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Systemic Lithium Chloride Administration Improves Tooth Extraction Wound Healing in Estrogen-Deficient Rats

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The purpose of this investigation was to evaluate the effects of lithium chloride (LiCl) on the socket healing of estrogen-deficient rats. Seventy-two rats were allocated into one of the following groups: Control, Ovariectomy and LiCl (150 mg/kg/2 every other day orally) + Ovariectomy. Animals received LiCl or water from the 14th day post-ovariectomy, until the completion of the experiment. On the 21st day after ovariectomy, the first molars were extracted. Rats were euthanized on the 10th, 20th and 30th days following extractions. Bone healing (BH), TRAP positive cells and immunohistochemical staining for OPG, RANKL, BSP, OPN and OCN were evaluated. The Ovariectomy group presented decreased BH compared to the LiCl group at 10 days, and the lowest BH at 20 days ($p<0.05$). At 30 days, the Ovariectomy and LiCl-groups presented lower BH than that of the Control ($p<0.05$). The number of TRAP-stained cells was the lowest in the LiCl group at 20 days and the highest in the Ovariectomy group at 30 days ($p<0.05$). At 10 days of healing, the LiCl group demonstrated stronger staining for all bone markers when compared to the other groups, while the Ovariectomy group presented higher RANKL expression than that of the Control ($p<0.05$). LiCl enhanced bone healing in rats with estrogen deficiency, particularly in the initial healing phases. However, as data on the effects of lithium chloride on bone tissue are still preliminary, more studies related to its toxicity and protocol of administration are necessary before its application in clinical practice.

Key Words: ovariectomy, lithium chloride, bone healing, rank ligand, estrogen.

Introduction

Tooth extraction is one of the most common dental procedures. The socket healing process has been the focus of recent attention due to the high interest in preserving and improving bone volume and preventing delayed repair, in order to achieve functionally and esthetically successful implant-based oral rehabilitation. Socket healing comprises successive and overlapping phases of bleeding, blood clot formation and stabilization, inflammation and cell recruitment, formation of granulation tissue and unmineralized new bone matrix, as well as mineralization (1). Intrinsic and extrinsic factors including lifestyles, medications, infections and diseases may interfere in the biological processes of bone healing and, ultimately, impair repair at the right time and reduce the quantity and quality of bone formation (2).

Osteoporosis is a common disease in the elderly that reduces bone quality and strength, and increases the probability of fractures. Estrogen deficiency is associated with increases in osteoclast number and activity, leading to post-menopausal osteoporosis, one of the major health problems associated with menopause (3). Animal models of ovariectomy satisfactorily mimic the state of post-menopausal osteoporotic bone. Previous studies using the ovariectomy model have reported a negative impact of estrogen deficiency/osteoporosis on the socket-healing process due to alterations in bone-healing markers and delayed complete-socket healing (4-10).

Despite the numerous medications available for the treatment of osteoporosis, new agents able to reduce bone resorption with high efficiency and minimal adverse effects are being developed by the pharmaceutical industry. Lithium is a drug that is generally prescribed for bipolar disorder and manic depression treatment (11); however, recent experimental investigations show that lithium chloride, a glycogen synthase kinase (GSK)-3β inhibitor, improves bone mass and bone regeneration by promoting osteoblast proliferation and inhibiting osteoclast differentiation (12,13). These findings suggest that lithium chloride may be a potential candidate for the treatment of osteoporosis and other bone loss-related diseases and reveal possibility of lithium repositioning; i.e. discovery of new therapeutic purposes for an existing medication. However, to date, studies on the effects of lithium chloride on bone tissue are still preliminary and, hence, its direct clinical applications based upon these initial studies remain unpredictable. A comprehensive evaluation of the disadvantages of the long-term lithium

therapy is mandatory for its adequate repositioning. The adverse effects of lithium (e.g. tremor, polyuria, weight gain, thyroid dysfunction, hypercalcemia, memory deficits and skin disorders) (14) and a safe administration protocol must be considered in future clinical studies on the effects of lithium chloride on bone-related disorders.

Pre-clinical dental studies have shown benefits of lithium chloride, in states of both estrogen sufficiency and deficiency, on bone formation during orthodontic retention, midpalatal expansion and surgically-created periodontal defects, for improving implant osseointegration and for the attenuation of bone loss in a model of induced periodontitis (15-20). A single previous study in estrogen-sufficient rats evaluated the effects of lithium chloride on the healing process of tooth sockets. Results demonstrated that lithium chloride enhanced bone repair in the extraction sockets when administrated before or after tooth extraction, but retarded bone healing when continuously administrated (21). However, the effects of lithium chloride on extraction socket healing in a condition of estrogen deficiency, which may adversely affect bone repair, have not been evaluated to date. Since osteoporosis is prevalent in the elderly, who are vulnerable to tooth extraction and dental implant placement, treatments for osteoporosis have generated great interest due to their potential for improving socket healing. Therefore, the aim of this study is to evaluate the effects of lithium chloride on the socket wound healing of estrogen-deficient rats.

