effects of D-P* are dependent on two effects: first to its slow release of H_2S and second through its upregulation of HO-1 and NQO1 and induction of CO production leading to impairment of inflammation. Taken together, these data suggest that D-P* might be considered a promising candidate compound primarily for H_2S research and secondly for the development of novel therapeutic drugs suitable for treatment of degenerative and inflammatory joint diseases.

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COMBINATION OF BONE MORPHOGENETIC PROTEIN 9 AND TRANSFORMING GROWTH FACTOR BETA-1 IMPROVES CARTILAGINOUS MATRIX FORMATION BY EQUINE CHONDROCYTES IN THREE-DIMENSIONAL CULTURE

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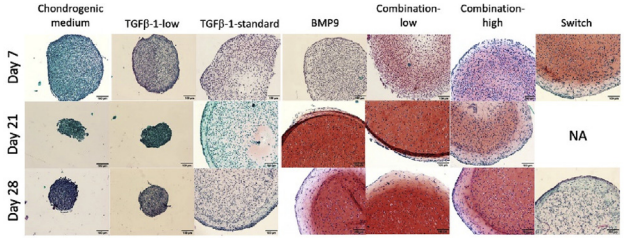
Purpose: Osteoarthritis is not only common in humans, but also highly prevalent in horses. *In vitro*, chondrocytes are used to study disease processes under controlled conditions and the effects of (new) drugs at the cellular level. Bone morphogenic protein 9 (BMP9) has been shown to be a potent modulator of cartilage development by bovine articular chondrocytes. The hypertrophic effect of BMP9 could be antagonized by a low dose of TGFβ-1. We compared the effects of BMP9 and TGFβ-1 on cartilaginous matrix deposition by equine chondrocytes in pellet culture and hypothesized that BMP9 would synergize with a low dose of TGFβ-1 to preserve the healthy chondrocyte phenotype.
Methods: Pellets from healthy equine chondrocytes (P1; n = 2 Shetland Pony donors) were cultured for 28 days with chondrogenic medium alone or supplemented with the following: TGFβ-1-low (0.1 ng/ml), TGFβ-1-standard (10 ng/ml), BMP9 (50ng/ml), combination-low (50 ng/ml BMP9 + 0.1 ng/ml TGFβ-1), combination-standard: (50 ng/ml BMP9 + 10 ng/ml TGFβ-1), or combination-low for one week, followed by TGFβ-1-standard (i.e. Switch group). Bern scoring and collagen type-2 (COL2) staining of histology sections and glycosaminoglycan (GAG) and DNA analsyis were performed at days 7, 21, and 28. Gene expression of chondrogenic markers (aggrecan, collagen type-2, TGFβ-1) and hypertrophic or fibrosis markers (ADAMTS5, collagen type-1, collagen type-3, MMP3, MMP13) were measured at day 7 and 28. Statistical analysis was conducted between TGβ-1-standard and the other groups.
Results: Analysis of Safranin-O/Fast-green stained sections demonstrated higher Bern scores for the BMP9-treated groups at all time points (fig.1). At day 7, COL2 staining was stronger for groups that received BMP9. GAG content normalized for DNA was significantly higher for the BMP9 and combination-low group at days 21 and 28. At both time points, aggrecan expression was significantly higher than standard in all groups that received BMP9 during the entire culture period. However, *COL2* expression was significantly higher for combination groups only, at day 7. *MMP3* was significantly upregulated in the BMP9 and combination-low groups at day 28, whereas *MMP13* expression was significantly downregulated in these two groups at the same time point.
Conclusions: BMP9 improved matrix deposition by equine chondrocytes based on pellet size, GAG/DNA, and intensity of Safranin-O and COL2 staining. Although the combination of BMP9 with low doses of TGFβ-1 upregulated *MMP3* expression, it downregulated the expression of the late hypertrophy marker *MMP13* while upregulating *aggrecan* and *COL2* expression. Further analysis of hypertrophy markers and underlying signalling pathways should be considered for a deeper understanding of the observed synergistic effect. Altogether, a combination of BMP9 with a low dose of TGFβ-1 significantly improved chondrogenesis of equine chondrocytes.



