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Original Research

Comparison of Synovial Fluid in Middle Carpal Joints Undergoing Needle Aspiration, Infusion with Saline, and Infusion with a Combination of *N*-Acetyl-D-Glucosamine, Hyaluronic Acid, and Sodium Chondroitin Sulfate

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A B S T R A C T

Synovial fluid white blood cell (WBC) count and total protein (TP) concentration were evaluated in the midcarpal joints of horses to not only determine the effects of needle aspiration, infusion with saline, and infusion with a combination of *N*-acetyl-D-glucosamine, hyaluronan, and sodium chondroitin sulfate (GHCS) at two different doses to evaluate the latter for safety, but to also provide information on saline injection as a control in joints. The midcarpal joints from 24 horses were used for this study. One midcarpal joint served as an untreated control, in which only synovial fluid was aspirated, whereas the opposite joint received either 2.5 mL isotonic saline (n = 8 horses), 2.5 mL of GHCS (n = 8 horses), or 7.5 mL of GHCS (n = 8 horses). Synovial fluid WBC and TP concentration were measured on days 1, 3, 5, 7, 14, and 21. Needle aspiration caused a transient increase in synovial fluid WBC and TP levels after 1 day. Instillation of fluid (2.5 mL), whether saline or GHCS, caused significantly higher WBC and TP concentrations. GHCS at a dose of 7.5 mL created an elevation in TP level for an additional 48 hours; however, after 48 hours, WBC and TP were at concentrations that were not statistically different from controls. Even though an increase in WBC and TP concentrations occurred because of intra-articular saline and GHCS administration, these results were transient demonstrating that GHCS is no different than saline on synovial fluid, WBC, and TP parameters and that as previously described short-term elevation in synovial fluid inflammatory parameters should be expected when saline is used as a control.

1. Introduction

Joint disease is a leading cause of disability in human beings leading to significant loss of days from work and to joint replacement [1]. Joint disease is also one of the most common causes of lameness in horses and has a notable effect on the equine industry [2-4]. There are numerous medications that can be used to manage joint disease, which have been experimentally tested in the past several years [5]. In those experiments, volumes of saline equivalent to those of the injected medication were used as controls. Although previous studies [6,7] have presented data on the effects of saline in joints, a direct comparison with simple needle aspiration within several days has not been performed.

In therapy studies, most of the medications have shown efficacy and safety, and some newer medications now contain

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several compounds that have been shown to be effective independently. However, as new medications are created for clinical use, especially those that contain several different types of chemical compounds, specific safety testing is needed to ensure that the compounds will not cause harm. One such product, a combination of *N*-acetyl-D-glucosamine, hyaluronan, and sodium chondroitin sulfate (GHCS), is composed of 250 mg *N*-acetyl-D-glucosamine (NAG), 12.5 mg of hyaluronan (HA), and 250 mg of sodium chondroitin sulfate. Each compound has shown effects that are considered efficacious. The effect of intra-articular HA has been studied for several decades in horses and in human beings. Intra-articular HA, NAG, and sodium chondroitin sulfate have been found to be safe and efficacious in human osteoarthritis trials [8-10], experimental animal models [11], and in vitro studies [12-14]. Recently Frisbie et al. also showed efficacious effects of sodium HA and polysulfated glycosaminoglycan when injected separately into equine joints [15]. Therefore, on the basis of this earlier in vivo and in vitro work, it appears that the combination of these compounds within one medication may be efficacious.

Although these medications have shown efficacy, safety is also of great concern. The effect of intra-articular HA has been studied intensively in human beings and found in most studies not only to be efficacious but also to be safe. However, a variable percentage of human beings do have transient local reaction to intra-articular HA administration. The percent of people suffering from local transient reaction to intra-articular HA administration ranges from 7% to 53% in these studies [8,16-19]. In addition, Reichenbach found that some forms of HA produced a higher incidence of transient reactions as compared with others [20]. In horses, a case of severe reaction in the fetlock joint 10 hours after injection has been reported [21]. This case resolved with both local and systemic anti-inflammatory medication, but the case demonstrates the need to best ensure that any new medication has been tested for safety.

