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Original Article

Tooth Extraction and Bone Drill Hole in Wistar Rats: Kinetics of Biochemical Markers During Wound Healing

Extraction Dentaire et Trou de Forage Osseux Chez les Rats Wistar : Cinétique des Marqueurs Biochimiques Lors de la Cicatrisation

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ABSTRACT

Introduction. Biochemical markers provide a dynamic picture of the bone remodeling process. The aim of this study was to determine the kinetics of remodeling markers during bone healing under two models of induced lesions in the Wistar rat. **Methodology.** Over a three-month period, from February to April 2024, we conducted an experimental study involving wistar rats weighing a minimum of 150g and aged eight weeks. The animals (N=24) were randomly divided into three groups of eight rats. Group I was the control group. In group II, the rats had undergone dental extraction, while in group III, a bone drill hole was made in the mandibular symphysis. The follow-up period was 45 days. Data analysis was performed using Graph pad sprim software version 8.0.1. l. Results were expressed as mean plus or minus standard error on the mean. **Results.** We included 24 rats. We observed weight loss in Group II females at weeks 2 and 6. PAL concentrations increased significantly in groups II and III at weeks 4,5,6. On the other hand, we observed a decrease in calcium concentration in female rats of groups II and III at week 4. **Conclusion.** At week 4 post-operatively, the determination of PAL and/or calcium could provide information on the level of consolidation.

RÉSUMÉ

Introduction. Les marqueurs biochimiques fournissent une image dynamique du processus de remodelage osseux. Le but de cette étude était de déterminer la cinétique des marqueurs de remodelage lors de la cicatrisation osseuse sous deux modèles de lésions induites chez le rat de wistar. **Méthodologie.** Nous avons mené sur une durée de trois mois, allant de Février à Avril 2024 une étude expérimentale incluant les rats wistar d'un poids minimal de 150g et d'un âge de huit semaines. Les animaux (N=24), étaient répartis de manière aléatoire en trois groupes de huit rats. Le groupe I représentait le groupe témoin. Dans le groupe II les rats avaient subi une extraction dentaire, tandis que dans le groupe III, un trou de forage osseux était réalisé sur la symphyse mandibulaire. Le suivi s'est fait sur 45 jours. L'analyse des données a été faite avec le logiciel Graph pad sprim version 8.0.1. l. Les résultats ont été exprimés sous forme de moyenne plus ou moins erreur standard sur la moyenne. **Résultats.** Nous avons inclus 24 rats. Nous avons observé une perte de poids des femelles du groupe ii aux semaines 2 et 6. Les concentrations de pal augmentaient significativement dans les groupes II et III aux semaines 4,5,6. Par contre on observait une diminution de la concentration de calcium chez les rats femelles des groupes II et III à la semaine 4. **Conclusion.** A la semaine 4 post-opératoire, le dosage de la PAL et/ou du calcium pourrait renseigner sur le niveau consolidation.

INTRODUCTION

Bone is a living material which constantly adapts according to the mechanical stresses it undergoes, in particular multiform lesions. The bone healing process therefore involves a complex biological process [1].
Biochemical markers initiate the remodeling phase after bone injury. They arise on the one hand from bone formation (bone alkaline phosphatase, osteocalcin, the c-terminal propeptide of type I procollagen, the N-terminal propeptide of type I procollagen) and on the other hand from the bone resorption (tartrate-resistant acid phosphatase, hydroxyproline, hydroxylysine glycosides and pyridinolines) [2]. However, the dosages of Alkaline Phosphatase (ALP), calcium and inorganic phosphorus are simple and accessible. These biochemical markers provide a dynamic picture of the underlying bone remodeling process, including its turnover, pathogenesis and can differentiate normal healing from delayed healing [3]. Among the bone lesions frequently encountered in our context, we have alveolar-dental lesions and fractures [4]. The objective of this work was to determine the activity of bone remodeling markers during healing in Wistar strain rats, in which dental extractions and symphyseal lesions by drilling were induced.

MATERIALS AND METHODS

Type and location of study

This was a prospective experimental study with a descriptive aim conducted over a period of 3 months.