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ASSESSMENT OF CARTILAGE MARKERS IN SYNOVIAL FLUID FROM THE FORWARD STUDY PROVIDE INSIGHT TO THE BIPHASIC EFFECT OF SPRIFERMIN

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Purpose: Sprifermin is a truncated form of fibroblast growth factor (FGF) 18 known to induce chondrocyte proliferation and type II collagen formation [1,2]. Data from preclinical investigations show that cartilage formation happens in different phases after therapy with sprifermin, starting with a phase of cartilage degradation during the induction of proliferation of chondrocytes followed by a phase of cartilage formation/production of extracellular matrix. The aim was to investigate the effect of intra-articular (IA) administrated sprifermin on cartilage turnover as compared to placebo by measurement of markers in longitudinal synovial fluid samples of patients participating in the FORWARD study.
Methods: Synovial fluids (SF) from participants with baseline and at least one follow-up sample (baseline (BL) at three consecutive weeks in six month intervals through to week (wk) 80, fig.A available from the phase II clinical trial evaluating the efficacy and safety of intraarticularly delivered sprifermin [3] were selected for the investigations. Biochemical markers were measured in available SF samples of the placebo (saline IA, n=38) and the highest sprifermin dose group (100 mg/IAx4, n=59). Each included patient had baseline and at least one FU sample available. Samples were pretreated with ultrasound and centrifugation to decrease viscosity. Markers measured were PRO-C2 (type II collagen formation), huARGS (aggrecan degradation), and FBN-C (fibronectin). Markers were technically validated for synovial fluid measurement. Data were normalized to baseline to investigate the median change over time.
Results: Baseline mean (SD) levels of the markers in SF at BL were: PRO-C2, 21.4 (13.6) ng/mL, huARGS, 1117 (516) pM and FBN-C, 2556 (1959) ng/mL. PRO-C2 was initially decreased (from BL to wk 2) after injection with sprifermin; however, the level was increased at the beginning of a new injection cycle followed by a decrease after injection of sprifermin (Fig.B). Overall PRO-C2 levels increased over time in therapy with sprifermin, while no change was observed for the placebo arm. huARGS showed a similar pattern as PRO-C2 - there was an overall increase in ARGS over time in the sprifermin group (fig.C). Interestingly ARGS continuously decreased over time in the placebo group. FBN-C is continuously increased after injection's cycles, whereas no effect was seen in the placebo group (fig.D).
Conclusions: Confirmatory of the preclinical investigations we saw a biphasic response on cartilage turnover after injection with sprifermin. Cartilage formation and chondrocyte proliferation was only modulated by sprifermin, and cartilage degradation (ARGS) was temporal induced and reduced by sprifermin and saline injections, respectively.*1. Gigout A, Guehring H, Froemel D, Meurer A, Ladel C, Reker D, et al. Sprifermin (rhFGF18) enables proliferation of chondrocytes producing a hyaline cartilage matrix. Osteoarthr Cartil. 2017;25. 2. Reker D, Kjelgaard-Petersen CF, Siebuhr AS, Michaelis M, Gigout A, Karsdal MA, et al. Sprifermin (rhFGF18) modulates extracellular matrix turnover in cartilage explants ex vivo. J*

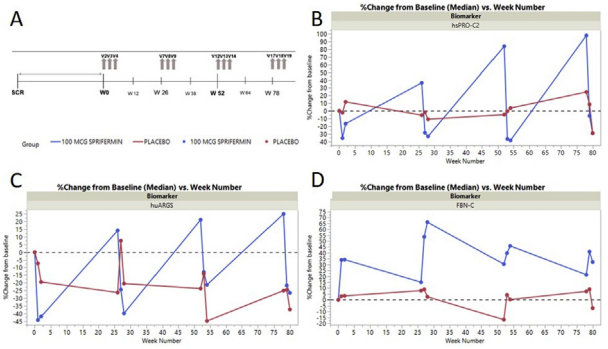

Fig. Assessment of cartilage markers in synovial fluid at the 4 cycles. A) Study overview. B) type II collagen formation, PRO-C2. C) Aggrecan degradation, huARGS. D) Fibronectin degradation, FBN-C. Data are shown as median of available samples at each timepoint.