Considering these findings, there were two goals of this study: (1) to evaluate the effects of synovial aspiration, synovial aspiration with 2.5 mL saline instillation, synovial aspiration with instillation of 2.5 mL GHCS, and synovial aspiration with 7.5 mL GHCS instillation on synovial fluid white blood cell (WBC) and total protein (TP) concentrations over 7 days; and (2) to compare these same parameters between synovial fluid aspiration, synovial fluid aspiration with 2.5 mL of saline, and synovial fluid aspiration with instillation 2.5 mL GHCS over 21 days. The hypothesis was that there would be no significant difference in objective synovial fluid parameters between the four treatment groups over 7 days or between the three groups over 21 days.

2. Materials and Methods

The use of live animals in this project was approved and monitored by the Institutional Animal Care and Use Committee at Colorado State University (IACUC Protocol #: 08-154A-01). On the basis of suggested dosing by the manufacturer, the 1× GHCS (2.5 mL Polyglycan, Arthrodynamic Technologies, LLC, Versailles, KY; dose 2.5 mL) was evaluated at doses administered three times, 7 days apart (days 0, 7, and 14) for 21 days, and the 3× GHCS (7.5 mL) dose only once on day 7. Therefore, the study was divided into two parts, with the study of the 3× dose concluding on day 7, and the remaining horses analyzed for an additional 14 days. The reason for this was to evaluate a single 3× dose and repeated dosing at the 1× dose. A total of 24 horses were divided into three groups (eight horses per group) , and one midcarpal joint of each horse served as the treated joint, randomly assigned either isotonic saline (2.5 mL) (0.9% NaCl, Hospira Inc., Lake Forest, IL; PH 5.6), 1× GHCS (2.5 mL), or 3× GHCS (7.5 mL). The opposite midcarpal joint served as an untreated control in which synovial fluid was aspirated without injection of any material.

All horses were evaluated for lameness on a 0-5 scale, synovial effusion (0-4 scale), and response to joint flexion (0-4 scale) on days 0 (before treatment) and 7 [15]. Synovial fluid was sterilely aspirated from each midcarpal joint from each horse on days 0 (before treatment administration), 1, 3, 5, and 7. At this point, the horses treated with 3× GHCS dose were released from the study, and the remaining 16 horses completed the 21-day portion of the study. In the remaining 16 horses, synovial fluid was aspirated on days 7 and 14, before saline or 1× GHCS administration, and again on day 21. Lameness examinations were again performed on days 14 and 21 before synovial fluid aspiration.

Synovial fluid (2 to 4 mL) was aspirated from each midcarpal joint of each horse using a sterile 20-gauge needle and syringe, and placed immediately into tubes containing ethylenediaminetetraacetic acid. Synovial fluid was analyzed for WBC count by using an automated cell counter and TP by using a refractometer [15].

Data analysis was divided into two parts. In all analyses, a commercial software package was used (SAS Institute Inc, Cary, NC). In the first analysis (analysis A), the effects of control, saline, 1× GHCS, and 3× GHCS were compared using a repeated measures analysis of variance between days 0, 1, 3, 5, and 7. The dependent variables were lameness, synovial effusion, response to joint flexion, synovial fluid WBC count, and TP concentration. The horse served as the random variable, and main effects (treatment and time) and interactions were determined. A least squares means procedure was used for individual comparisons. The value of $P < .05$ was considered significant. In the second analysis (analysis B), the effects of control, saline, and 1× GHCS were compared using the same methods between days 0, 1, 3, 5, 7, 14, and 21.

3. Results

The various treatments caused no adverse effects in clinical observations of the patients. There was no evidence of increased lameness, synovial effusion, or painful response to joint flexion after the administration of either the dose of GHCS or saline compared with controls.

In analysis A, synovial fluid WBC increased significantly on day 1 (24 hours after initial aspiration) in all groups (Fig. 1). However, there was no significant difference in values for control joints between day 0 and days 3, 5, and 7. In addition, synovial fluid WBC was significantly higher in saline, 1×, and 3× treated joints as compared with untreated control joints on day 1, but this was not significantly different on days 3, 5, and 7, resulting in no significant difference from control joints on these days (Fig. 1).

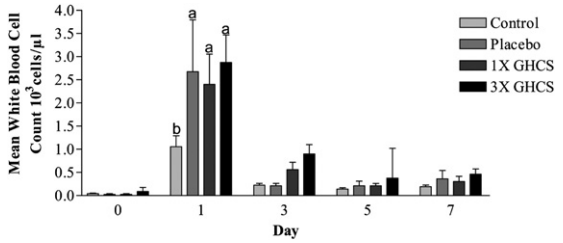

Fig. 1. Mean and standard error of white blood cell counts in synovial fluid on day 0, 1, 3, 5, and 7 for the control, placebo, 1×, and 3× *N*-acetyl-D-glucosamine, hyaluronan, and sodium chondroitin sulfate (GHCS) groups (n = 8 joints per group per time).