Material and Methods

Sample and Power Calculation

Based on a single study (21) that evaluated the effect of lithium on alveolar repair, it was initially estimated that five animals should be included in our study. However, it was decided to include eight animals per group, considering the possibility of animal loss due to the surgical procedures and the length of the lithium administration protocol.

Animals

The current study protocol followed the 'NC3Rs ARRIVE Guidelines, Animal Research: Reporting of In Vivo Experiments' and was accepted by the Institutional Committee for Animal Care and Use of the Guarulhos University (Guarulhos, São Paulo, Brazil) (#028/16). Seventy-two female Wistar rats were taken from the University of São Paulo (São Paulo, SP, Brazil) and maintained in individual cages in the Bioscience Laboratory of Guarulhos University during the five days of acclimatization and during the entire experimental phase. Animals were 90 days of age and weighed ~235±30 g at baseline. Non-ovariectomized animals had food and drinking water ad libitum; however, the diet of the ovariectomized rats was controlled to allowing matching of their body weights to those of non-ovariectomized rats (22). Rats were maintained in an area with a controlled temperature (22 °C to 24 °C) and a 12-h light/dark sequence. Animals were continuously weighed each 5 days.

Experimental Groups

Seventy-two animals were randomly allocated into one of the following groups: Control: animals that had sham-surgery and water administration (n=24); Ovariectomy: animals that had ovariectomy and water administration (n=24); Lithium chloride: animals that had ovariectomy and lithium chloride administration (n=24). Eight animals per group were also assigned to be euthanized at 10, 20 and 30 days post-extractions.

Ovariectomy

Animals received xylazine (0.125 mL/250 g of body intraperitoneally) and ketamine hydrochloride Anasedan, Sespo Indústria e Comércio LTDA, Paulínia, SP, Brazil (0.3mL/250g of body intraperitoneally). Two-sided ovariectomies were performed in the rats of the Ovariectomy and Lithium chloride groups as previously reported (22). The animals of the Control group received sham surgeries, in which the ovaries were exposed and repositioned. Immediately after surgeries, the animals had single intramuscular doses of analgesic Tramal (Pfizer LTDA, New York, NY, USA) (0.2 mL/kg/body) and antibiotic medication Pentabiótico Veterinário (Zoetis LTDA, Parsippany, NJ, USA) (1 mL /kg/body). The estrous cycle was verified during the 14 days after the sham and ovariectomy surgeries and to check for the success of ovariectomy and the status of estrogen depletion, as previously described (23). Both the ovariectomized groups exhibited continuous diestrous cells in the vaginal smears, with reduced number of cells and predominance of polymorphonuclear cells. The Control group showed the four phases of the estrous cycle (estrus, diestrus, proestrus and metaestrus). Therefore, during this phase of the experiment, none of the animals were excluded due to failure of ovariectomy.

Administration of Lithium Chloride

On the 14th day after sham or ovariectomy surgeries, animals in the Lithium chloride groups received 150 mg/kg lithium chloride (Labsynth, Diadema, SP, Brazil) by gavage every other day (17). The drug was diluted in water. Animals in the Control and Ovariectomy groups received water on the same days by the same via.

Extractions

Tooth extractions were performed on the 21st day after sham and ovariectomy surgeries using a micro-chisel and

a micro-forceps, under the same protocol of anesthesia as described above. After extraction, bleeding was contained by local compression with gauze. Animals were euthanized at 10, 20 and 30 days post-extractions by CO_2 breathing.

Histological Procedures

Maxillae were fixed for 24 h in formalin solution (neutral buffered 10%). The specimens containing the extraction sockets were decalcified in 4.13% ethylenediamine tetraacetic acid for approximately 75 days, dehydrated in increasing series of solutions of ethanol and embedded in paraffin. Sequential sections presenting 5 µm of thickness were acquired in a transversal direction. Fifteen sections (5 µm apart) of the central third part of sockets of the disto-buccal root of the maxillary left first molar were selected for analyses. The first six sections per animal/group/time (10^{th}, 20^{th} and 30^{th} days) were stained with hematoxylin and eosin (HE) and used for histometric analysis. The subsequent three sections were employed for TRAP staining. Additional sections were taken from the 10^{th} day for immunohistochemistry.

Examiner Calibration

For intra-examiner calibration, the examiner inspected ten non-study sections twice within 48 h. The Lin's concordance correlation coefficient was used to calculate examiner reproducibility. A calibrated and blinded examiner (TSM), who demonstrated a reproducibility of 97% for bone healing (BH) measurement, completed the histometric analyses. A blinded and calibrated examiner (FSM), who demonstrated a reproducibility >90%, completed the TRAP and immunohistochemical analyses.

