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Lithium Chloride rescues Monensin-potentiated Wnt signaling inhibition in inflammatory bone loss in rats

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Research Article

Keywords: Lithium. Monensin. WNT pathway. Periodontitis. Bone Resorption

Posted Date: June 28th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4559888/v1

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Additional Declarations: No competing interests reported.

Abstract

The aim of the study was to evaluate the effect of Lithium Chloride (LiCl) on animals submitted to Monensin-potentiated periodontal bone loss. For that Wistar rats were submitted to experimental periodontitis (EP) and received either 0.1ml/200g corn oil (vehicle), daily; or Monensin (Mon), receiving 10 mg/kg of Mon daily; or LiCl, receiving 150mg/kg of LiCl on alternate days; or Mon + LiCl. Naïve (N) group was not submitted to any treatment. After euthanasia, maxillae were collected for macroscopic and histological analyses, and for expression of bone markers and Wnt signaling. Molecular docking assays were used for assessing the affinity between Mon and WNT pathway components. It was seen that EP caused bone loss and inflammation in the periodontium which was potentiated by Mon. LiCl protected bone and rescued the deleterious effects of Mon marked by reduction on bone loss, increase on osteoblasts number (39%), and Runx2 (70%) and OPG (68%) gene expressions, concomitant with reduction of osteoclasts in number (32%) and function (27%). LiCL increased the gene and protein expressions of beta-catenin in animals with EP receiving Mon. In summary LiCl rescued the deleterious effect of Mon on bone in animals with EP and stands as pharmacological tool to counteract the strong inhibition of Wnt pathway.

INTRODUCTION

Periodontitis is a highly prevalent chronic inflammatory disease. Its etiology is multifactorial and complex, where periodonto pathogenic bacteria stimulate inflammation leading to bone loss [1]. This inflammatory bone loss can be explained by the effect of inflammatory infiltrate and cytokines unbalancing molecular pathways, for instance, Wnt signaling [2].

WNT/beta-catenin pathway is a well-known pathway, related to cell proliferation and cancer, that has also been highlighted as a regulator of bone metabolism, involved with the differentiation of osteoblasts [3]. The interaction between WNT proteins and their receptors (FZD and LRP5/6) inhibits the action of Glycogen Synthase Kinase 3 Beta (GSK3b) favoring the accumulation of β -catenin in the cytoplasm which can then translocate to the nucleus, stimulating the expression of genes such as Runx2 and osteoprotegerin (OPG) related to osteoblastogenesis [4]. Physiologically, this pathway is inhibited by Dickkopf-1 (DKK-1) and Sclerostin (SOST) [5]. Our group has already reported that the inflammatory process increases DKK-1 expression, reducing bone formation in models of periodontitis and osteonecrosis of the jaws [6–8].

Knowing, therefore, the importance of the WNT pathway for bone metabolism, it becomes interesting to evaluate the effect of drugs that modulate this pathway. LiCl has been drug used to prevent mood swings and suicide, and also used as an adjunct in the treatment of depression [9], but has also shown to be associated with the formation of bone tissue [10], precisely due to the inhibition of GSK3b, favoring the accumulation of β -catenin in the nucleus and, therefore, stimulating osteoblastogenesis [11]. Monensin (Mon), in the other hand, is a natural compound isolated from *Streptomyces cinnamonensis* and belongs to the group of ionophore antibiotics that bind to cations such as Na+, K + and Li+. Mon

exhibits a broad spectrum of biological activities such as antimicrobial, antiproliferative, antiparasitic and antiviral [12]. However, more recently, Monensin has been studied as a drug with anti-cancer activity¹², showing positive effect both *in vitro* and *in vivo* against melanoma cells [13], leukemia acute myeloid [14] and prostate cancer [15]. Monensin blocks the phosphorylation of LRP6 inducing its degradation, and more especially it inhibits β -catenin, thus turning off the WNT pathway [16].

Therefore, knowing that the blockage of Wnt signaling contributes to inflammatory bone loss and that bone resorption can be potentiated by the use of Wnt inhibitors, as Monensin, we have decided to investigate if LiCl can rescue Wnt pathway activation since it was previously reported that other types of GSK3b inhibitors have failed [16].

