RADIOLOGICAL ANALYSIS OF EXPERIMENTAL DISC DEGENERATION IN RABBITS

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ABSTRACT

Objective: To validate radiographic evaluation of a rabbit model for disc degeneration. Methods: Lumbar intervertebral discs of New Zealand rabbits were stabbed three times with a 18G needle at a limited depth of 5mm, through lateral approach. Serial radiographic images were taken on the early pre-and postoperative periods, and after four, eight and 12 weeks of the procedure, with subsequent analysis of disc height, osteophyte formation, endplate sclerosis, and presence of disc degeneration. The statistical analysis of data was validated by the Kappa coefficient, with a confidence interval (CI) of 95%. Results: A significant reduction of disc space was found on AP X-ray images after 12 postoperative weeks, with Kappa = 0.489 for CI 95% (0.25-0.72) with p < 0.001. X-ray signs of disc degeneration also presented Kappa = 0.63 for CI 95% (0.39-0.86) with p < 0.001. The remaining assessed criteria showed positive results, but with a lower Kappa value. Conclusion: The disc degeneration model using rabbits as proposed in this study was shown to be feasible, with positive X-ray correlation between pre- and postoperative images, validating the potential to induce disc degeneration in this animal model for future studies.

Keywords – *Spondylosis; Intervertebral disk; Radiology; Rabbits*

INTRODUCTION

Disc degeneration involves the changes of the structural, biological, and biochemical features of the intervertebral disc (IVD)[1], characterized by significant loss of the proteoglycan network and water in the disc core. This can lead to clinical evidence of degeneration – the ratio of decrease in disc height – resulting in increased loads on structures near the spine and, ultimately, changing its mechanical relationships[2]. Histologically, there is increased cell proliferation with the formation of clusters and, paradoxically, increased cell death by apoptosis and tissue necrosis[3]. Molecular changes also occur,

such as an increase in the rate of production of cytokines and matrix-destroying enzymes (matrix metalloproteinases [MMPs]), interleukin-6, nitric oxide, and prostaglandin E2[4], and a decrease in its intracellular components (proteoglycans), leading to the gradual loss of intradiscal fluid[5]. This entire pathophysiologic process is also characteristic of normal human aging; there are reports of a gradual reduction of the formation of cartilage in the growth plate starting at 16 years of age[6], characterizing the beginning of discopathy without, however, causing any clinical manifestations. With its natural evolution, the column presents progressive instability in the affected area due to degeneration of the intervertebral disc,

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leading to a series of other events in other elements of the functional unit[7]. Note also that the presence of symptoms related to degenerative disc disease (DDD) depends on the cumulative interaction of a large set of factors, linked to the environment and lifestyle habits, such as micro and macrotrauma, occupation, smoking and atherosclerosis that are capable of modifying, to some degree, the natural progression of degeneration – which is genetically determined[8-11].

At the polar opposite, when degenerative disc disease is symptomatic, it is manifested by chronic lumbar back pain, and is extremely important epidemiologically, generating a significant negative social impact. Several studies have shown its substantial prevalence; it is the second leading cause of medical consultation in the United States[12-16]. Worldwide, about 60 to 80% of people have back pain during their lifetime, resulting in up to 13 million doctor's appointments per year citing it as a complaint in the United States alone[17]. Furthermore, according to U.S. data, US$20 billion are spent each year on the direct treatment costs of chronic back pain, which, in addition to overhead, generate an amount that exceeds US$100 billion[18].

In order to alleviate this alarming picture, several biological models of treatment have been proposed, seeking future applications of new therapeutic technologies and/or effective preventive technologies. However, many doubts and flaws remain in approaches to DDD, requiring scientific progress in this area. In this context, it becomes important to use animal models that, though they do not reflect human characteristics exactly, show many biochemical and pathological changes identical to those occurring in humans[19]. In contrast, in vitro models preclude analysis of the process of degeneration as a complex event involving several structures that act in an interrelated manner and play key roles in the final result, and are useful only for the identification of processes of a short duration[20].

There is the possibility of applying different animal models, and choosing one depends on the objective to be attained. Each has its own advantages and drawbacks, and it is up to the researcher to evaluate them and consider them[21].

By combining the best available methodologies (type of animal used, mode of induction of degenera-tion, lesion depth, period of and method of monitoring evolution, and sample size), the objective of this study is to validate the radiographic evaluation of experimental disc degeneration in rabbits, based on the interobserver reliability of the analysis, hitherto not carried out in previous studies.

