, also in combination with other drugs. Robust clinical trials [27-33,37,40,42,43] confirm its efficacy in pain reduction, soft tissue repair and improvement of joint function in humans as well as animals. ACS implements and promotes healing responses where metabolic/biochemical dysregulation has overcome the natural healing mechanisms. In addition to pain relief, observed effects of ACS injections include normalization of synovial fluid viscosity, increase of intrinsic i.a. IL-1Ra, reduction of synovial membrane hyperplasia and joint effusion. Also important is reduction of i.a. radical load footprints such as nitrate and dienes. This coincides with an observed long-term improvement of pain and function in osteoarthritic knees (human) and coffin joints (equine) (Table 2). These studies demonstrate symptom modifying effects and disease modifying properties of ACS.
Clinical and experimental results imply a restoration of joint homeostasis, likely involving a switch of tissue macrophages from M1-type (aggressive, inflammatory, NO* producing) to an M2-type (protective, inflammation resolving).

Ingredient	**Effect**
Cytokines (incl. IL-1Ra, IL-10, IL-4)	Anti-inflammatory
Growth Factors (incl. PDGF, VEGF, IGF)	Regenerative (incl. cell division, vascularization)
Lipid Mediators (incl. SPMs)	Inflammation resolving (incl. Macrophage shift M1 → M2)
Extracellular Vesicles (incl. Exosomes)	Immune-modulatory, Inflammation resolving

Tab.1 Contents of ACS and it's Effects. IL-1Ra = Interleukin 1 Receptor antagonist, IL = Interleukin, PDGF = Platelet Derived Growth Factor, VEGF = Vascular Endothelial Growth Factor, IGF-1 = Insulin like Growth Factor, SPM = Specialized Pro-Resolving Mediators

Study type	**Effect**	**Reference**
Human OA	Pain relief over 6 months	Baltzer [27]
Equine OA model	Improved lameness, Reduced synovial hyperplasia	Frisbie [28]

Equine, naturally occurring tendinopathy	Increased Coll I synthesis	Geburek [30]
Mouse, muscle contusion Human, muscle injury	Increased stem cell presence, Accelerated healing	Wright Carpenter [31,32]
Human ACL reconstruction	Reduced bore tunnel widening	Darabos [33]
Human adhMSC in vitro	Chondrocytic differentiation of adhMSC, enhanced CD90 expression	Blazquez [34]
Human in vitro cartilage culture	Decreased GAG release	Rey-Rico [35]
Canine primary chondrocytes in vitro	Increase of Col II mRNA	Soontararak [36]
Prospective, Controlled Open-Label Clinical Study	Superior to PRP clinically and biochemically	Shirokova [28]
Clinical observational	Clinical effect extends to 2 years	Baselga [37]

Tab. 2 Selected clinical and experimental studies illustrate the observable effects of ACS.

Adjunct Therapy

Complementing intra articular Glucocorticoid with Autologous Conditioned Serum combines two well-studied treatment methods for articular pain, function, and joint disease. Autologous Conditioned Serum has an inflammation resolving as well as regenerative/ protective effect. With regards to joint pathologies a series of i.a. ACS injections is usually applied to build up a solid effect on the joint's homeostasis. This period of build-up may take several weeks but improvement can last for six to 12 months and longer depending on indication and severity of the underlying disease. Glucocorticoids on the other hand have an effect of rapidly calming down inflamed environments, leading to a rapid analgesic effect lasting several weeks. With regards to joint inflammation a careful dosing of GC is advisable and long term, repetitive use is not advocated.

The rationale of combining GC with ACS lies in the mutual support between the two components. GCs deliver "instant pain relief", where ACS has a lasting beneficial effect on joint environment, including longstanding pain resolution and improvement of joint *function. ACS rebuilds balanced joint homeostasis.* The local environment is thus enabled to regenerate, and disease progression may be prevented. Additionally, ACS confers protection from cytotoxicity of GCs (Figure 1). Complementing GC with ACS allows for low injection numbers and frequency.

The treatment is thought to have symptom- as well as disease-modifying effects and a higher effect size than other OA treatments so far. This procedure is protected by worldwide patents.

