Article
https://doi.org/10.61900/SPJVS.2023.02.13

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

Ioan HUTU[1,3], Bianca Cornelia LUNGU[1,2*], Bratu Daniel[1], Spătaru Ioana-Irina[1,2], Torda Iuliu[1,2], Calin MIRCU[1,2]

* 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 an 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 an indicators / control variables in chronic osteo-articular animal model (OA).

.

[1] *Horia Cernescu* Research Unit – USV Timisoara, RO.
[2] University of Life Science "*The King Michael I*" – Faculty of Veterinary Medicine, 119th Calea Aradului, Timisoara, 300645, RO.

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 a osteoarthrosis 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 (LxlxH): standard (S) cages (713×716×476 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) doubled cages (846×610 ×256+256, Techniplast®) with plastic floor with square holes, in three rooms: rabbits room (14.69 m^3) , guinea pig room (10.52 m^3) and rats room (11.35 m^3) .

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±1.96x√2xSD 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 reapeted 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.

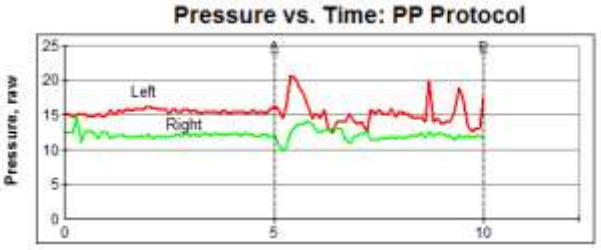

Figure 1. **Pressure distribution on left** (red) **and right** (green line) **during 10 seconds of measurement (Group B, rabbit no 15)**

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.

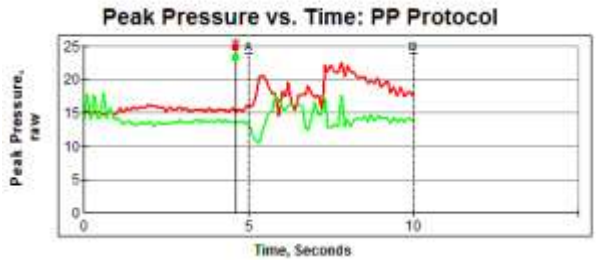

Figure 2. **Peack pressure distribution on left** (red) **and right** (green line) **during 10 seconds of measurement (Group B, rabbit no 15)**

Regarding the results of *Mann-Whitney Test* the type of cage did not impact significant the level of pressure of legs (FSX-R, *p*=0.574 and FSX-L, *p* = 0.847). Also, the differences between peak pressures of legs in terms of absolute value (*p*=0.661) and percent (*p*=0.646) 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 peak pressure on left (*p*=0.217) or right legs (*p*=0.069). .

A significant *Pearson correlation* was observed between the peak pressure exerted by the left and right hind limbs, with a correlation coefficient of *r*=+0.694 and a *p* =0.000. The left and right hind limbs was positively correlated with body mass (*r*=+0.432 and a *p* =0.007 and respectively, *r*=+0.531 and a *p* =0.001). 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).

For the peack 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 gentile 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.

ACKNOWLEGMENTS

The research was financed by Extension Unit, ONG, in Research Contract no 6584 from 19.10.2015 – *Const analysis and evaluation of needs in Horia Cernescu Reseach* and Contract no. 8359 of 26.06.2018 conducted under condition of Ethical Statement no. 87 07.05.2018 and Project authorization no. 002 25.06.2018.

REFERENCES

Benato L., Rooney N. J., & Murrell J. C., 2018 - Pain and analgesia in pet rabbits within the veterinary environment: a review. Veterinary Anaesthesia and Analgesia. doi:10.1016/j.vaa.2018.10.007

Davidson J. Spreadb d., 1975 - Nutrition of the New Zealand White rabbit. Proceedings of the Nutrition Society, 34(1), 75-83, doi: 10.1097/PNS19750013

Go EJ, Kim SA, Cho ML, Lee KS, Shetty AA, Kim SJ. A Combination of Surgical and Chemical Induction in a Rabbit Model for Osteoarthritis of the Knee. Tissue Eng Regen Med. 2022 Dec;19(6):1377-1388. doi: 10.1007/s13770-022-00488-8.