Study duration/period

The study was carried out over a period of three months, from February to April 2024. The follow-up time was 45 days.

Population studied

We had a population of 24 rats, all of the Wistar strain, which met the inclusion criteria (rats aged 08 weeks with a minimum weight of 150g) and exclusion criteria (death or weight loss of 10% of initial weight). . They were randomly divided into three groups of 08 rats each (04 females and 04 males), according to the absence and type of lesions to be induced.
The first group (G1) constituted our control group. The second group (G2) included rats undergoing dental extraction of the lower incisor. The third group (G2) consisted of rats requiring a 1.6mm bone drill hole at the level of the mandibular symphysis.

Data collection

The 24 rats received a phosphocalcium blood test (calcium, inorganic phosphorus), measured using the colorimetric method. Blood samples were taken in the morning on an empty stomach and the dosage was carried out the same day. These samples were taken by inserting a glass micro-hematocrit capillary tube into the retro-orbital area. The blood was then collected using dry tubes. Weight gain was taken every two days using an electronic scale. The ALP assay was carried out by a kinetic technique using the BIOLABO commercial kit. These dosages were carried out for the three groups before the start of the experiment (W0) then every week until week 6 (W6).

Study variables

The variables of interest in our study were: the weight of our animals, the activity of alkaline phosphatase, calcium and inorganic phosphorus.

Statistical analysis

Data analysis was carried out using Graph pad sprim version 8.0.1 software. L. Results related to quantitative variables were expressed as mean plus or minus standard error of the mean.

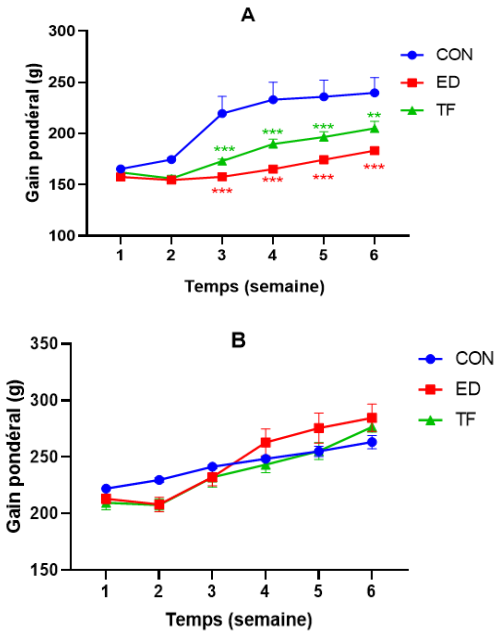
Ethical considerations

Our study previously obtained ethical clearance (N:854 CIER/UY1/FMSB/VDRC/DAASR/CSD). The Marshall Hall Principles, Principles of Laboratory Animal Care and the 3R Rule were also followed

RESULTS

Anthropometric data

Anthropometrically, there was a weight loss, exclusively in female rats in groups 2 and 3, from week 1 to week 3 of the experiment.
This weight loss was greater in rats that had dental extractions than those that had drill holes. (Figure 1)



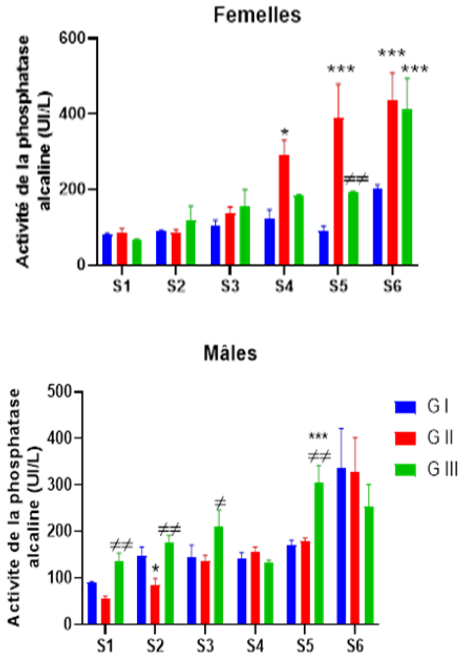
* p < 0.05; ** p < 0.01; *** p < 0,001: ≠≠ p < 0.01
Con= control group, ED= group of tooth extraction, TF= group of drill hole