Histometric Analysis

The percentage of newly-formed bone, defined as BH, was evaluated at 10, 20 and 30 days post-extraction in images at 40× magnification. A checkered diagram was overlapped on the images to count the quantity of intersections in each socket. Afterwards, the number of intersections in which new bone was created was calculated. The proportion of newly-created bone was estimated using to the formula: BH = number of intersections in new bone x 100/ entire number of intersections in each socket.

TRAP Analysis

TRAP staining was used to detect osteoclast-like cells in the socket wound at 10, 20 and 30 days post-extraction. The sections were firstly deparafinized and incubated in a solution containing naphthol AS-BI (Sigma Chemical Co., St Louis, MO, USA - 4 mg), red violet salt (Sigma Chemical Co. - 24 mg), acetate buffer (30 mL; pH 5.2) and tartrate (Sigma Chemical, USA) (0.3 mmol/L; pH 5) at 37 °C during 30 min. The slices were then rinsed in distilled water. Subsequently, the sections were counter-stained with fast green (Sigma Chemical Co., 1%). Successive sections were incubated in medium free of substrate to be used as a negative control. The number of cells presenting particles of TRAP staining with ≥ 3 nuclei was totaled within the limits of the healing socket using images of 100× magnification and an appropriate software (Image J, National Institute of Mental Health, Bethesda, Maryland, USA). Results were reported as quantity of TRAP-stained cells present in each mm^2 of newly-formed bone.

Immunohistochemical Analysis

Staining for receptor activator of NF-κB ligand (RANKL), osteoprotegerin (OPG), bone sialoprotein (BSP), osteopontin (OPN) and osteocalcin (OCN) were assessed at 10 days post-extraction, i.e. in the earliest time of the healing. Sections were set on glass slides treated with 3-aminopropyltriethoxy-silane. In order to eliminate endogenous peroxidase, the slides were treated with hydrogen peroxide (3%) for 30 min. Afterwards, the sections were blocked with PBS (1%) bovine serum albumin for 30 min. Subsequently, the slides were incubated with the primary antibodies (GeneTex, Inc., Irvine, CA, USA) (polyclonal antibodies for RANKL [1:100], OPG [1:100]), BSP [1:600], OPN [1:200] and OCN [1:200]) for 3 h and, then with biotinylated secondary antibody for 45 min. Finally, the sections were treated with the streptavidin peroxidase conjugate (GeneTex) for 30 min. The specific reaction for each antibody was observed by means of 3,3'diaminobenzidine. Sections were counter-stained with Mayer's hematoxylin, dehydrated through graded ethanol, cleared in xylene, and mounted on slides with the aid of Permount mounting media (Permount mounting media, Thermo Fisher Scientific, Waltham, MA USA). Negative controls were done by exclusion of the primary antibodies. Staining intensity was counted within the borders of the healing socket using images at 100× magnification and an image analysis software (Image J). Briefly, a uniform checkered diagram was placed on the healing socket. The intensity of labeling in each glass slide was assessed using a semi-quantitative ranking with 0 indicating lack of brown immunoreactivity as the negative control, 1 representing a very light brown color, 2 indicating a slight brown and 3 reflecting intense dark brown staining. Therefore, the dominant staining intensity in each square was categorized as no staining [0], weak [1], moderate [2], and strong [3] (24,25). The concluding score was obtained by distinguishing the predominant staining intensity in a given image.

Statistical Analysis

The normality of the data was inspected using the Shapiro–Wilk test. Parametric tests were used, since data

indicated normality. Body weights were compared among groups by ANOVA and Tukey's test. The BH and the amount of TRAP-positive cells were calculated for each socket at 10, 20 and 30 days, while the ranks of staining for RANKL, OPG, BSP, OPN and OCN were calculated at 10 days and, averaged across each group. BH and TRAP were compared among groups at each time by ANOVA and Tukey's test and between times within each experimental group by the repeated measures ANOVA and Bonferroni test. The Chi-square test was employed to compare the categories of immunostaining for RANKL, OPG, BSP, OPN and OCN. The level of significance determined for the analyses was 5% ($p< 0.05$).

Results

The success of the ovariectomy was established by the examination of ovaries after euthanasia. Ovaries were nonexistent, and the uterine horns were atrophic, thin, pale and lacking a blood supply in all of the ovariectomized rats. On the other hand, the ovaries were preserved, and the uterine horns presented a normal blood supply in the animals under sham surgery. There was some loss of animals, specimens and sections during the study due to anesthesia or technical difficulties, but all groups had at least six rats per group and time at the end of the study. Therefore, a post-hoc power analysis was accomplished after study completion to define the actual power of the histometric analysis presented in this investigation, with at least six rats per group. A minimum of six animals per group provided a 75% power with an α of 0.05, when considering a difference of 10% in newly-produced bone between the Lithium chloride and the Ovariectomy groups at 10 days post-extraction, and a standard deviation of 7.6% (Fig. 1). There were no differences in body weights among groups at any experimental time ($p>0.05$) (Table 1).