MATERIALS AND METHODS

Study design and ethical aspects

This is an experimental study using animal models of rats subjected to periodontitis. The experiments had their protocols carried out based on the recommendations presented in the ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments guidelines) and began shortly after approval by the Ethics Committee for the Use of Animals of the UFC (CEUA n°7128020620) (ANNEX A), which is governed by the Universal Declaration of Animal Rights (UNESCO – 27 January 1978) and the International Ethical Guidelines for Biomedical Research Involving Animals (Council for International Organizations of Medical Sciences – CIOMS).

Ligature-induced periodontitis model and Experimental groups

This was a preclinical randomized and blinded study. For this, ninety male Wistar rats (*Rattus novergicus*), with a body mass of approximately 200 grams in the 12th week of life were used for the study. All animals received balanced commercial food and water *ad libitum* and remained under the same environmental conditions of light/dark cycles of 12 hours and room temperature of 22°C throughout the experiment.

Periodontitis was induced by placing a 3.0 nylon suture around the 2nd upper left molar [17] in a rat previously anesthetized with Ketamine (80 mg/kg) and Xylazine (10 mg/kg), intraperitoneally. After the placement of the ligature, a surgical knot was tied facing the buccal surface of the animal's oral cavity. At the end of the experiment (11th day), the animals were euthanized by an overdose of Ketamine (240 mg/kg) and Xylazine (30 mg/kg) administered IP.

The animals were initially divided into 5 groups (n = 6 per group):

• Naive Group (N): animals were not submitted to any treatment or procedure;

- Experimental periodontitis (**EP**) group: animals received corn oil (vehicle), by gavage, 30 min before periodontitis induction, and daily for 11 consecutive days, until euthanasia;
- Monensin (Mon) group: animals received 10mg/kg of Monensin (Sigma-Aldrich-San Luis, Missouri, USA- No. M5273), by gavage, 30 min before the induction of periodontitis, and daily for 11 consecutive days, until euthanasia [16];
- Lithium chloride (LiCl) group: animals received 150mg/kg of LiCl every other day by gavage for 11 days until euthanasia (Cequímica-Fortaleza, Ceará, Brazil) [18];
- Monensin + Lithium Chloride Group (Mon + LiCl): animals received daily 10mg/kg of Mon and 150 mg/kg of LiCl every other day, by gavage, for 11 days until euthanasia.

For this study, 3 sets of experiments (n = 30 animals/set) were performed. The first set was used for macroscopic analysis; 2nd set for histopathological histomorphometric studies and immunohistochemistry assays; 3rd set for determination of gene expression by RT-PCR. In all sets of experiments blood samples were collect for further dosage of serum levels of DKK1 and CTx.

Macroscopic analysis of the alveolar bone

After euthanasia, the maxillae were removed and fixed in 10% buffered formalin for 48 hours. They were then dissected and separated into hemiarches, clarified in 2.5% sodium hypochlorite for 1 minute and stained in 1% methylene blue for 10 seconds, in order to differentiate the bone tissue from the teeth (Adapted from Goes et al.[19]).

Subsequently, the hemimaxillae were photographed. Bone resorption was measured considering the difference between the area of cementum-enamel junction until bone crest in the region between the first molar and third molars from the left and right sides, using the Image J® software (NIH, Bethesda, Maryland, USA) [17].

Histopathological analysis of the periodontium

For this analysis, a new set of experiments was performed. After the euthanasia, the jaws were removed and fixed in 10% neutral formaldehyde for 24 hours. Then, they were decalcified in 10% EDTA, neutral pH [19], for four weeks. Subsequently, the material was embedded in paraffin and 4 µm thick sections were collected and stained with Hematoxylin-Eosin (HE).

For the microscopic analysis, the region between the 1st and 2nd molars was considered, assigning scores ranging from 0–3, according to the intensity of the findings, considering the following aspects: presence/intensity of cellular infiltrate and state of preservation of the alveolar process and cementum [20]. So that 0 was classified as absent and 3 as high intensity of alteration.