METHODS

Induction of disc degeneration

The research design of this study was approved by the Ethics Committee on Animal Use at the Pontifícia Universidade Católica do Paraná under Case #252, Opinion 180/07, and was implemented in accordance with the provisions of the Declaration of Helsinki of the World Medical Association.

For this study, we selected 13 white male New Zealand rabbits weighing between 3.5 and 4.5 kg, of about one year of age.

Each animal underwent preoperative anesthesia with intramuscular ketamine hydrochloride 5% (20-25 mg/kg) – changed to intravenous administration through an auricular vein access in case the anesthesia needed to be maintained for more than 40 minutes – and intramuscular xylazine (0.15 mg/kg). After anesthesia, ventridorsal and lateral radiographs were obtained preoperatively[22-24] to determine the base values for the height of the IVD.

After the induction of anesthesia, the rabbits were placed in a right lateral decubitus position and underwent a left lateral retroperitoneal approach (about 10 cm), exposing the lateral surface of five consecutive lumbar IVDs (L2-L3 to L6-L7). As a control, the first and second IVDs (L1-L2 and L2-L3) were left intact. After palpation, under direct visualization of the intervertebral space to determine the level of the discs, the areas to be punctured (1-2 mm in diameter) were carefully exposed (Figures 1 and 2).

The three caudal IVDs were punctured three times by an 18G needle (Figure 3) at an exact depth of 5 mm, controlled via the bayonet shape (Figure 4). The needle was inserted into the central height of the disc through the annulus fibrosus into the nucleus pulposus, and remained there for five seconds. The injury resulting from the surgical procedure was intensely irrigated throughout its length with sterile saline and closed with sutures. After the suture, the rabbits were subjected to new lumbar spine radiographs.
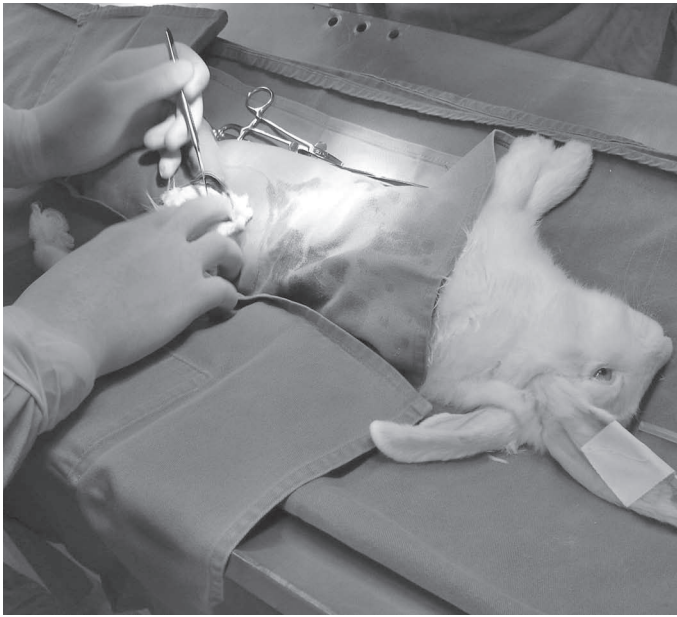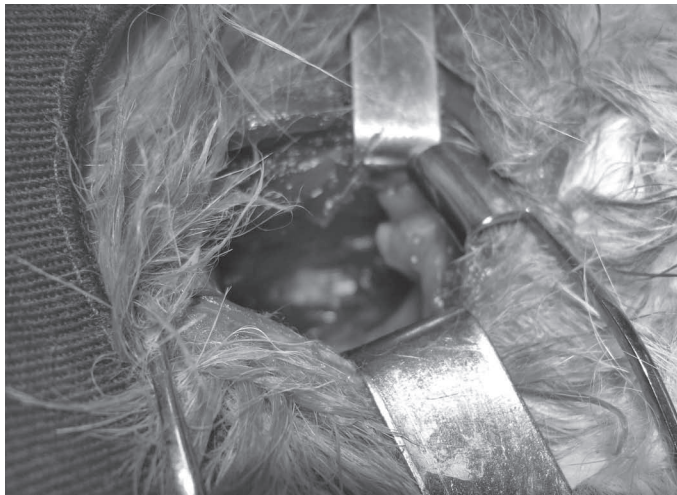
Figure 1 – Beginning of surgical intervention

