CLINICAL AND METABOLOMIC EVALUATION OF DOGS AFFECTED BY OSTEOARTHRITIS TREATED WITH UNDENATURATED COLLAGEN TYPE II

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INTRODUCTION

- **Osteoarthritis** (OA) is a chronic disease that affects the synovial joints, characterised by inflammation, metabolic imbalance and tissue damage. Pain and mobility impairment are the most important symptoms related to OA. (1)
- **Undenaturated type II collagen (UC-II)** is a feed material, which has demonstrated its benefits in human and animals suffering from OA. UCII reduces inflammation acting on the modulation of immune response (T cells) toward cartilage constituents (oral tolerance).(2)
- **High resolution ^{1}H Nuclear Magnetic Resonance (NMR)** spectroscopy was proposed as valid analysis to characterise the metabolomes of biofluids, able to detect changes associated with osteoarthritis. In veterinary science, studies have been carried out on dogs in experimental condition. (3)
- **The aim of this study** was to evaluate clinical and joint metabolic changes determined by 30 days UC-II supplementation in a spontaneous canine model of OA. Our hypothesis was that UC-II could be effective to improve joint condition, improve clinical signs and metabolic pathways, detectable by ^{1}H-NMR analysis.

Figure 1: Mobility and clinical scores before and after treatment with UC II. * P<0.01

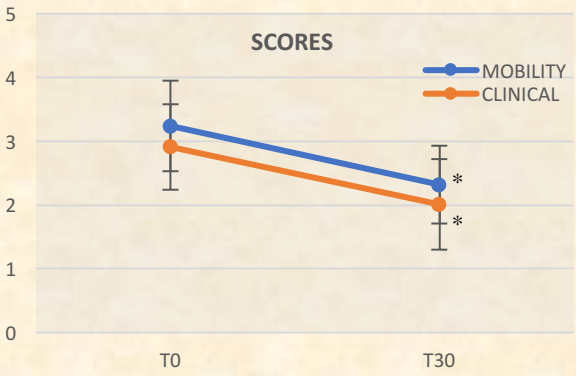


Figure 2: Stacked plot of ^{1}H NMR spectra of pre and post therapy synovial fluid samples, showing difference in the metabolic profile.

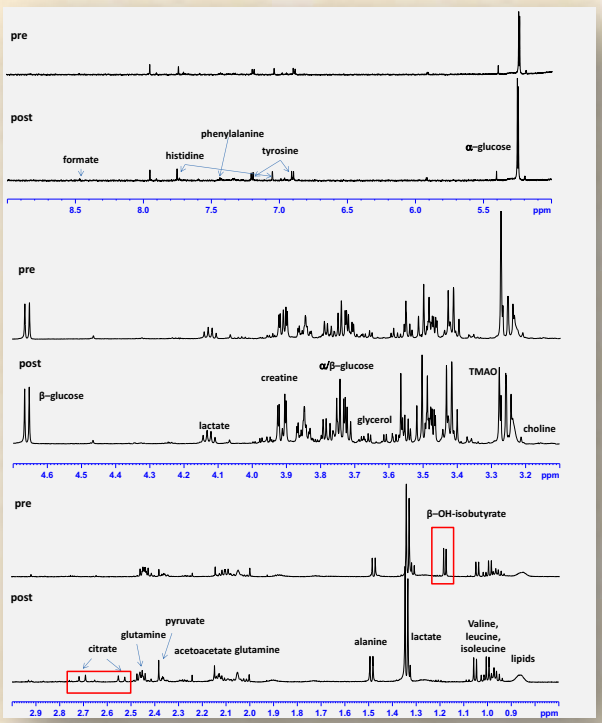
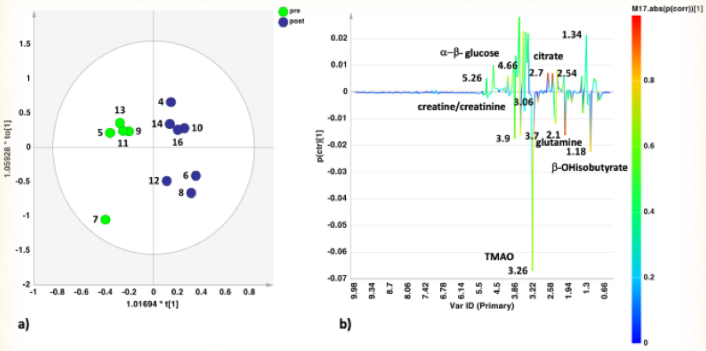