Indications	**Treatment goals**
Osteoarthritis	Resolution of pain
	Improvement of quality of life
	Improvement of function
	Maintenance of joint mobility
	One injection with long clinical effect
	Deceleration of disease progression
Acute Synovitis	Resolution of inflammation
i.a. ligament or tendon injury	Accelerated healing

Tab. 3 Indications and treatment goals for concomitant use of GC and ACS

Experimental data

(Figure 1) shows protective effects of ACS in *in vitro* culture of primary human chondrocytes in the presence of variable concentrations of triamcinolone. A vitality assay (CCK-8) shows the high impact of Triamcinolone on cells cultured with standard Bovine Calf Serum supplement (10% FCS). The impact was diminished when FCS was replaced with 10% ACS. Then, not only do Triamcinolone-challenged cells show greater viability but also the non-challenged cells. ACS aids chondrocyte proliferation. This protective effect supports the use of ACS adjunct to TA.

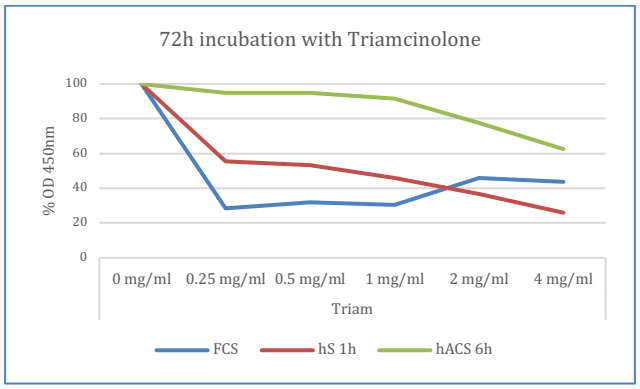

Figure 1 Vitality Assay of human primary chondrocytes cocultured with Triamcinolone (TA) in ascending concentrations and without TA as control. Human ACS (hACS) generated by extended coagulation duration of 6 hours was used as supplement and FCS and human serum (hS) of the same voluntary donors as control. The experiment shows that (a) FCS cannot protect primary chondrocytes, (b) with rising TA concentration viability of chondrocytes decreases, (c) the protective effect of hACS on chondrocyte vitality increases with extended coagulation, during which more regenerative mediators are produced and released into ACS. Each data point represents the means of 3 wells.

Human primary chondrocytes and CCK-8 kit were purchased from Sigma Aldrich. *Cell Counting Kit–8* is for quantitation of viable cell number in proliferation and cytotoxicity assays. Cells were initially proliferated with DMEM/F12 supplemented with standard 10% bovine calf serum (FCS 10%). Subsequently replated in 96well plates with 4000 cells per well with same medium. The assay procedure generates a dye, the OD at 450nm of which is positive correlated to the vitality and number of cellular mitochondria. The scale is normalized to baseline of each group as 100 and the OD decline as per cent % change. OD= optical density; nm=nanometer

Clinical Study Results

<u>Knee Osteoarthritis</u>

Damjanov et al. evaluated the use of Triamcinolone+ACS or Triamcinolone+Placebo (Saline) in human knee OA in 40 patients (20:20). Forty individuals with advanced knee osteoarthritis (Kellgren&Lawrence Grades III-IV [38]) received either 5ml ACS or 5ml Saline (Placebo) adjunct to TA 40mg. Outcomes were recorded by NRS pain and Knee Injury and Osteoarthritis Outcome Score (KOOS [39]) at baseline and at weeks 3, 6, 12 and 24. At week 24, the TA+ACS group demonstrated significant superiority over the TA+placebo group. Pain relief at 3 weeks was similar between groups. This shows that ACS does not impair short-term GC-mediated pain-relief but selectively enhances long term results. [40, in press]. Both treatment arms were well tolerated.

The adjunct use of ACS aims to reduce the overall frequency of OA injections and enable doctors to administer patients according to label guidance and guidelines, thereby reducing intervention to a minimum. The present clinical trial required immediate sequential injections to minimize risk and burden for the study patients involved. It is possible however to perform the sequential treatment separated by several days, should routine procedures require such an approach. E.g.: first injection TA on day 0 to ease patients pain and process ACS. 2nd injection with ACS on one of the following days.