Figure 2 – Exposure of intervertebral discs

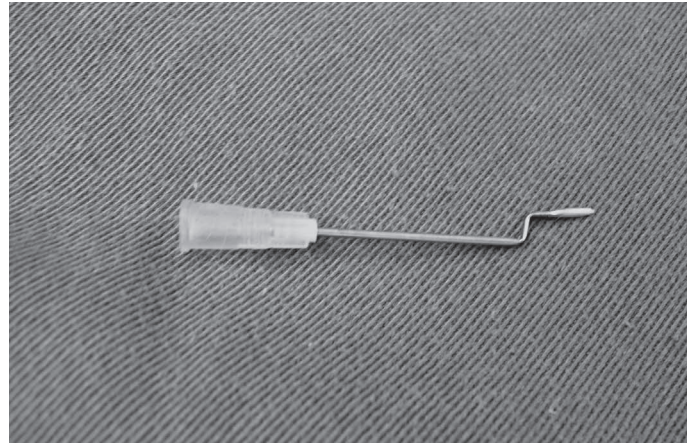**Figure 3** – Punctures of the IVD (arrow)

Figure 4 – 18G needle molded into bayonet shape

Radiological evaluation

To assess the height of each disc, ventridorsal (AP) and lateral (profile) radiographs of the lumbar spine were taken soon after surgery, and four, eight, and 12 weeks after the intervention (Figure 5).

Care was taken to maintain a consistent level of anesthesia during the radiography of each animal in order to obtain a similar degree of muscle relaxation, which could affect disc height during imaging. Consequently, the preoperative as well as postoperative radiographs (both at a high level of muscle relaxation) were always used as the basis of measurement. To reduce the errors due to axial rotation of the column and beam divergence, radiographs were repeated at least twice in each animal in lateral decubitus position, with the beam centered 4 cm from the iliac crest of the rabbit.

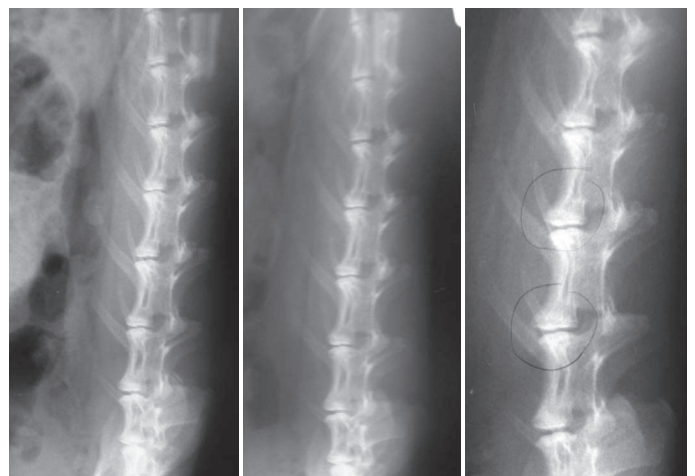**Figure 5** – Profile radiographs in the immediate postoperative period (IPO), unchanged; after four weeks (4 wk), with evidence of disc space narrowing; and eight weeks (8 wk) showing formation of osteophytes and sclerosis of the vertebral plate

After 12 weeks of surgery, the rabbits were euthanized with an overdose injection of pentobarbital (90 mg/kg), and their spinal columns collected for histological analysis and determination of the degree of degeneration.

For the radiological analysis of changes in disc height, four researchers involved in the study assessed the following variables: disc space narrowing on AP radiographs[25,26]; disc space narrowing on profile radiographs; anterior osteophyte formation; vertebral plate sclerosis and disc degeneration. The height of the experimental discs was compared with the control and the pre- and postoperative radiographs and expressed as a ratio. The measurements were performed individually and recorded so that the evaluators did not have access to further analyses.

Statistical analysis

After the individual analysis of the radiographs, statistical validation of the results for the evaluation and grading of the disc degeneration model in rabbits was performed using the kappa coefficient adjusted for prevalence through the chi-square test for goodness of fit. The kappa is a measure of interobserver agreement and measures the degree of agreement beyond what would be expected merely by chance. This measure of agreement has values ranging from 0 to 1, 0 representing no agreement beyond chance alone and 1 representing perfect agreement[27]. The agreement measured by kappa follows the interpretation guidelines published by Landis and Koch[28]: kappa < 0 – absent, from 0 to 0.19 – mild, from 0.20 to 0.39 – weak, from 0.40 to 0.59 – moderate, from 0.60 to 0.79 – substantial, and from 0.80 to 1.00 – almost perfect agreement.