BH

The percentage of newly-formed bone was greater at 20 and 30 days than at 10 days for all experimental groups ($p<0.05$). At 10 days, the Ovariectomy group (52.69±7.54%) had a lower percentage of newly-formed bone than the Lithium chloride group (62.76±12.97%) ($p<0.05$) although there was no difference with control group (60.10±7.38). At 20 days, the Ovariectomy group (63.71±9.83%) exhibited a lower percentage of newly-formed bone than the control (78.57±6.56%) and Lithium chloride (73.66±10.38%) ($p<0.05$). At 30 days, the Ovariectomy (70.30±11.35%) and Lithium chloride (77.78±5.66%) groups had lower percentages of newly-formed bone than the Control group (87.87±6.67%) ($p<0.05$). Figure 1 shows the newly-formed bone inside healing sockets after tooth extractions. Notably, the proportion of newly-formed bone improved over time within the sockets, and the Ovariectomy group presented, overall, larger gaps between the formed trabeculae.

TRAP

The numbers of TRAP-stained cells were higher at 20 and 30 days than at 10 days for all experimental groups ($p<0.05$). There were no differences among groups in the number of TRAP-stained cells in the healing wound at 10 days post-extraction ($p>0.05$); however, the amount of TRAP-stained cells was lower in the Lithium chloride group at 20 days than in the other groups ($p<0.05$). At 30 days, the number of TRAP-stained cells was higher in the healing socket of the Ovariectomy group, when compared to the other experimental groups ($p<0.05$). Figure 2 shows the TRAP reaction in the healing sockets after extraction. Weak TRAP staining (red) can be detected in the Lithium chloride group at 20- and 30-days post-extraction.

Immunohistochemistry

After 10 days of socket healing, the Lithium chloride group exhibited stronger staining for RANKL, OPG, BSP, OPN and OCN, when compared to staining for the Control and Ovariectomy groups ($p<0.05$). Furthermore, the Ovariectomy group presented higher RANKL expression than the Control group ($p<0.05$). The photomicrographs shown in Figure 3 depict a strongly positive reaction for all markers in the Lithium chloride group and a robust positive reaction is detected for RANKL in the Ovariectomy group.

Discussion

To the best of author's knowledge, this is the first study to investigate the effect of lithium chloride on socket healing, following tooth extraction, in estrogen-deficient rats. Our results show that lithium chloride administration improved alveolar bone formation after tooth extraction, particularly during the early and intermediate stages of healing. Furthermore, lithium chloride stimulated the expression of bone-related markers and reduced the expression of TRAP, a marker of osteoclasts. Findings indicate an overall beneficial effect of this medication on the socket healing of bone presenting an osteopenic/osteoporotic

Table 1. Mean (± SD) body weights at baseline and at 10, 20 and 30 days

Group	Baseline	10 days	20 days	30 days
Control	235.0 ± 18.8	243.4 ± 25.0	253.4 ± 16.8	259.8 ± 19.3
Ovariectomy	249.8 ± 23.4	254.5 ±11.3	260.2 ± 13.1	264.1 ± 22.4
Lithium chloride	229.3 ± 19.3	245.4 ± 13.2	249.0 ± 22.5	257.0 ± 18.1

There were no differences between groups in any of the experimental times ($p<0.05$).

phenotype.

In the current study, untreated ovariectomized rats exhibited less new bone formation inside the healing socket, when compared to non-ovariectomized control animals, especially during the middle to last stages of healing (Fig. 1). These findings indicate an adverse effect

