HIND LIMBS PRESSURE ANALYSIS IN CHRONIC OSTEO-ARTICULAR MODEL OF RABBITS

Ioan HUTU¹,², Bianca Cornelia LUNGU¹,²*, Bratu Daniel¹, Spătaru Ioana-Irina¹,², Torda Iuliu¹,², Calin MIRCU¹,²

* e-mail of corresponding author: bianca.lungu@fmvt.ro

Abstract

Osteoarthritis (OA) is the most common joint disease. Animal models provide a clinically relevant way to study the efficacy and toxicity of potential treatments for OA. The aim of paper was to study the impact of some variables (housing conditions, treatments) on pressure exerted by the hind limbs of the rabbits as indicators / control variables in chronic osteo-articular animal model (OA). A number of 38 (3-31/2 month old) rabbits in 4 groups (non-OA, OA-control, OA-treatment 1 and OA-treatment 2) where observed for 8 weeks period. Pressure and peak pressure were measured with MobileMat™ device. For both the left (FSX-L) and right hind limb (FSX-R), positively correlated (r=+0.693 and a p =0.000) the Mann-Whitney Test indicating a significant difference (p=0.028 and p=0.023) in the pressure exerted by those limbs depending on the post-operative or non-operative state of the rabbits. The peak pleasure for the right hind limb (FSX-R), was significant (p=0.019) in OA and non-OA comparison. Pressure exerted by this limb depending on the post-operative or non-operative state of the rabbits. The most relevant correlation is between peak pressure of left (FSX-L) and right (FSX-R) which are negative and significant (r=-0.425 and a p =0.008). In conclusion, the results of the study were not influenced by cage types and treatments but body mass and OA model are clearly associated with raw pressure and peak pressure on hind limbs.

Key words: osteo-articular rabbit model, pressure and peak pressure

Osteoarthritis (OA) is the most common joint disease (Sharma, 2021). As there is no proven disease-modifying treatment, it often results in chronic pain, physical disability, and impairment of life quality. It is a multifactorial chronic degenerative disease involving both genetic and environmental components (Goldring, 2006; Primorac et al, 2020). Due to an imbalance between cartilage cell catabolism and anabolism, proteoglycan at the cartilage surface is progressively lost, followed by collagen Type II degradation. As a result, cartilage fissures and cracks occur at the cartilage surface. As OA progresses, increased area of calcified cartilage and vascular invasion into the articular cartilage contribute to the decrease in articular cartilage thickness. Osteophyte formation and thickening of the subchondral bone are also hallmarks of OA (Goldring, 2006, Krasnokutsky et al 2007).

Animal models provide a clinically relevant way to study the efficacy and toxicity of potential treatments for OA. The rabbit model has been used in the past to evaluate the efficacy of various compounds in OA treatment (Rebai et al, 2020; Yan et al, 2021; Go et al, 2022; Wang et al, 2022). The rabbit offers the advantages of being easy to use and to have a similar gross knee appearance as humans (Pritzker et al 2010, Gregory et al 2012).

The aim of paper was to study the impact of some variables (housing conditions, treatments) on pressure exerted by the hind limbs of the rabbits as indicators / control variables in chronic osteo-articular animal model (OA).
MATERIAL AND METHODS

The Experimental Units of Horia Cernescu Research Unit are an authorised research infrastructure for using animal in the procedure of research projects where a number of 38 New Zealand micro-chipped females and males rabbits, aged 3 - 31/2 months, were used in an osteoarthritis study. The study took place over a period of 8 weeks in the Experimental Units of the University of Life Science “King Michel I” from Timisoara under the Ethical Statement no. 87 07.05.2018 and Project authorization no. 002 25.06.2018. A number of 38 animals were divided into 4 groups: A non-OA (12 rabbits), B – control OA (no treatments-9 rabbits), C – OA- treatment 1 (9 rabbits) and D – OA-treatment 2 (9 rabbits). After the accommodation period, in the first week of study the animal model was performed, followed in 2nd week by first intra-articular treatment and in 7th week by last treatment.

The rabbits were kept individual in four different types of cages (LxH): standard (S) cages (713x716x476 mm, Techniplast®) with plastic floor with holes, cats (C) and dog (D) stainless steel cages (1490x640x1580 mm) with steel floor with holes and Guinea Pig (GP) double cages (846x610 x256x256, Techniplast®) with plastic floor with square holes, in three rooms: rabbits room (14.69 m²), guinea pig room (10.52 m²) and rats room (11.35 m²).