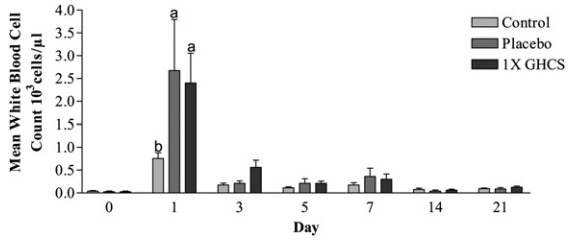

Fig. 3. Mean and standard error of white blood cell in synovial fluid on day 0, 1, 3, 5, 7, 14, and 21 for the control, placebo, and 1× GHCS groups (n = 8 joints per group per time).

However, there was a trend ($P = .0641$) for 3× GHCS to be greater than controls on day 3. The same was true for synovial fluid TP, as it too increased significantly on day 1 in all groups (Fig. 2) and increased significantly on day 1 in all 3 treatment groups as compared with untreated control (Fig. 2). Again, there was no significant difference in control values between day 0 and days 3, 5, and 7. TP in the joints that were treated with 3× GHCS was significantly higher than the joints treated with saline ($P < .0001$) on day 1, and control joints ($P < .0001$) and joints treated with 1× GHCS ($P < .0001$) on day 3.

In analysis B, the WBC increased significantly on day 1, then decreased over time, and WBC was significantly higher in treated joints compared with controls at day 1 (Fig. 3). In addition, the same was true for synovial fluid TP, in that it too increased at day 1; however, there was no significant treatment-by-study day interaction, and for pooled treatment data, saline remained significantly higher than controls over the duration of the study (Fig. 4).

4. Discussion

It seems that there were no detrimental acute clinical effects of GHCS administered at 1× dose once a week for 3 weeks, or GHCS administered at 3× dose only once when compared with intra-articular saline injection. There were no effects on clinical parameters, and those changes in synovial fluid parameters were mostly reflective of fluid administration, based on the results in the placebo group (saline). Although intra-articular administration of saline caused an increase in WBC and TP levels in the joints as compared with control joints, the effects were transient and short-lived. Therefore, the investigators considered this as an appropriate substance to which GHCS could be compared for consideration of safety. However, these effects were compared in a short period and a long-term study would be needed to ensure that no long-term effects were evident.

Needle insertion alone and removal of 2 to 4 mL of synovial fluid created a transient increase in WBC and TP levels in the joints. The magnitude of the rise was increased by instillation of fluid into the joint, regardless of whether it was saline or GHCS. This too was transient because outcome measures were not different between groups by day 3. Pooled WBC data for all treatment groups over time showed significant elevation, mostly because of the increase seen in 3× GHCS. This corresponded to a trend for increased WBC in 3× GHCS over control on day 3.

Other studies have also shown a significant rise in synovial fluid WBC and TP levels after exogenous sterile saline administration. Wagner et al. showed a significant increase in leukocyte count and TP concentrations within 24 hours after intra-articular administration of saline [7]. However, they also noticed that those levels returned to baseline after 7 days. Another study showed similar changes with intra-articular saline [6]. Although they did not report that the levels of TP had significantly changed, the magnitude of change is similar to the results of the present study. The effect of saline on the joint environment has been controversial. In vitro studies on articular cartilage proteoglycan synthesis and content have shown upward decrease of 20% in proteoglycan synthesis and content when articular cartilage is incubated in normal saline [22,23]. However, in vivo lavage studies have shown no significant difference between normal saline and unirrigated controls on both proteoglycan synthesis and electron microscopy results of chondrocytes [24,25]. This

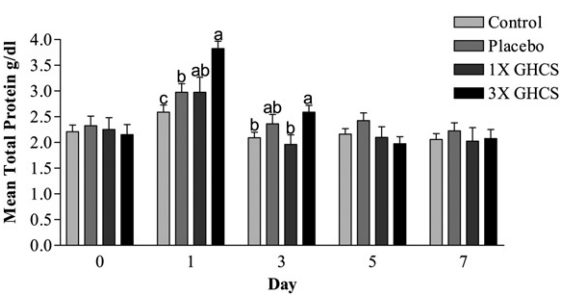

Fig. 2. Mean and standard error of total protein concentrations in synovial fluid on day 0, 1, 3, 5, and 7 for the control, placebo, 1×, and 3× GHCS groups (n = 8 joints per group per time).