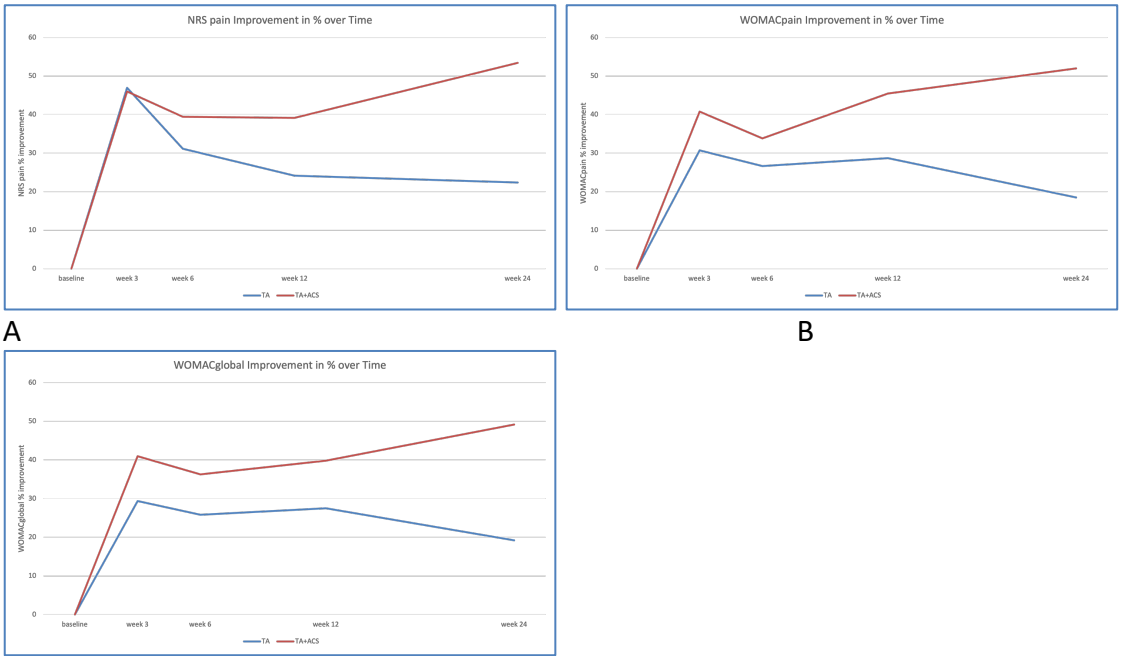

Figure 2 (A) Numeric Rating Scale (NRS), (B) WOMACpain and (C) WOMACglobal [40] rating of clinical effect. The scale is normalized to baseline of each group as 0 and the progress as per cent % change (improvement) of each group over time. WOMAC questions were extracted from KOOS scores to generate Figures (B) and (C).

Temporomandibular Joint OA

A clinical study by Romero-Garcia compared 1 injection each of ACS (2.25mL) n=5 vs Triamcinolone (TA, 0.25mL=10mg) +ACS (2.25mL) n=5. The patients were injected under local anesthesia and were evaluated at baseline and weeks 1, 3, 6, 12 and 24. [42]. This study shows a benefit of TA+ACS until 24 weeks. At 1st and 3rd week the adjunct group improves faster and stronger. The combination of TA with ACS treatment leads to higher pain reduction than with ACS treatment alone. Also, the measured mouth opening was wider under the combination treatment. No safety issues were observed during the study.