RESULTS

Of the 13 rabbits selected for the study, 12 completed all phases of the study. One died during the postoperative follow-up, its results are not considered.

After the experiment was concluded and the animals were euthanized, four researchers individually analyzed the variables: disc space narrowing on AP and profile radiographs, anterior osteophyte formation, vertebral plate sclerosis, and the presence of disc degeneration, through affirmative or negative ("yes" and "no") responses for each category evaluated.

The statistical analysis was validated by the kappa coefficient of agreement, with a 95% confidence interval (95% CI).

The narrowing of disc space on AP radiographs showed a kappa value of 0.489 with a 95% CI (0.25-0.72) with p < 0.001. The disc space narrowing on profile radiographs presented a kappa value of 0.261 with a 95% CI (0.03-0.49) with p < 0.027. The formation of anterior osteophytes presented a kappa value of 0.385 with a 95% CI (0.15-0.61) with p < 0.001. Vertebral plate sclerosis presented a kappa value of 0.329 with a 95% CI (0.09-0.56) with p < 0.005. Disc degeneration showed a kappa value of 0.63 with a 95% CI (0.39-0.86) with p < 0.001.

Using the Landis and Koch interpretation[28] for the kappa values, we found the radiographic evaluation of disc degeneration by the observers in the study to have substantial agreement, that is, having reliable correlation with significant statistical value. The narrowing of disc space on AP X-rays showed moderate agreement, also showing itself to be a good parameter for the evaluation and validation of results. The narrowing of the disc space on profile radiographs, the formation of anterior osteophytes, and vertebral plate sclerosis showed weak agreement, although they were appropriate positive results, they constituted lower interobserver agreement in the validation of the rabbit model of disc degeneration (Table 1).

Table 1 – Radiologic agreement in the rabbit model of disc degeneration

Variable	Kappa (95% CI)	Agreement
AP narrowing of disc space	0.489 (0.26-0.72)	MODERATE
Profile narrowing of disc space	0.261 (0.03-0.49)	WEAK
Anterior osteophyte formation	0.385 (0.15-0.61)	WEAK
Vertebral plate sclerosis	0.329 (0.09-0.56)	WEAK
Disc degeneration	0.63 (0.39-0.86)	SUBSTANTIAL

Source: Spinal Cord Injury and Experimental Trauma Laboratory

DISCUSSION

Even after numerous studies[22-25] using animal models, there is no consensus among authors as to the ideal animal model for the study of DDD, as well as the necessary modes of analysis for checking the effectiveness of the model, which would allow us to begin extrapolating results to humans. Rousseau

et al.[25] used the lumbar and caudal IVDs of rats, causing injury by incisions made with a No. 11 scalpel blade, and analyzed their histology, cytokine production, functional capacity, and biomechanical properties. Kim *et al.*[22] compared various methods of inducing degeneration in rabbits, using intradisc injections of camptothecin (an apoptotic agent), aspiration of the nucleus pulposus, and puncturing the annulus fibrosus with No. 18 and 21 needles at different lumbar levels, analyzing the final liquid content of the core and disc height through magnetic resonance imaging. Masuda *et al.*[24] formed small experimental groups, comparing the degeneration generated from punctures with Nº. 16, 18, and 21 needles, and punctures made by a Nº. 11 blade, evaluating the results by means of X-rays, MRI, and histologic features. Finally, Kroeber *et al.* [23] used an external intersomatic device to generate loads in the lumbar spine of rabbits, assessing the IVDs in terms of rigidity, height, morphology, and cell viability. Yet another important point in relation to DDD animal models is based on the depth of the lesion generated in the IVD, which would significantly alter the results, since it directly correlates with the generation of pain, similar to human pathophysiology. Aoki *et al.*[29] compared the effects of puncture of different depths, analyzing the results immunohistologically and by magnetic resonance.

Thus, it appears that even with the existence of animal models of disc degeneration, there is no consensus among studies, as there are large variations in methodology and results.