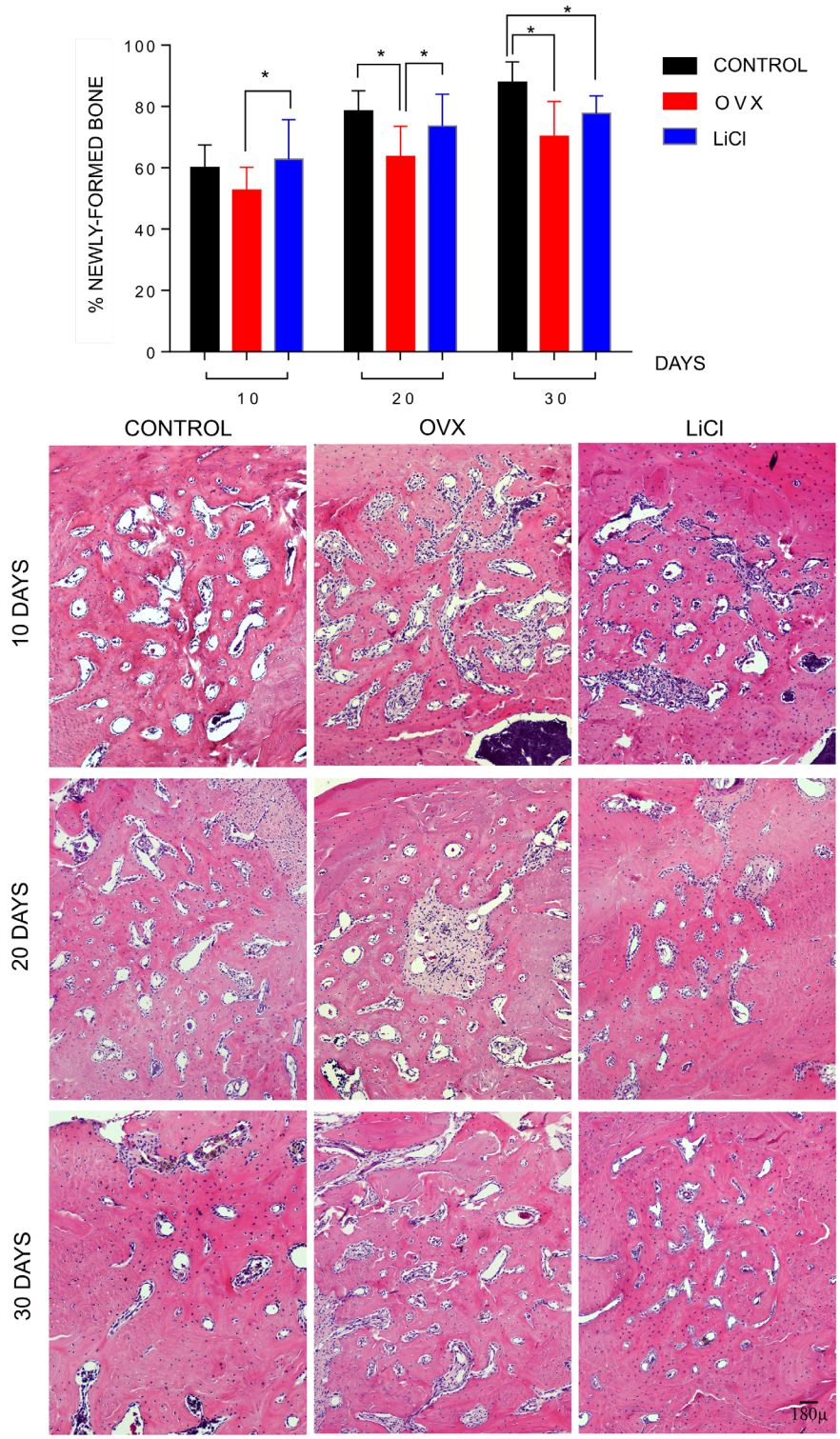

Figure 1. Bar chart demonstrating the percentage of newly formed bone (BH) within the limits of the tooth-extraction wound healing at 10, 20- and 30-days post-extraction (mean ± SD). *Significantly different, by ANOVA and Tukey's test. Photomicrographs showing newly formed bone into the sockets at 10, 20 and 30 days after tooth extraction (40× magnification). Note that BH improved over time within the alveolar sockets, and that the Ovariectomy group presented overall larger spaces between the formed trabeculae.

of estrogen deficiency/osteoporosis on socket healing after tooth extraction, consistent with data from previous investigations (4-10). Most importantly, the ovariectomized rats treated with lithium chloride presented improvements