Histometric analysis of alveolar bone

For this analysis, we used the same slides used for histological study. The slides that presented, in the same histological section, dental root, interdental papilla and interproximal bone were selected. Images were obtained from the interproximal region at 400x magnification [21]. The images were launched in the

Image J® software (NIH, Bethesda, MD, USA) and an observer blinded to the groups performed the osteoblast and osteoclast count by bone perimeter (N.Ob/B.Pm and N.Oc/B.Pm respectively) using the Image J® software [8].

Immunohistochemistry for beta-catenin

Immunohistochemistry for beta-catenin was performed using the streptavidin–biotin–peroxidase method in formalin-fixed, paraffin-embedded tissues sections (4µm thick) and mounted on poly-L-lysinecoated microscope slides. The sections were deparaffinized and rehydrated through xylene and graded alcohols. After antigen retrieval, endogenous peroxidase was blocked (30 min) with 3% (v/v) hydrogen peroxide and washed in phosphate-buffered saline (PBS). Sections were incubated overnight (4°C) with anti-beta-catenin (1:200 ABCAM®,Cambridge, MA, USA). The slides were then incubated with the secondary antibody diluted 1:200 in PBS–BSA. After washing, the slides were incubated with avidin–biotin–horseradish peroxidase conjugate for 30 min, following the manufacturer's instructions. Beta-catenin was visualized with the chromogen 3,3 diaminobenzidine (DAB), after 2 min of incubation. Negative control sections were processed simultaneously as described above but with the first antibody being replaced by 5% PBS–BSA. Slides were counterstained with hematoxylin, dehydrated in a graded alcohol series, cleared in xylene, and coverslipped. The immunostained osteoblasts for beta-catenin of five different areas of each section (from four specimens per group) were quantified at 400x magnification.

RNA isolation and quantitative PCR

In the third set of experiments, after euthanasia, the maxillae were collected, the gingival tissue removed and the bone tissue was macerated in liquid nitrogen using Trizol (Thermo Fischer-Waltham, Massachusetts, USA). The extracted mRNA was quantified using Nanodrop (Thermo Fischer-Waltham, Massachusetts, USA) and then transcribed using Superscript II (Invitrogen). Subsequently, the RT-PCR assay was carried out using SYBR_green as a reference (ABI 7500 Fast; Applied Biosystems). The PCR condition was 50°C for 2 minutes and 90°C for 10 minutes, then 40 cycles at 95°C for 15 seconds and 60°C for 1 minute, where the RT-PCR system at 7900HT from Applied Biosystems. To calculate the results obtained, the threshold cycle method (Livak & Schmittgen [10]) was used, where they were presented as an x-fold increase related to beta-actin. Primer sequences were as following (Table 1)

Table 1								
Primer sequences								
ß-actin s: TGAGCTGACCAGTTCCCTCT	ß-actin as: AAGCTCGCTCCTGTGAGTTC							
ß-catenin s:TGAGCTGACCAGTTCCCTCT	ß-catenin as: AAGCTCGCTCCTGTGAGTTC							
Runx2 s: CCTTCCCTCCGAGACCCTAA	Runx2 as: ATGGCTGCTCCCTTCTGAAC							
OPG s: CAAAGGCAGGGCATACTTCCT	OPG as: CCAGTGCTAGGTGCTTCTCTG							

Blood collection and biochemical analysis

At the time of euthanasia of all experimental sets, after anesthesia, 2ml of blood was collected from each animal, by cardiac puncture, for biochemical analysis of Dickkopf protein 1 (DKK1) and C-telopeptide of collagen (CTx) by means of ELISA according to the manufacturer's guidelines Abebio® (Wuhan, China).

Molecular docking with Wnt pathway components

The molecular docking technique was employed to analyze the interaction mode between monensin and the proteins involved in WNT pathway. This computational method can efficiently predict the binding mode and affinity between two molecules [23, 24].

Monensin and proteins' structure preparation

The chemical structure of monensin was obtained from the PubChem database, identified by ID 441145 (Fig. 1). Subsequently, the protonation state was determined using the MarvinSketch program^{© 25}. The β -catenin and LRP6 proteins from the WNT pathway were chosen as targets for molecular docking calculations based on literature references¹⁶. Their three-dimensional structures were obtained from the Protein Data Bank (PDB) with codes 1qz7 (resolution of 2.2 Å) and 3s8v (resolution of 3.1 Å), respectively.