Figure 3 - a) OPLS-DA t1/to[1] scores plot (1+2+0 components give R2X = 0.849; R2Y = 0.971; Q2 = 0.947). **b)** S-line for the model visualizing the p(ctr)1 loading colored according to the absolute value of the correlation loading, p(corr)[1].



METHODS

- **Ethics committee protocol number:** (DETO/223/III/13/2018)
- **Study design:** 30-day, open-label, controlled clinical study.
- **Animals:** client-owned **dogs with naturally occurring osteoarthritis**.

Protocol of the study:
At the time of first presentation (T0), all dogs underwent a complete physical, orthopaedic and X-ray examination. Clinical evaluation of posture, lameness, range of motion and pain on manipulation of the affected joint were scored from 1 to 4 based on severity (***CLINICAL SCORE***). Owners were asked to complete the Liverpool Osteoarthritis in Dogs (*LOAD*) survey to assess mobility alteration (***MOBILITY SCORE***).
A synovial fluid sample (at least 0.3 ml volume) from the affected joint was collected at T0 and stored at a temperature of -20 °C, until the NMR measurements. All measurements were performed on a Bruker Avance III 600 Ascend NMR spectrometer, operating at 600.13 MHz for ^{1}H observation. The NMR spectra were segmented in fixed rectangular buckets of 0.04 ppm width.
Supplementation: UC-II (Flexadin® Advanced), a tablet (40 mg) per day orally for 30 days.
Follow up: the MOBILITY and CLINICAL scores were reassessed and synovial fluid was resampled in all dogs after 30 days (T30).

Statistical analysis For the demographic and clinical data, the mean and standard deviation were calculated. Clinical data were compared between T0 and T30 with the one way ANOVA for repeated measures. A $P < 0.05$ was considered statistically significant.
After the characterization of the metabolites, a multivariate statistical analysis (MVA) was performed, using Simca-P version 14 software.
In particular, Principal Components Analysis (PCA), and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) were performed.

RESULTS

- **Fifteen dogs were included** in the study and considered for the clinical evaluation and UC-II supplementation.
- In **8** cases we were able to obtain the **synovial fluid samples** at T0 and T30.
- Dogs had an overage age of 62 ± 24 months and weight of 32.6 ± 12.7 kg. The *MOBILITY* score was lower ($P < 0.01$) at T30 (2.32 ± 0.61) compared to T0 (3.24 ± 0.71). *CLINICAL* score was lower ($P < 0.01$) at T30 (2.01 ± 0.71) compared to T0 (2.91 ± 0.67). (Figure 1)
- Visual inspection of the synovial fluid spectra showed typical signals ascribable to different metabolites. (Figure 2)
- **Supervised OPLS-DA analysis performed on synovial fluid samples spectra gave a model (1 + 2 + 0) with excellent fit and predictive parameters (R2X= 0.849; R2Y= 0.971 and Q2 = 0.947) that highlighted the marked separation between pre and post therapy samples**. (Figure 3)
- The group of post therapy samples was characterised by a higher relative content of **citrate**. The pre-therapy samples were characterised by a higher relative content of **β-hydroxybutyrate, glutamine, trimethylamine-N-oxide (TMAO) and creatine/creatinine.**

CONCLUSIONS

The results of this study confirmed (4) that UC-II supplementation is able to improve the clinical and mobility scores in dogs affected by OA. The application of ^{1}H-NMR analysis revealed a marked separation between synovial fluid samples obtained from dogs pre and post therapy. The metabolic changes relative to content of β-hydroxybutyrate, glutamine, TMAO and creatine/creatinine in pre-therapy samples and citrate in post therapy ones, proved that methabolic pathways related to glucose and lipid metabolism, hyaline cartilage collagen and proteoglycan destruction and muscle break-down are influenced by UC-II.

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