Experimental studies based on animal models need to consider several factors in order for their results to be reliable. Initially, the choice of animal in creating the model is crucial, and, in this case, consider points such as: availability, acquisition cost, ease of handling, IVDs with easy surgical access and with the appropriate size for the proposed procedure, as well as metabolic characteristics that result in very rapid onset of disc degeneration produced to allow for safe posterior analyses[22]. In our reality, two animal models fit these conditions: mice and rabbits. While considering them, it was observed that rabbits have greater advantages due to their larger discs, easier surgical access, and greater ease in the evaluation of injuries. Even though mice have higher number of discs – a very important feature for this type of study

– the biomechanics of the caudal discs precludes their use due to the extreme difference in comparison to that of humans[22-24]. The animal model adopted for this research showed advantages over the use of larger or smaller animals, such as sheep or rats[25]. In contrast to the rat study, the biomechanical behavior of the lumbar spine of the rabbit is more similar to the human due to the presence of facet joints and paravertebral muscles[23]. Moreover, the growth plates of the vertebral bodies of mature New Zealand breed rabbits are closed, unlike rats[23], which also present greater technical difficulty for performing reliable radiographs[24]. When compared to larger animal species, the rabbit also has advantages, such as its ease of acquisition, and its low acquisition and maintenance costs[22].

As for the induction of disc degeneration, from a comparison of studies, 18G needle punctures were shown to produce models of slow degeneration and moderate intensity[22,24]. Following a predetermined condition, and coupled with the limited depth of 5 mm described as ideal by Aoki *et al.*[29], the generation of changes evident in the control X-rays found in this study were consistent with the changes observed in human intervertebral disc degeneration[3,30-33].

All of the radiographic findings in this study showed positive statistical correlation, validating the rabbit model of disc degeneration. The narrowing of disc space on AP radiographs was the most evident change in the images 12 weeks after the induction of disc degeneration by needle – results similar to other studies in the literature[24,33].

Radiographic analysis at 12 weeks postoperatively showed significant alterations in the structure of the intervertebral disc, such as the narrowing of disc space, formation of osteophytes, vertebral plate sclerosis and evidence of vertebral disc degeneration, agreed upon among the observers with significant statistical correlation. Kim *et al.*[22], Masuda *et al.*[24], and Sobajima *et al.*[33] obtained similar results in the induction of disc degeneration in rabbits by needle puncture, demonstrating it to be an efficient, easy to perform method that has a lower rate of complications when compared with other techniques described[23,34,35].

The use of a series method for evolutionary monitoring stands out in the literature. Since DDD exhibits characteristics according to its various stages, there is great need for periodic evaluations. MRI has been

used in this process in other studies[22,33]. However, X-ray analysis allowed for a statistically reliable differentiation between the presence and absence of disc degeneration, using a technology that is more accessible, less expensive, and that requires less infrastructure. In a similar manner, the uniform period of serial evaluation allowed for the final verification of significant results regarding the presence of degeneration.

This type of study has a special feature due to its sample size, in that it is based on the absolute number of discs and not the number of animals used, with each disc considered an independent variable in relation to the absolute criterion of the presence or absence of degeneration. Many of the results presented in DDD animal models are based on small samples, which are sometimes subdivided into groups according to the different methods of induction of disc degeneration applied to each segment. Kim *et al*.[22] used a total of nine IVDs for each experimental methodology employed. Kroeber *et al*.[23] developed their model with 33 animals. However, five comprised the control group and the remaining 28 were divided into groups, undergoing serial euthanasia, with only seven samples undergoing the complete evolutionary period of two months, decreasing the significance of evolutionary analysis. Rousseau *et al*.[25] also divided their sample by serial euthanasia, with a maximum period of 28 days, even though they employed a large number of experimental animals (rats). In contrast, this study conducted an analysis of 60 IVDs, all undergoing a uniform procedure of induction of disc degeneration, and were followed serially under identical conditions, generating a significant number in relation to that presented in the literature.

Therefore, the results produced allow for future studies aimed at therapeutic strategies for DDD, with emphasis on their potential direct application in the study of cellular therapies.

CONCLUSION

The rabbit model of disc degeneration proposed in this study proved to be feasible, with positive correlation between the pre- and postoperative radiological images, validating the possibility of inducting disc degeneration in this animal model for future studies and confirming the role of radiography in identifying disc degeneration.

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