The environment temperature and humidity were continuously monitored (every half an hour) by multi-functional wireless digital device Weather Station PCE FWS 20. The lighting program was 14 hours light/10 hours dark.

Each rabbit received daily 160 g of pelleted feed and water ad libitum.

During the trial, the body mass, feed consumption, average daily gain, feed efficiency pain scoring, the pressure of legs was measured weekly and clinical signs, telemetry temperature (Hutu si col. 2018) was observed daily.

Currently, the assessment of pain, suffering or distress in animals used in procedures is based on the physiological responses and behavioral changes that the animal exhibits. (Hutu, 2017; Mota-Rojas et all, 2020). SOPs, clinical observation or Welfare Committee controls together with principal investigator. In addition of pain scoring chart, when the trend of body weight is decreasing, below the lower action limit (LAL = X±1.96xSVx2x5 for two consecutive measurements in ≤15 days) the animal is takeout form the study group.

The modified MobileMat™ device was the instrument for measuring the i) pressure and ii) peak pressure exerted by the hind limbs of rabbits (Hall et al, 2022). Data were recorded in 10-second intervals, thereby ensuring a detailed capture of pressure variations. A number of 2 to 6 measurements were made for each animal during the last two weeks of trial.

In the data analysis, were considered the variable: the post-operative or non-operative state of the rabbits, housing conditions, administered treatment and the number of measurements taken for each individual. The statistical tests used were: ANOVA, t-test, GLM Analisys (Test of Equality of Covariance, Mauchly's Test, followed by Greenhouse-Geisser and / Huynh-Feldt) with repeated measures using SPSS Statistics for Windows, Version 17.0. (Chicago: SPSS Inc. USA). A P-value of <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSIONS

Pressure results (Figure 1). The results of the statistical analysis revealed the impact of the OA animal model (operated at right knees) vs. Non-OA pressure exerted by the limbs of the rabbits. For both the left (FSX-L) and right hind limb (FSX-R), the Mann-Whitney Test indicating a significant difference (p=0.028 and p=0.023) in the pressure exerted by those limbs depending on the post-operative or non-operative state of the rabbits.

By the results of Mann-Whitney Test the type of cage did not impact significant the level of pressure of legs (FSX-R, p=0.652 and FSX-L, p = 0.743. Also, the differences between pressures of legs in terms of absolute value (p=0.745) and percent (p=0.674) was not significant.

In complementing the previous analysis, it is essential to mention that the variable related to the treatment administered to the rabbits did not have a significant impact on any of the studied pressure on left (p=0.119) or right legs (p=0.133).

A significant Pearson correlations was observed between the pressure exerted by the left and right hind limbs, with a correlation coefficient of r=+0.693 and a p = 0.000. The left and right hind limbs was positively correlated with body mass (r=+0.420 and a p = 0.009 and respectively, r=+0.549 and a p = 0.000).

For the FSX-L - FSX-R difference, the Box’s test of equality of covariance indicates that the assumptions of homogeneity of covariance was
met (p=0.480). The multivariate test demonstrated that the main effect of treatment was not statistically significant Wilks’ Lambda = 0.892, F=1638 at p=0.213 on FSX-L - FSX-R but the time x group (treatment) interactions was statistically significant Wilks’ Lambda = 0.813, F=3316 at p=0.050.

Peak pressure results (Figure 2). The results of the statistical analysis revealed the impact of the OA animal model (operated at right knees) vs. Non-OA peak pressure exerted by the limbs of the rabbits. For the right hind limb (FSX-R), the study finds a significant difference (p=0.019), in the pressure exerted by this limb depending on the post-operative or non-operative state of the rabbits. In the case of the left hind limb (FSX-L), peak pressure test, showed a p=0.053, nearly reaching the threshold for statistical significance. The pressure difference between FSX-L and FSX-R (p=0.447) and the percentage difference between pressure (p=0.269) of the two limbs showed no significant differences.

For the peak pressures the GLM analyses did not show any effects in our study.

One of the limitations of study was related with the short period of using pad pressure – last two weeks of the study. Perhaps, the longer periods between measurements will give more significant results. Thus, the pressure appear to be a better indicator than peak pressure in OA rabbit models but a real challenge is to keep the animals motionless on the pressure pad, which is possible with gentle training to avoid any pain in OA models.

CONCLUSIONS

Both pressures and peak pressure exerted by the limbs of the rabbits can be use for monitoring the OA model in rabbits. In a chronic model the operating (right in our case) leg is less used and the peak pressure of the body is greater on left part but the investigator have to chose a strong statistical model, recommended repeated measures in order to find the effects of treatments.

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