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Clinical experience with ACS/Orthokine/IRAP in horses

With compliments

ORTHOCEN
VETERINARY
Clinical experience with ACS/Orthokine/IRAP in horses

THOMAS WEINBERGER

Introduction

Joint diseases have always been the biggest healthcare concern in equine orthopedics. The introduction of new imaging techniques, such as magnetic resonance imaging and computed tomography, and the further development of existing imaging systems, such as digital radiography and digital ultrasound, has improved the accuracy of diagnoses. This refinement of diagnostic potentials has enabled the development of new therapeutic methods in equine medicine. One group of novel therapeutic strategies for the treatment of joint diseases is referred to as regenerative therapy. Regenerative therapies aim to induce or promote tissues and cartilage healing using biological materials and agents, most of which are manufactured under biotechnological processing conditions. The following article will describe the use of the IRAP autologous therapy system, a biotechnology product for intra-articular treatment of joint disease, in more detail.

The ORTHOKINE / IRAP (Orthogen Veterinary Ltd., Düsseldorf, Germany) system for autologous conditioned serum (ACS) therapy has been used for treatment of osteoarthritis in horses since 2001. This biotechnological treatment strategy has been utilized successfully in human medicine since the 1990s. In equine medicine, hyaluronic acid and glucocorticoids were and are the product groups traditionally used for treatment of joint diseases in horses. However, the action of hyaluronic acid is confined to its positive effect on the joint metabolism. Short-term, long-term and extended-release glucocorticoid preparations are only able to reduce acute inflammation symptoms within the scope of the production and secretion of inflammatory factors. They have a positive effect on acute synovialitis and joint effusion, resulting in the alleviation or elimination of pain in the affected joint. However, hyaluronic acid and glucocorticoids do not have a beneficial effect on potential cartilage damage or potential further breakdown of the cartilage via interleukin-1. Moreover, glucocorticoids are also reported to have negative effects on cartilage metabolism and, if cartilage damage exists, further mechanical degeneration can be expected after their injection. Previous strategies for treatment of osteoarthritis in humans and horses were characterized by their focus on the alleviation of pain symptoms but not on the elimination of the actual causes of osteoarthritis pathogenesis.

In arthritis, the response to inflammatory stimuli results in the local release of cytokines, provoking the degeneration of hyaline cartilage and its matrix. Independent of the actual lesion, this stimulus leads to the renewed local secretion of destructive factors that further damage the matrix. This fuels a negative chain of reactions resulting in alteration of the cartilage matrix metabolism. Interleukin-1 (IL-1) is known to be a major mediator in these reactions in both humans and horses. The interleukin-1 receptor antagonist (IL-1Ra) is also endogenously produced. IL-1Ra blocks interleukin-1 receptors on the cartilage surface, thus averting the harmful effects of

Fig. 1. IRAP kit for production of ACS from horses.

Fig. 2: Coated glass beads that stimulate the production of target cytokines in equine blood.
this cytokine. In human medicine, the rationale for injecting IL-1Ra is that the endogenous concentration is too low to prevent cartilage destruction, particularly in chronic arthritis.

**Methods**

Autologous conditioned serum (ACS) is manufactured by selectively stimulating the production of anti-inflammatory cytokines and growth factors. With the IRAP system (Fig. 1), blood from the horse is drawn into a large syringe containing coated glass beads (Fig. 2). Incubation of the blood/bead mixture for 24 hours at body temperature stimulates the peripheral blood leukocytes to produce large quantities of IL-1Ra and other endogenous anti-inflammatory cytokines (Fig. 3).

The individual steps of the ACS production procedure are illustrated in Figures 4 and 5. First, 50 ml of venous blood from the equine patient is drawn into a special syringe under sterile conditions (autologous method). The syringe is then sterilely sealed, placed upright in a rack, and deposited in an

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**Fig. 3:** Schematic representation of the adhesion of monocytes to the glass beads and the production of positive cytokines.

**Fig. 4:** Schematic representation of the individual steps in ACS production. Blood is first drawn into a syringe, incubated for 24 hours, and subsequently centrifuged.

**Fig. 5:** After centrifugation, the serum is extracted and injected through a sterile filter into 2ml syringes. The filled syringes can be used immediately or stored at -18°C.
incubator set to 37°C. After 24 hours of incubation, the entire syringe unit is placed in a special centrifuge and centrifuged for 10 minutes at 3700 rpm to separate the serum from the blood. After centrifugation, a second syringe is used to carefully extract the serum under sterile conditions and subsequently inject it through a sterile filter (0.2 μm) into 2 ml syringes (Fig. 6). Once the syringes have been properly labeled, the harvested ACS can be either stored at be injected through a sterile filter again before it is injected into the joint. The intra-articular injection is subject to the usual provisions of equine medicine.

**Materials**

An analysis of the results of our study on the use of ACS for treatment of osteoarthritis in 262 horses (87 dressage horses, 45 show jumpers, 73 pleasure horses, 39 racing horses (thoroughbreds), and 18 quarter horses) will be presented in the following. A total of 110 coffin joints, 87 fetlock joints, 26 carpal joints, 33 hock joints, and 6 hip joints were treated. The age distribution ranged from 20 months to 14 years. Of the 262 horses treated, 12 were stallions, 123 were mares, and 127 were geldings.