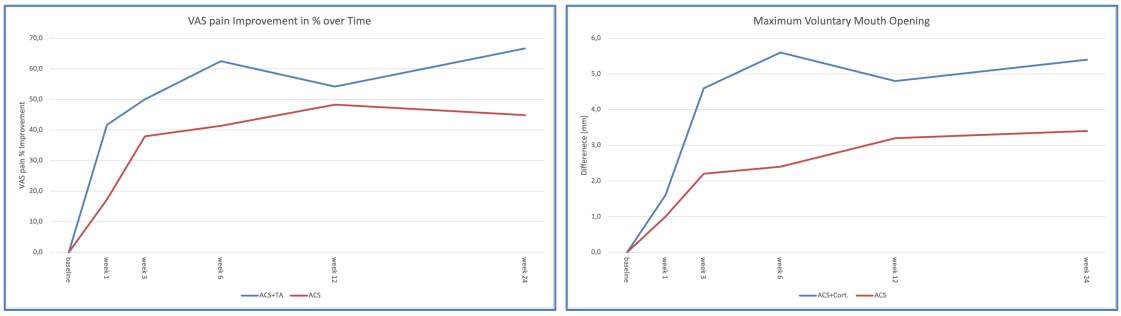

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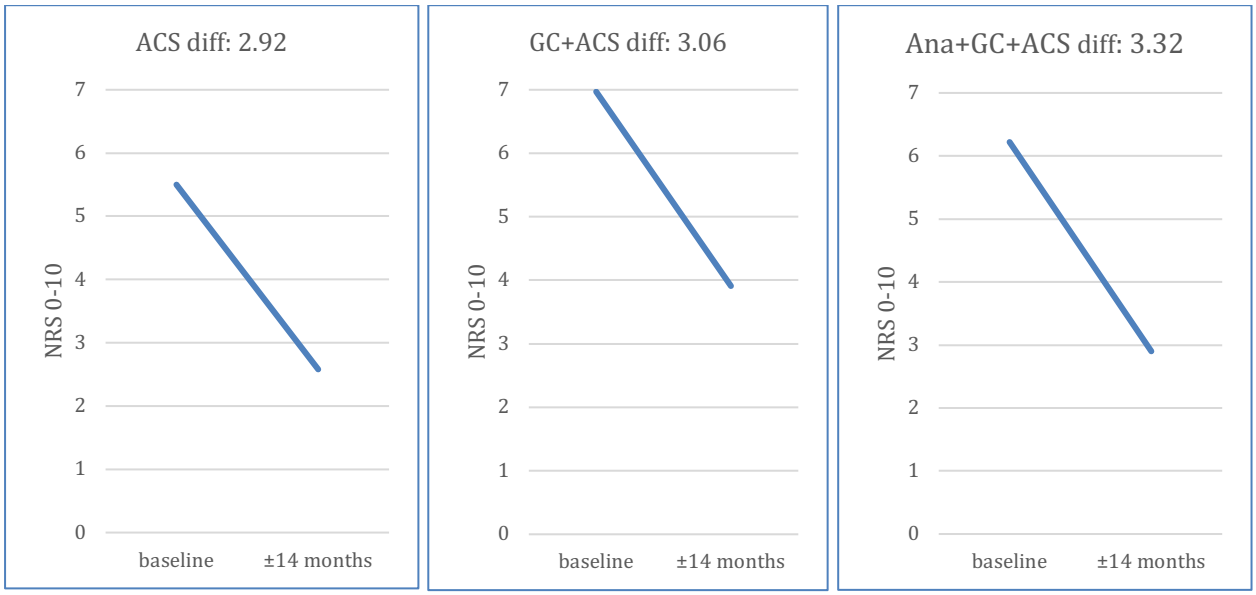
Figure 3 (A) Visual Analog Scale (VAS), (B) Maximal Voluntary Mouth Opening (MVMO) rating of clinical effect. The scale is normalized to baseline of each group as 0 and the progress as per cent % change (improvement) of each group over time.

Hip Osteoarthritis

A retrospective comparison by Baltzer et al. evaluated the effects of ACS vs ACS+GC (triamcinolone) vs ACS+GC+rIL-1Ra (recombinant interleukin-1 receptor antagonist, Anakinra (Ana)) on pain in hip OA (n=150). Measurements were conducted prior to injection treatment and post treatment (in average 14 months post injection). This study demonstrated that ACS efficacy is similar when injected alone or as add-on in hip OA. The study did not evaluate fast onset pain relief. In this study 5-6 ACS; 1-3 GC and up to 4 injections Ana were performed. At 14 months, the two groups - “GC+ACS” and “Ana+GC+ACS” did not show inferior efficacy when compared to injection with ACS alone. No Adverse Events were observed. [44].



A B C

Figure 4 Numeric Rating Scale pain (NRS 0-10) of Hip OA treated with (A) ACS, (B) GC+ACS, (C) Ana+GC+ACS. Aim of this study was to evaluate if added GC or added Ana+GC did change the clinical efficacy of treatment. Mean Pre/post difference in the groups was 2.92, 3.06 and 3.32 respectively.