Goldring, S. R. & Goldring, M. B. Clinical aspects, pathology and pathophysiology of osteoarthritis. *J Musculoskelet Neuronal Interact* **6,** 376–378 (2006).

Gregory, M. H. *et al.* A review of translational animal models for knee osteoarthritis. *Arthritis* 2012, 764621–14 (2012).

Hall P., Stubbs C., Anderson D.E., Greenacre C., Crouch D.L. 2022. Rabbit hindlimb kinematics and ground contact kinetics during the stance phase of gait. PeerJ 10(1):e13611 DOI: 10.7717/peerj.13611

Huțu I., 2018 – Manual de bune practice în unitățile experimentale. Editura Agroprint Timișoara, Vol. II, cap. VI, 207-234. ISBN: 978-606-785-036-9

Hutu, I., Patras,I Gherghel D., Lungu, B., Mircu, C, Application of infrared thermography in rabbit

orthopaedic models , Lucrări științifice - Medicină veterinară, 2018, Vol. 61. Partea a 3-a.9-14, Editura “Ion Ionescu de la Brad”.

Krasnokutsky, S., Samuels, J. & Abramson, S. B. Osteoarthritis in 2007. **65,** 222–228 (2007).

Laverty, S., Girard, C. A., Williams, J. M., Hunziker, E. B. & Pritzker, K. P. H. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the rabbit. in *Osteoarthr. Cartil.* 18 Suppl 3, S53–65 (Elsevier, 2010).

Mota-Rojas D, Olmos-Hernández A, Verduzco-Mendoza A, Hernández E, Martínez-Burnes J, Whittaker AL. The Utility of Grimace Scales for Practical Pain Assessment in Laboratory Animals. Animals (Basel). 2020 Oct 9;10(10):1838. doi: 10.3390/ani10101838. PMID: 33050267; PMCID: PMC7600890.

Pelletier J.P., Kapoor M., Martel-Pelletier J. 2015. 174 - Animal models of osteoarthritis. Rheumatology (Sixth Edition),Volume 2, Pages 1454-1461

Primorac D, Molnar V, Rod E, Jeleč Ž, Čukelj F, Matišić V, Vrdoljak T, Hudetz D, Hajsok H, Borić I. Knee Osteoarthritis: A Review of Pathogenesis and State-Of-The-Art Non-Operative Therapeutic Considerations. Genes (Basel). 2020 Jul 26;11(8):854. doi: 10.3390/genes11080854.

Rebai MA, Sahnoun N, Abdelhedi O, Keskes K, Charfi S, Slimi F, Frikha R, Keskes H. Animal models of osteoarthritis: characterization of a model induced by Mono-Iodo-Acetate injected in rabbits. Libyan J Med. 2020 Dec;15(1):1753943. doi:10.1080/19932820.2020.1753943.

Sharma L. Osteoarthritis of the Knee. N Engl J Med. 2021 Jan 7;384(1):51-59. doi: 10.1056/NEJMcp1903768.

Wang L, Zhao L, Shen L, Fang Q, Yang Z, Wang R, Wu Q, Xie Y. Comparison of the effects of autologous and allogeneic purified platelet-rich plasma on cartilage damage in a rabbit model of knee osteoarthritis. Front Surg. 2022 Jul 15;9:911468. doi: 10.3389/fsurg.2022.911468.

Yan F, Zhao X, Duan S, Maimaiti A, Qi Y, Li M, Maimaiti M, Li W. High fibular osteotomy ameliorates medial compartment knee osteoarthritis in a rabbit model. J Biomech. 2021 Nov9;128:110734.doi:10.1016/j.jbiomech.2021.110734

Yoshioka **M., Coutts R.D., Amiel D., Hacker S.A.** Characterization of a model of osteoarthritis in the rabbit knee Osteoarthritis Cartilage. 1996 Jun;4(2):87-98. doi:10.1016/s1063-4584(05)80318-8.

*****FELASA** working group on revision of guidelines for health monitoring of rodents and rabbits; Mähler Convenor M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, Raspa M. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim. 2014 Jul;48(3):178-192. doi: 10.1177/0023677213516312. Epub 2014 Feb 4. Erratum in: Lab Anim. 2015 Jan;49(1):88. PMID: 24496575.