A prior treatment attempt with a conventional intra-articular therapeutic agent, such as hyaluronic acid or glucocorticoids, was a prerequisite for inclusion in this study. The clinical relevance of the affected joint was confirmed in a lameness examination prior to initiation of ACS treatment. Positive joint anesthesia and clinical findings consistent with joint disease were determining criteria for inclusion in the study. Radiographic studies and, in some cases, MRI and ultrasound examinations were additionally performed. Horses with considerable joint remodeling or extreme cartilage defects with partially complete destruction of weight-bearing cartilage areas were excluded from ACS treatment.

IRAP treatment procedure: The horses received a total of two to three intra-articular ACS injections separated by 8 to 12 day intervals, with 2 days of box rest after each injection, hand walking until the next injection, and training according to an individualized plan after the last injection. Corrective/orthopedic shoes were used in some cases. In the clinical lameness examination, the degree of lameness was

<table>
<thead>
<tr>
<th>Indications</th>
<th>Treatment protocol</th>
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<tbody>
<tr>
<td>Poffin joint</td>
<td>D: 4 – 6 ml; N: 2 – 3 times; l: 8 – 14 days</td>
</tr>
<tr>
<td>Pastern joint</td>
<td>D: 2 – 4 ml; N: 2 – 3 times; l: 8 – 14 days</td>
</tr>
<tr>
<td>Fetlock joint</td>
<td>D: 4 – 6 ml; N: 2 – 3 times; l: 8 – 14 days</td>
</tr>
<tr>
<td>Carpal joint (one section)</td>
<td>D: 2 – 4 ml; N: 2 – 3 times; l: 8 – 14 days</td>
</tr>
<tr>
<td>Elbow joint</td>
<td>D: 4 – 6 ml; N: 2 – 3 times; l: 8 – 14 days</td>
</tr>
<tr>
<td>Shoulder joint</td>
<td>D: 4 – 8 ml; N: 2 – 3 times; l: 8 – 14 days</td>
</tr>
<tr>
<td>TMT joint (bone spavin)</td>
<td>D: 1 – 2 ml; N: 2 – 3 times; l: 8 – 14 days</td>
</tr>
<tr>
<td>Hock joint</td>
<td>D: 6 – 8 ml; N: 2 – 3 times; l: 8 – 14 days</td>
</tr>
<tr>
<td>Stifle joint (three sections)</td>
<td>D: 4 – 8 ml; N: 2 – 3 times; l: 8 – 14 days</td>
</tr>
<tr>
<td>Hip joint</td>
<td>D: 4 – 8 ml; N: 2 – 3 times; l: 12 – 21 days</td>
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Fig. 7: Treatment protocol developed by the author for use of IRAP for treatment of joint disease in horses. D = Dose; N = Total number of doses; l = Interval between doses.
graded according to the AAFP scoring system, ranging from 0 (no lameness) to 5 (minimal weight bearing in motion and/or at rest; inability to move). The horses were re-evaluated approximately 6 weeks and 12 weeks after treatment. The basic lameness assessment was performed in a clinical examination. In cases without clear clinical improvement or elimination of lameness, a second joint anesthesia test was frequently performed to confirm the basic diagnosis.

Results
Approximately 6 weeks after treatment, ACS therapy had led to resolution of lameness in 199 of the 262 horses treated and to improvement of lameness in 22 of the remaining 63 horses. Roughly 12 weeks after treatment, 178 of the 262 horses still showed no signs of lameness and were back to normal training. No adverse treatment-related events or reactions were detected after any of the intra-articular injections.

Discussion
More than 1000 ACS injections have been administered at my veterinary treatment facility since 2001. Due to the fact that relevant data from preliminary trials in animals were not available, I developed a treatment protocol for horses based on the available clinical study data and experiences in humans (Fig. 7). By direct extension from human medicine, the assumption that ACS can be used as a treatment alternative for up to Grade 3 (34) arthritis is generally applicable. If complete cartilage destruction is present, clinical improvement cannot be excluded, but any improvement would not be permanent (Figs. 8 and 9). Because the interleukin-1 receptor antagonist competes with interleukin-1 for the same receptor sites on the affected joint, it is important to remove as much joint effusion as possible before injecting ACS into the joint. In complicated cases, joint lavage / arthroscopy is performed before the first ACS injection. The aftercare protocols used following the injection are similar to those used after intra-articular injection of other products.

Based on current knowledge, it must be assumed that the beneficial therapeutic effects of IRAP / ACS are not attributable to the action of a single agent, but to a combination of different endogenous components, such as interleukin-1 receptor antagonist and other endogenous cytokines and growth factors. Because no assays for quantitative determination of the active substances have been developed to date, unambiguous detection is still difficult.

From our perspective, IRAP treatment has produced very satisfactory results. Previous experience has shown that ACS/IRAP is useful for treatment of treatment-resistant joint diseases and moderate cartilage damage and for supportive therapy after joint arthroscopy. Therefore, biotechnology-based ACS / IRAP therapy appears to be a useful addition to the existing treatment armamentarium for joint diseases.

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References