Canine Hip Osteoarthritis

In a randomized double blinded study on canine hip OA (cross over model) dogs were treated with a single injection of either Saline+3mL ACS or 20mg TA+3mL ACS [43]. The final group size was 12 dogs per arm. Briefly, dogs were followed at days 0, 8, 15, 30, 60, 90, 120, 150, 180. Scores recorded were Canine Brief Pain Inventory (CBPI), Liverpool Osteoarthritis in Dogs (LOAD), and Canine Orthopedic Index (COI).

Significant differences were observed between groups in PSS scores from day 8 after treatment and in both scores (PSS and PIS) of the CBPI from the +15d evaluation up to the +60d evaluation, with TA+ACS showing lower scores. The same was observed with the LOAD score. With the stiffness and function scores, a difference was observed from the +15d to +60d period. At the +90d to +120d period, the two groups experienced a similar level of improvement. At the +120d evaluation, TA+ ACS had better LOAD and function scores and at the +150d had better PIS scores. Clinically significant improvements were observed in both groups compared to baseline. No additional treatment or medications were administered, and no adverse events were observed. The intra-articular administration of ACS was able to improve the overall condition of OA patients. TA+ACS lead to a faster and longer-lasting improvement in pain scores.[45]

Dogs in this study are highly trained as e.g. police or fire brigade dogs. They are worked after treatment with no change in duties.

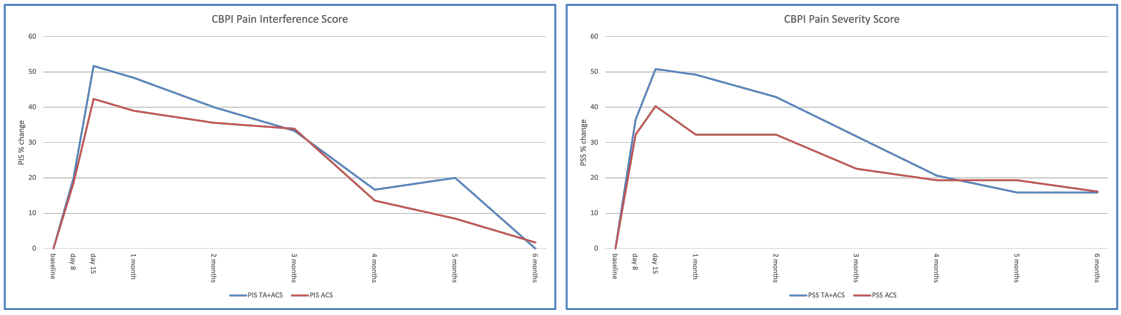


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Figure 5 Canine Brief Pain Inventory (CBPI) (A) Pain Interference Score (PIS) and (B) Pain Severity Score (PSS) rating of clinical effect. The scale is normalized to baseline of each group as 0 and the progress as per cent % change (improvement) of each group over time.

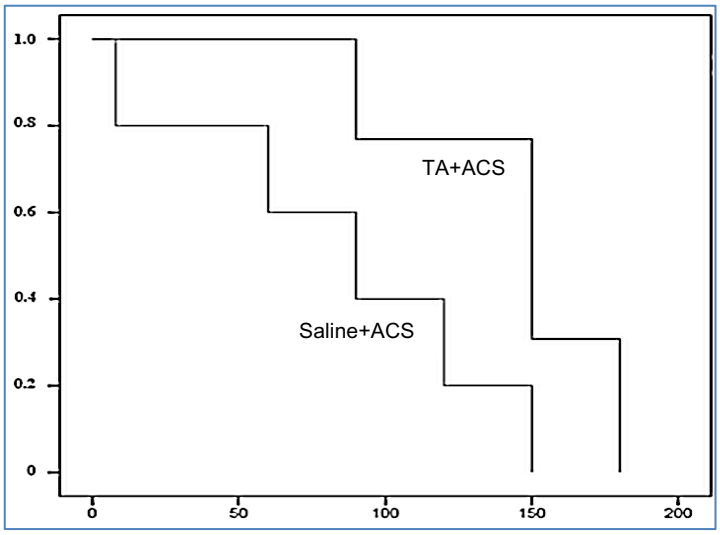

Figure 6 Kaplan Meier graph ("survival plot") shows the time elapsed until relapse. Y axis: proportion recording improvement measured by Canine Orthopaedic Index (COI) function subscore, X axis: time elapsed in days.

