INTRODUCTION:
Osteoarthritis (OA) is a painful progressive joint disease characterized by cartilage degeneration and inflammation. The pro-inflammatory mediator prostaglandin E2 (PGE2) plays a critical role in the pathogenesis of the disease (1). PGE2 stimulates pain fibers innervating the joint, activates cartilage degrading metalloproteinases and aggrecanases, and promotes the production of other pro-inflammatory mediators including cytokines and chemokines. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to reduce pain and inflammation; however, life-threatening gastrointestinal and cardiovascular side effects have prompted a search for alternative and complementary strategies for the management of OA (2). Beneficial effects have been observed with non-pharmacologic agents such as avocado/soybean unsaponifiables (ASU), glucosamine (GLU), and chondroitin sulfate (CS). These agents have been used individually and in combination to promote joint health in humans and animals. In vitro studies have demonstrated that the [ASU+GLU+CS] combination inhibits pro-inflammatory gene expression, production of PGE2, and activation of metalloproteinases (3-4). Another common non-pharmacologic agent used for the management of OA is the glycosaminoglycan hyaluronan (HA). Its viscoelastic properties are associated with attenuation of NF-kB activation. This display of potent anti-inflammatory activity suggests that the combination may offer additional benefits in reducing pain associated with joint inflammation. The use of HA and [ASU+GLU+CS] combination offers a complementary approach for the management of joint disorders.

METHODS:
Equine articular chondrocytes were isolated from knee joints of mature horses and grown in monolayer culture at 37°C, 5% CO2 until confluent. Cells were seeded onto 6-well plates (5x10^5 cells/well), and incubated for 24hrs with control media alone, HA (125 μg/ml) alone, the combination of [ASU+NMX-1000M, 25μg/ml] + GLU (FCHG90, 15μg/ml) + CS (TRHI22a, 20μg/ml), or the mixture of HA and [ASU+GLU+CS]. Chondrocytes were activated with IL-1β (10ng/ml) 24hrs later or at the same time of treatment. Supernatant from chondrocyte cultures following 24hr IL-1β activation was analyzed for PGE2 production by ELISA. Chondrocytes after 1hr IL-1β activation were analyzed for NF-kB translocation by immunostaining or by Western blot of nuclear fractions. Pair-wise multiple comparisons were carried out using one-way ANOVA, Tukey post-hoc with SigmaStat statistical software (Windows Version 3.11) where P<0.05 was considered statistically significant.

RESULTS:
Equine chondrocytes proliferated in monolayer and produced the cartilage-specific extracellular matrix components type II collagen and aggrecan up to passage four. Chondrocytes responded to cytokine activation with a ten-fold increase in PGE2 production. There was no reduction of PGE2 production by chondrocytes pretreated with HA alone however some inhibition was observed with [ASU+GLU+CS]. In contrast, treatment with the combination of HA and [ASU+GLU+CS] before IL-1β activation significantly reduced PGE2 production (P=0.001, Figure 1). Similarly, treatment with the combination of HA and [ASU+GLU+CS] at the same time as IL-1β activation significantly reduced PGE2 production (P=0.001, Figure 2). Western blots of isolated nuclear fraction (Figure 4, left panel: electrophoretogram and Figure 4, right panel: scanned pixels).

DISCUSSION:
The principal finding of this study is that the combination of HA and [ASU+GLU+CS] significantly inhibited PGE2 production associated with a reduction in NF-kB activation. This display of potent anti-inflammatory activity suggests that the combination may offer additional benefits in reducing pain associated with joint inflammation. The use of HA and [ASU+GLU+CS] combination offers a complementary approach for the management of joint disorders.

REFERENCES:
3. Au R, 11th World Congress OARS. Prague, Czech Republic, 2006.

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