Figure 1: effects of tooth extraction and drilling on weight gain of female (A) and male (B) rats

Effect of induced bone lesions on alkaline phosphatase activity

A significant increase in ALP activity was noted at the 4th week (289.33IU/L) ($p < 0.01$), 5th week (387IU/L) ($p < 0.001$) and 6th week (437IU/L) L) ($p < 0.001$) in females of groups II and III. The ALP activity of male rats having undergone the drill hole was significantly higher (135.67IU/L) than that of the rats having undergone dental extraction at the first (56.75IU/L) ($p < 0, 01$), the second

(177UI/L and 84UI/L) ($p < 0.01$), the third (211.25 and 135.5UI/L) ($p < 0.05$) and the fifth week (303.67 and 177.75UI/L) ($p < 0.01$) of the experiment. (Figure 2)



* p < 0.05 ; ** p < 0.01 ; *** p < 0,001: ≠ p < 0.01
G I= control, G II= tooth extraction, G III= drill hole

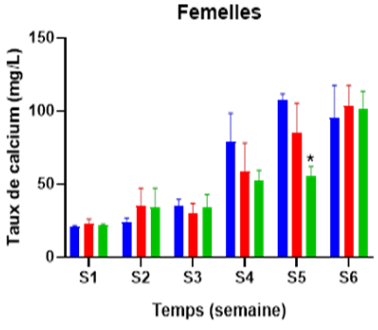
Figure 2: Effects of tooth extraction and drilling on alkaline phosphatase activity in female and male rats

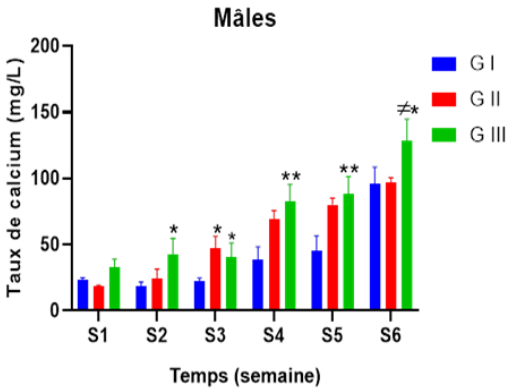
Effect of induced bone lesions on calcium activity

The drill hole caused a significant decrease ($p < 0.05$) in the calcium level in female rats at the 5th week (55.5 mg/l) compared to the control group (107.67 mg/l).

In male rats, dental extraction led to a significant increase ($p < 0.05$) in the calcium level (24.3 mg/l) in the second week (W2) compared to the control group (18.33 mg/l).

The borehole, for its part, led to a significant increase in the calcium level in the 2nd (42.67mg/l) i.e. ($p < 0.05$), 3rd (40.5mg/l) ($p < 0.05$), 4th (82.25mg/l) ($p < 0.01$), 5th (82.25mg/l) ($p < 0.01$) and 6th ($p < 0.05$) week. However, the calcium level of male rats having undergone the drill hole was significantly higher ($p < 0.05$), (128.25 mg/l) than that of the rats having undergone tooth extraction (96.67 mg/l) at the sixth week (S6) of the experiment. (Figure 3)



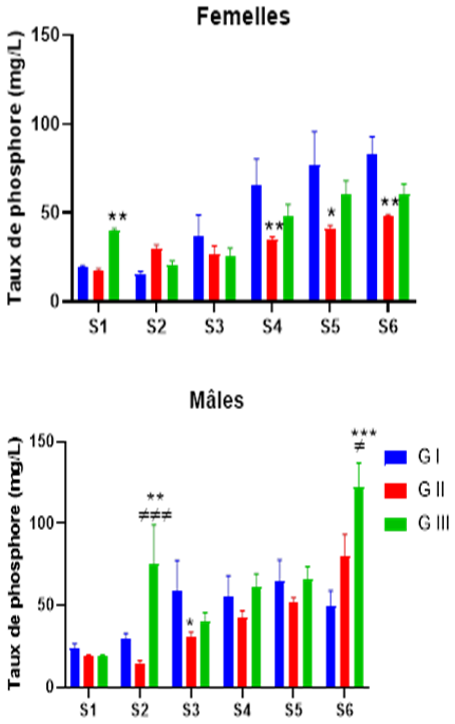


* p < 0.05; ** p < 0.01 ; *** p < 0,001: ≠ p < 0.01
G I= control, G II= tooth extraction, G III= drill hole