Discussion

Up to now single-substance therapies for chronic diseases have failed to “solve the problem” and rather created another chronic situation, namely chronic dependency on pharmaceuticals. GCs are excellent drugs for instant treatment of inflammation and pain. This has led to uninhibited use in medicine. As an intra articular injectate for OA repetitive use of GCs has gained a reputation for inducing cartilage degeneration and (at least in hip) osteonecrosis. Short duration of effect is another characteristic of i.a. GCs. ACS has accumulated a reputation to aid tissue regeneration and restoration of homeostasis. ACS clinical onset of effects however is slower than GCs. Most published ACS protocols require repetitive injections (3-6) and often the clinical onset becomes visible at/after 2nd injection. To support the benign properties of GCs, ACS provides characteristics that prolong the clinical effect (of GC) while at the same time providing growth factors and other mediators buffering the catabolic side effects of GC (Figure 1). In exchange ACS slow onset is hastened by GC. We predict that i.a. therapy will profit from requiring fewer GC and ACS joint injections while achieving the combined clinical benefits. (Figure 7) It is also envisaged that Triamcinolone dosage of 40mg (e.g. for a knee) may be reduced to 10-20mg since the effected downturn of inflammation is sufficient to enable a more rapid initiation of ACS induced mechanisms.

This is a different approach than chosen by other products that aim to increase the retention time of GC in the joint or combine it with other substances such as hyaluronan which have little regenerative properties. We opt to postulate that a simple anti-inflammatory, lubricant or growth factor treatment is less likely to succeed in a degenerative tissue/organ that has entered a senescent downward slope.

All clinical studies presented here are characterized as safe and efficacious with a reasonable duration of 15 weeks improvement in dogs that did not have a post injection rest period and >24 weeks in human applications.

The multiple beneficial components of GCs and ACS lead to an overall improved outcome of therapy in respect to joint pain and function. The concept of “joint homeostasis” has gained traction in the past 10 years with >3000 hits in google scholar. We are convinced that this concept has merit, and it may apply also to other organs/tissues and -in fact- whole organisms. This topic however deserves a separate publication.

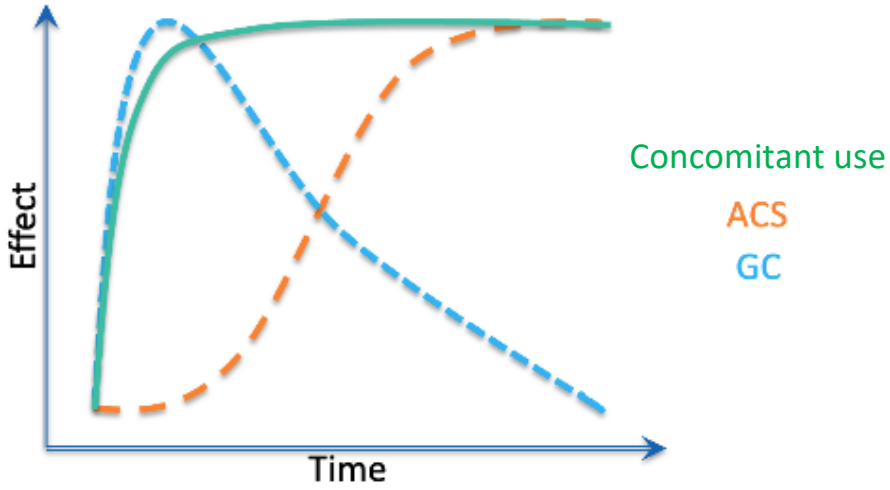

Figure 7 Model of the clinical effect seen with GC, ACS, and the respective adjunct use. Effect as a function of time comparing Autologous Conditioned Serum (ACS), Glucocorticoid (GC) and the concomitant use of GC and ACS.

Conclusion

The concomitant use of ACS with GC combines the fast onset of anti-inflammatory effects of GCs with the long lasting regenerative and preventive effects of ACS in a single injection. Repeated injections at short intervals can be avoided, also, high, potentially harmful doses of corticosteroids can be reduced, and serial injections of ACS can be spared. Figure 7 suggests a model of clinical efficacy based on the data shown for ACS, GC+ACS and GC.

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