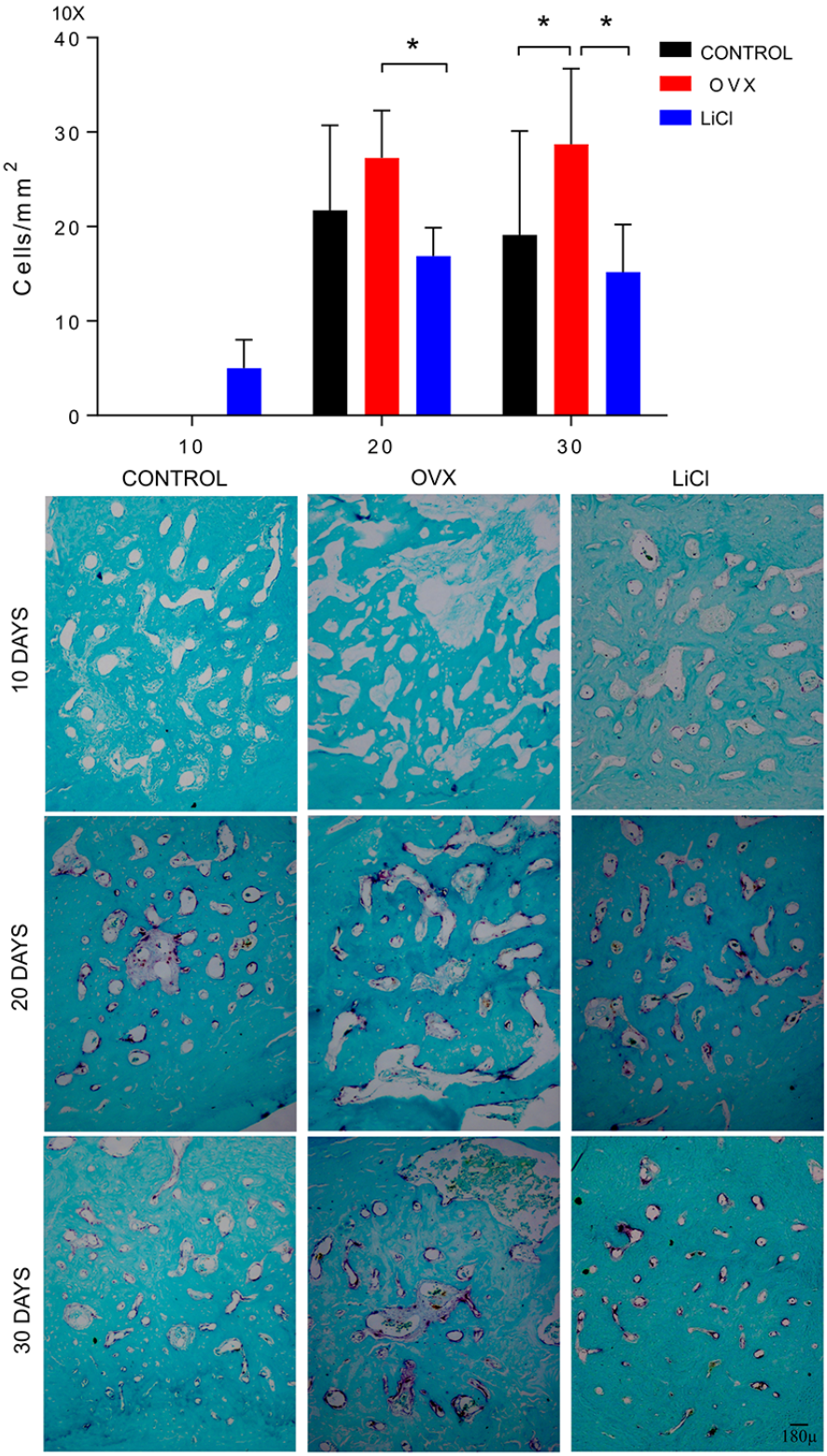

Figure 2. Bar chart demonstrating the number of TRAP-stained cells within the limits of the tooth-extraction wound healing at 10, 20- and 30-days post-extraction (mean ± SD). *Significantly different, by ANOVA and Tukey's test. Photomicrographs showing TRAP staining in the sockets at 10, 20 and 30 days after tooth extraction (40× magnification). Note the weak TRAP reaction products (red staining) in the Lithium chloride group at 20- and 30-days post-extraction.

in BH of ~10% and 8% at 10- and 20-days post-extraction, respectively, when compared to untreated estrogen-deficient rats. Furthermore, at these experimental time points, BH in the lithium chloride-treated rats was similar