As a pre-processing step for molecular docking, the protonation state of the proteins at pH 7.4 was determined using the PDB2PQR software [26]. Additionally, hydrogen atoms, water molecules, and small existing molecules present in the original PDB files were removed. Furthermore, only the E3 and E4 domains of the extracellular portion of the LRP6 protein were taken into consideration.

Molecular docking calculations

Docking molecular assays were performed using the DockThor software [27, 28]. The structural regions were constrained to known interaction sites of other inhibitors reported in the literature, with the grid centered on these regions, the coordinates, and other parameters of which are presented in Table 2. A total of 24 poses were generated and ranked by the binding affinity score provided by DockThor, which utilizes the DockTScore program for this purpose [29]. The best poses were manually inspected using Pymol and Discovery Studio [30].

PDB	Grid center			Grid Size		
	Х	Y	Z	Х	Y	Z
1QZ7	19.648	1.249	7.772	24	24	24
3S8V	-14.51	8.27	-7	23	24	25

Table 2					
Box's dimensions and positioning parameters.					

Statistical analysis

Quantitative data were submitted to the Shapiro-Wilk normality test. Parametric data were submitted to the ANOVA test, followed by Tukey and expressed as Mean \pm SEM. Non-parametric data were submitted to the Kruskal-Wallis test followed by Dunn and expressed as a median (minimum-maximum). All analyzes were performed using the statistical software GraphPad Prism 6.0®, considering a significance level of 95% (p < 0.05).

RESULTS

Lithium Chloride mitigates bone loss potentiated by Monensin

The model of experimental periodontitis (EP) induced by ligature was marked by bone loss, root exposure and furcation lesion (Figs. 2A and D), with increase on osteoclast number (Fig. 2B) and on CTx serum levels (Fig. 2C). It was also seen an important inflammatory infiltrate on the periodontium of these animals (Table 3). The treatment with LiCL significantly attenuated bone loss (Figs. 2A and D) with reduction on the number and function of osteoclasts (Figs. 2B and C), compared to EP. LiCl also reduced periodontal inflammation (Table 1). Meanwhile Monensin potentiated bone loss (Fig. 2D), increasing the number of osteoclasts (Fig. 2A) as well as CTx levels (Fig. 2C) (p < 0.05). A greater inflammatory infiltrate on periodontium was observed in these animals (Table 1). However, the use of LiCl in the group of animals with EP receiving Mon was able to significantly mitigate bone loss by 28% (p < 0.05), reducing the number of osteoclasts by 32% (Fig. 2B) and CTx serum levels by 27% (Fig. 2C) compared to Mon group. LiCL also improved the histological aspects of the periodontal tissue with Mon was used (Table 3). Taken together, LiCl was able to protect bone tissue by reduction on osteoclast number and activity in animals with periodontitis receiving Monensin.

Escore (0-3)	Experimental groups (n = 6/group)					
	Ν	EP	Mon	LiCl	Mon + LiCl	
(1) Absence	0	0	0	0	0	
(2) Discrete	0	0	0	4	1	
(3) Moderate	0	2	1	2	4	
(4) Intense	0	4	5	0	1	
Median	0 (0-0)	3(2−3) ^δ	3(2-3)	1(1-2)*	2(1-3)#	
(extreme values)						

Values presented in absolute number of animals/score and in median (extreme values).

N = Naive; PE = experimental periodontitis; LiCl = Lithium chloride; Mon = Monensin.

 $(^{\delta})$ Indicates the statistical difference compared to the Naive group (N);

(*) Indicates the statistical difference compared to the experimental periodontitis (EP) group;

 $(^{\#})$ Indicates statistical difference compared to Monensin (Mon).

Kruskal-Wallis test followed by Dunn (p < 0,05).

Lithium chloride rescues the deleterious effect of Monensin on osteoblasts

EP significantly reduced the number of osteoblasts on the periodontal tissue (Figs. 3A and D) (p < 0.05). LiCL reversed the low number of osteoblasts (Fig. 3A) with the increase on Runx2 and OPG gene expression compared to EP (p < 0.05). Monensin significantly reduced the number and function of osteoblasts (Fig. 3A-C). In the other hand, when LiCl was used in the animals with EP receiving Mon, it was seen an increase on osteoblast count by 39% marked by increase on Runx2 (+ 70%) and OPG (+ 68%) gene expressions. Confirming that LiCl was effective in stimulating osteoblasts even in animals with periodontitis receiving Mon.