Figure 3: Effects of tooth extraction and drilling on calcium activity in female and male rats

Effect of induced bone lesions on phosphorus activity

In group III females, a significant increase in phosphorus levels was observed in the first week. However, in males of the same group, the increase occurred between weeks 2 and 6.



* p < 0.05; ** p < 0.01 ; *** p < 0,001: ≠ p < 0.01
G I= control, G II= tooth extraction, G III= drill hole

Figure 4: Effects of tooth extraction and drilling on phosphorus activity in female and male rats

DISCUSSION

Evolution of the weight of the animals during the experiment

Weight loss was noted in both females who underwent tooth extraction and those who underwent drilling. This from the 2nd week until the 6th week of the experiment.

This weight loss observed only in females could be explained by the absence of testosterone. Indeed, testosterone, the masculinity hormone, would have led to two mechanisms in males, namely: a more rapid inhibition of the inflammation created by the trauma and a stimulation of the appetite. It therefore promotes the synthesis of muscle proteins, thus contributing to muscle mass gain [5].

Effect of induced bone lesions on alkaline phosphatase activity

In the present study, serum alkaline phosphatase (ALP) activity increased in group II and III in rats of both sexes. This increase which occurred in weeks 4.5 and 6 was significant. Our results are similar to those of Nilajagi et *al.* in India in 2021 which observed a significant increase in ALP from the 3rd to the 30th postoperative day [6]. According to these authors, this could be due to osteoblastic proliferation at the fracture site, and to the contribution of the periosteum of the destroyed bone, a rich source of alkaline phosphatase.

Effect of induced bone lesions on calcium activity

The increase in serum calcium level was observed in males of group II at week 3. In group III this increase was observed between week 2 and week 6. This could be explained by the fact that calcium would have been secreted into the blood and delivered to the fracture site for consolidation and stiffness of the damaged bone.

Indeed, the mineral matrix is responsible for the rigidity of the bone, the minerals attach to the protein framework of the osteoid for its mineralization [7]. Mineralization (third phase of fracture consolidation) of the soft callus occurs through the deposition of minerals such as calcium and phosphorus on the newly formed matrix [8]. Additionally, calcium and phosphorus are necessary elements for bone formation and are stored in bone in the form of hydroxyapatite. They give the bone its rigidity. Serum calcium level decreased non-significantly at week 4 in females in groups II and III. This is in agreement with Nilajagi et *al.* in India in 2021 which observed a non-significant drop in serum calcium level until D30, then a non-significant increase until D60 [6].

Effect of induced bone lesions on phosphorus activity

The results obtained in the present study on serum phosphorus levels showed in females of group III a significant increase in this parameter at the first week. In males of the same group the increase occurred between weeks 2 and 6. This observation is also made in group II of our experiment. Indeed, high levels of calcium and phosphorus in the blood and extracellular fluids trigger the deposition of calcium phosphate crystals in the osteoid and make the bone harder [9]. Phosphorus is therefore an essential bone component necessary for the proper mineralization of the skeleton. Most of the body's phosphorus is stored in bones [10].

CONCLUSION

During induced bone lesions, the kinetics of biochemical markers mimicked those of our control group. These simple-to-use, low-cost markers could make it possible to monitor the evolution of bone healing.

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DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that might appear to influence the work reported in this article.

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AUTHOR CONTRIBUTIONS

Nkolo Tolo Francis Daniel designed the study.

Tanetchop Nelly and Tcheucthoua Yannick collected the data.

Nkeck Jan Rene and Mame Momo Edwige Lea carried out the statistical analysis and drafted the manuscript.

Ama Moor Vicky Jocelyne critically read the manuscript.

All authors have given their consent for publication.

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