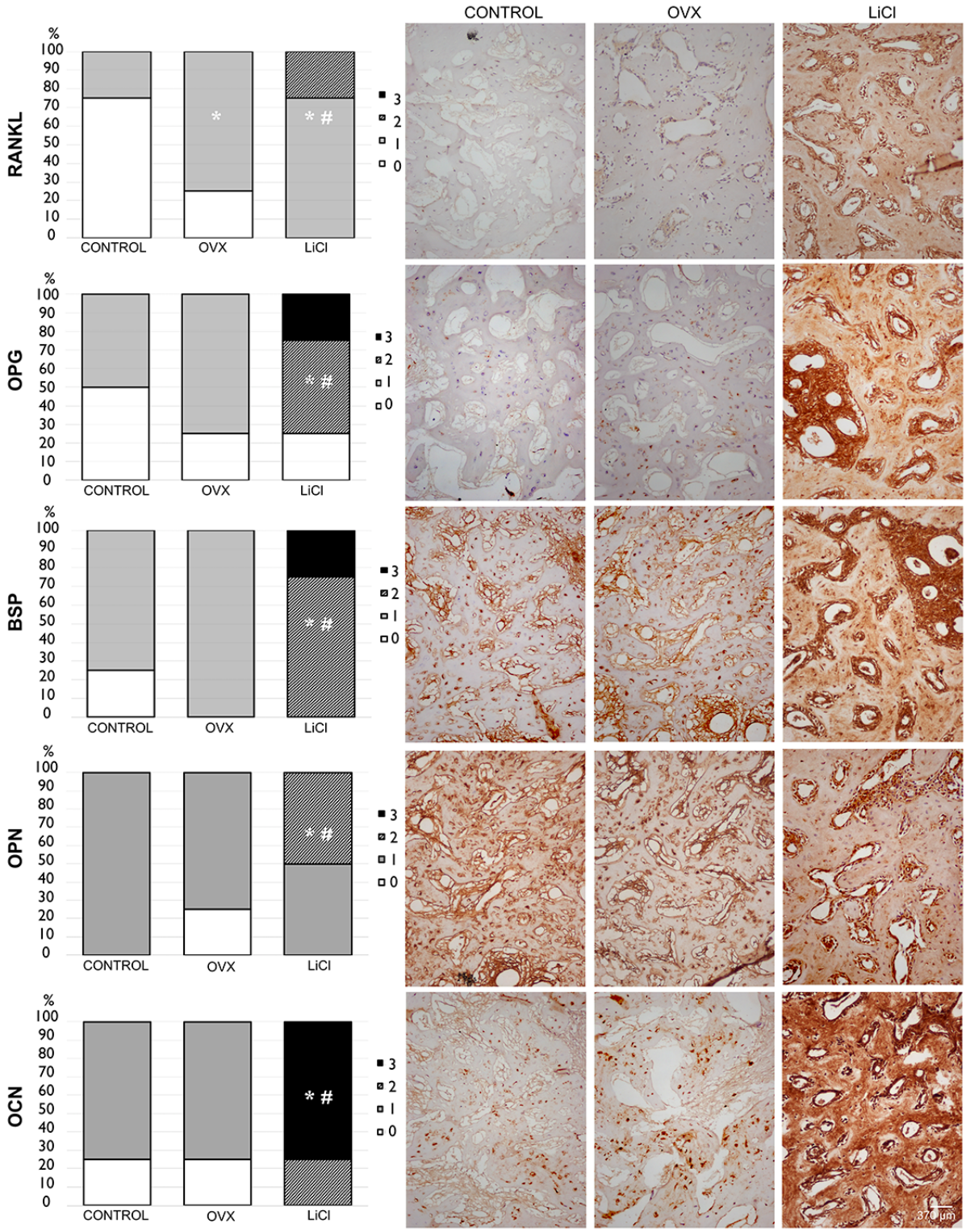

Figure 3. Stacked bar chart showing the percentage of ranks of staining for RANKL, OPG, BSP, OPN and OCN in the sockets at 10 days post-extraction. Photomicrographs illustrating immunohistochemical staining for all markers in the healing alveolar socket (100× magnification) at 10 days after extraction. 0- no staining, 1- weak staining, 2- moderate staining, 3- strong staining *Significantly different when compared to the Control group (Chi-square test; p<0.05). #Significantly different when compared to the Ovariectomy group (Chi-square test; p<0.05). RANKL: receptor activator of NF-κB ligand, OPG: osteoprotegerin, BSP: bone sialoprotein, OPN: osteopontin, OCN: osteocalcin.

to that of the Control group, indicating that this medication was able to restore bone healing at a level approaching to that of estrogen-sufficient rats in the early stages of the socket healing.

In support of these findings, previous investigations have demonstrated potential benefits of lithium chloride on fracture healing (26,27) and alveolar bone repair (16-21). Han et al. (16) demonstrated that local administration of lithium chloride stimulated greater alveolar bone volume and mineral density during the healing of surgically-created periodontal defects in estrogen-sufficient rats. Zeng et al. (21) reported that the pre-extraction and post-extraction administration of lithium chloride enhanced new bone formation in tooth sockets, but that continual administration of lithium for 21 days delayed healing. These data are somewhat conflicting with the results of the current study, which revealed significant benefits of continual lithium administration on socket repair. Possible explanations for such differences might be attributed to the dose of medication used and to the fact that the abovementioned study (21) evaluated socket healing in estrogen-sufficient rats. It is possible that continual lithium chloride administration may have different impacts on socket healing during estrogen sufficiency and estrogen deficiency. These mechanisms of action should be further investigated in future studies.

This study also explored the impact of lithium chloride administration on the expressions of some bone-related markers (RANKL, OPG, BSP, OPN and OCN) in the wound sockets of estrogen-deficient rats during the early phase of healing (10 days) (Fig. 3). The RANKL/OPG signaling system constitutes an axis from osteoblasts to osteoclasts that regulates the balance of bone formation and resorption. Osteoblasts express RANKL, which bind to the RANK receptor in osteoclast precursors and stimulate osteoclastogenesis. OPG is a soluble decoy receptor for RANKL that inhibits osteoclastogenesis by blocking the RANKL-RANK interaction (28). In our study, lithium chloride increased the expressions of both OPG and RANKL (Fig. 3). In support of these findings, previous investigations also reported that lithium chloride can increase the expression of RANKL (20,29). Nonetheless, the increased expression of RANKL that was observed, herein, during lithium treatment is somewhat surprising as bone healing was improved in the Lithium chloride group even though RANKL is a marker of osteoclastogenesis. Moreover, the number of TRAP-stained cells was lower in the Lithium chloride group than in the Ovariectomy group at both 20- and 30-days post-extraction (Fig. 2). Therefore, we speculate that this finding may indicate a feedback mechanism that controls the intense anabolic activity induced by this medication during the initial phase of healing.