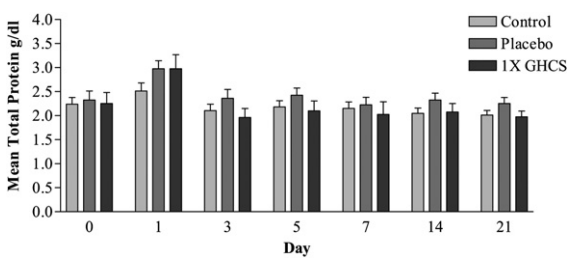

Fig. 4. Mean and standard error of total protein concentration in synovial fluid on day 0, 1, 3, 5, 7, 14, and 21 for the control, placebo, and, 1× GHCS groups (n = 8 joints per group per time).

study further demonstrates that in the equine midcarpal joint, the effects of intra-articular saline administration are transient and synovial fluid parameters return to baseline levels after 24 hours.

Recently a controlled in vivo study has shown a strong efficacious effect of intra-articular GHCS at 1× dose [26], and the current study shows that at this dose there is no significant effect on clinical and synovial fluid parameters in normal joints compared with saline alone. Although efficacy is unknown at 3× dose, the results of the present study demonstrate that in the short term it is safe to administer it intra-articularly. However, more long-term studies are needed to further prove efficacy and safety. The goal of testing the one-time 3× GHCS dose was to test its safety so that further efficacy testing could be performed in the hopes of having a one-time treatment. It is interesting to note that at 3× dose, three times the volume of GHCS was administered as compared with the 1× dose and had some mild effects on synovial fluid parameters. There was a trend for WBC levels in joints treated with 3× GHCS to be higher than controls at day 3, and TP levels in horses treated with 3× GHCS were significantly higher than saline-treated horses on day 1, as well as higher than control levels on day 3. This gives some indication that injected volume may have a mild effect, especially on TP levels. However, this change could be an effect caused by the medication at this dose and should be investigated further. Comparison of 3× GHCS (7.5 mL) to 7.5 mL of saline is needed.

The presence of exogenous fluid in a joint can have an effect on normal joint homeostasis. The issue of fluid volume on joint homeostasis is of concern because high pressure in the joint has the potential of exceeding capillary perfusion pressure, which can lead to transient ischemia [27]. Jawed et al. found that the presence of acute effusion alone does not necessarily result in increased intra-articular pressure [27]. In their study, they found that in patients with acute joint effusion, intra-articular pressure did not increase significantly as compared with those patients who had pre-existing joint disease. Further, in those patients with acute effusion, the volume of fluid did not correlate with pressure. In other words, in normal or acutely damaged joints with no history of disease, increasing fluid volumes have little effect on intra-articular pressure. It has also been shown in an experimental model of joint effusion in the midcarpal joint of horses that the normal synovial membrane is compliant and should be able to maintain relatively normal homeostatic parameters even at high intra-articular pressures [28]. Therefore, although it is unlikely that the presence of exogenous fluid (whether it be saline, 1× GHCS, or 3× GHCS) led to the changes in WBC and TP, a progressive increase in parameters was seen with increased volume. Therefore, a further study is needed to clarify the role of fluid volume and GHCS on joints; in vitro studies maybe useful for clarifying these issues.

There is some concern that aspiration of treated joints at 24 hours may reduce the effective volume of drug remaining in the joint. Although this is a concern for the first 7 days of this study, the 1× dose was reinjected at 7 and 14 days and no significant changes in synovial fluid WBC or TP values were seen 7 days after each injection. Given the fact that there were no negative effects as seen in the efficacy study, the authors conclude that the 1× GHCS is safe for use.

On the basis of the results of this study and the efficacy study reported previously [26], 1× GHCS appears safe and efficacious for intra-articular use. However, 3× GHCS should be studied more extensively to determine chronic effects.

5. Conclusion

The results of this study show that intra-articular administration of GHCS was safe in an acute setting at both 1× and 3× doses. Considering the positive clinical effects in an osteochondral fragment model in horses [26], this product should be safe for use.

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