In fact, lithium chloride treatment increased the expression of the three markers of osteogenesis including OCN, a middle stage marker, OPN, a late stage marker and BSP, a marker of osteoblast differentiation (30). Previous studies have already revealed similar trends towards the increased expression of osteogenic/osteoblast markers in the presence of lithium chloride (16,20,31-33). Taken together, these histometric and immunohistochemical findings suggest that lithium chloride may represent a promising pharmacological agent for bone repair in estrogen deficient status, particularly in the early to the middle healing stages. An intriguing finding is that the Lithium chloride group did not differ significantly from the Ovariectomy group regarding socket bone filling at 30 days, and both ovariectomized groups presented lower percentage of bone than the Control group. Therefore, it seems that lithium chloride was beneficial during the period in which healing was actively taking place, but not in healed extraction sockets. These findings could be attributed to the fact that different cellular and molecular interactions take place in healing and already healed bone. Furthermore, the lithium chloride treatment protocol used in this study may not be efficient to contain the loss of already consolidated bone structure under severe and long-term estrogen deficiency. Long-term studies are needed to confirm this hypothesis.

The mechanisms underlying the positive effects of lithium on bone seem to be predominantly mediated by canonical Wingless (Wnt)/β-catenin signaling (12,34). The activation of the Wnt/β-catenin pathway through the binding of Wnt to specific receptors downregulates the activity of GSK-3β, preventing β-catenin phosphorylation. The stabilized hypophosphorylated β-catenin aggregates in the cytosol and translocates to the nucleus, where it interacts with transcription factors, leading to the expression of different genes, including those related to osteoblastic activities and bone formation (35-36). Lithium can activate the Wnt/β-catenin pathway by suppressing GSK-3β and, hence, preventing β-catenin phosphorylation, increasing β-catenin nuclear translocation and promoting osteoblastogenesis-related gene expression (37-39). Since lithium chloride increased the expression of osteoblast markers (OPN, OCN and BSP) in the current study, it is supposed that lithium may have countered the deleterious effects of estrogen deficiency on socket healing by acting on the Wnt/β-catenin pathway. However, further investigations to explore the signaling transduction cascades associated with the Wnt/β-catenin pathway in socket healing are required to prove this hypothesis.

This study presents some limitations; firstly, a particular protocol of lithium chloride administration was tested (150 mg/kg/2 every other day orally) (17). The side effects

and toxicity of lithium (e.g. hyperparathyroidism, memory debits, skin disorders, hypothyroidism, hypercalcemia and hypermagnesemia) might vary according to doses and duration. Therefore, different protocols need to be tested in order to find a lithium dose that has beneficial effects on bone healing, but mild or no adverse systemic effects (24). Furthermore, immunohistochemical evaluation was restricted to five biomarkers just for the early phase of the bone healing (10 days post-extraction). Therefore, the assessment of a larger number of bone biomarkers, particularly those related to the Wnt/β-catenin pathway, during the different phases of healing would be of importance to better understand the impact of this medication on socket repair process.

In conclusion, lithium chloride administration enhanced bone healing and stimulated an overall expression of bone markers in healing sockets after tooth extraction in rats with estrogen deficiency, particularly in the initial healing stages. These preliminary findings suggest that lithium chloride administration may represent a promising strategy for improving socket repair in osteopenic/osteoporotic patients.

Resumo

O objetivo deste estudo foi avaliar os efeitos do Cloreto de Lítio (ClLi) na cicatrização de alvéolos de ratas deficientes em estrogênio. Setenta e duas ratas foram alocadas em um dos seguintes grupos: Controle, Ovariectomia e Cloreto de Lítio (150mg/kg/ oralmente a cada 2 dias) + ovarectomia. Os animais receberam ClLi ou água a partir do 14º dia pós-ovariectomia, até a conclusão do experimento. No 21º dia após a ovariectomia, os primeiros molares foram extraídos. As ratas foram sacrificadas nos dias 10, 20 e 30 após extrações. Foram avaliadas a cicatrização óssea (BH), células positivas para TRAP e coloração imuno-histoquímica para OPG, RANKL, BSP, OPN e OCN. O grupo Ovariectomia apresentou BH diminuída em comparação ao grupo LiCl aos 10 dias e a menor BH aos 20 dias ($p<0,05$). Aos 30 dias, os grupos Ovariectomia e LiCl apresentaram menor BH do que o Controle ($p<0,05$). O número de células positivas para TRAP foi menor no grupo ClLi em 20 dias e o maior no grupo Ovariectomia em 30 dias ($p<0,05$). Aos 10 dias de cicatrização, o grupo ClLi demonstrou imunomarcação mais intensa em todos os marcadores testados quando comparado aos outros grupos, enquanto o grupo Ovariectomia apresentou maior expressão de RANKL do que a do controle ($p<0,05$). O ClLi melhorou a cicatrização óssea em ratos com deficiência de estrogênio, particularmente nas fases iniciais do reparo. No entanto, como os dados sobre os efeitos do cloreto de lítio no tecido ósseo ainda são preliminares, mais estudos relacionados à sua toxicidade e protocolo de administração são necessários antes de sua aplicação na prática clínica.

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