Lithium chloride counteracts Mon-induced Wnt signaling inhibition

Considering Wnt signaling play an important role on osteoblastogenesis and function and that LiCl and Mon are modulators of Wnt signaling, we have decided to investigate the behavior of Wnt pathway under administration of LiCl + Mon.

Dkk-1 is a Wnt antagonist and it has already been shown, by our group, its role on periodontal bone loss [9]. However, there was no difference between the groups treated with either LiCl and/or Mon (p > 0.05) (Fig. 4A). indicating lack their lack of action over this component.

Beta-catenin is an effector key molecule of Wnt pathway. LiCl increased both Beta-catenin gene and protein expressions (Figs. 4B - D). Mon drastically reduced the expression of Beta-catenin of both gene and protein. The use of LiCl in animals with EP receiving Mon restored the expression on Beta-catenin compared to Mon group.

The molecular interaction mode between monensin and the β -catenin and LRP6 proteins

Initially, concerning the β -catenin protein, poses 12 and 9 assumed by monensin were identified as the most favorable at the binding site, displaying binding energies of -9.3 kcal/mol and – 7.5 kcal/mol, respectively. All poses exhibited the same conformation, with an RMSD of 0.3 Å, therefore, the pose 12

was chosen (Fig. 5A). Monensin binds to an exposed cavity located within the armadillo repeat domain. Within this site, crucial interactions occur with the residues Lys508 and Arg469. Lys508 forms a hydrogen bond with the oxygen adjacent to the carbon of the ligand's pyran ring end, while Arg469 interacts with the adjacent oxygen of the ligand's central pyran ring. Additionally, the residues Cys429 and Cys466 establish alkyl-type interactions with the carbons linked to the furan and pyran rings.

In the LRP6 protein, it was observed that poses 2 and 7 were the most favorable, displaying binding energies of -8.2 kcal/mol and – 7.8 kcal/mol, respectively. These poses showed different conformations, with an RMSD of 2.7 Å. Following a manual analysis, pose 2 was selected (Fig. 5B). Monensin binds to the interface of the E3 and E4 domains, establishing hydrogen interactions between the carbonyl oxygens and the adjacent oxygen, specifically with Arg638 and Arg639. Additionally, alkyl interactions occur between the pyran ring carbon at the end and the residues Ile681 and Tyr706, along with π -alkyl interactions involving residues His834, Tyr875, and Ala640.

DISCUSSION

In this study, the model of ligature-induced periodontitis was effective due to the intense bone resorption caused, confirming the previous findings of our group[8, 21–22, 31–33]. LiCl, a Wnt agonist, protected bone tissue, reduced osteoclast count and increased the number of osteoblasts. While Mon, a Wnt antagonist, potentiated bone loss and inflammation, marked by the increase on the number of osteoclasts, and reduction in osteoblast counts, also affecting their function. However, when the use of LiCl in animals with periodontitis receiving Monensin was able to reverse their deleterious effect on bone tissue.

In this study, Mon potentiated bone loss induced by periodontitis. Mon is an ionophore antibiotic that has recently been indicated as a drug with anti-cancer action [12], precisely because it inhibits the canonical WNT pathway by blocking of the LRP5/6 receptor and beta-catenin [16]. Molecular docking analyses revealed the high affinity of monensin for the above-mentioned proteins. Furthermore, it was identified that monensin forms a hydrogen bond with Lys508 of beta-catenin, a hotspot known to interact with other inhibitors [34]. With the LRP6 receptor, it was observed that monensin interacts with residues Ile681, Tyr706, and Tyr875, components of a hydrophobic patch crucial for interactions with its biological inhibitor, DKK-1[35, 36].

In bone tissue, the blockage of Wnt is related reduction on OPG leading to higher interaction between RANK/RANKL [37] improving osteoclastogenesis and bone resorption [8], as confirmed our findings. However, from the best of our knowledge, this is the first time that the effect of Monensin has been evaluated on periodontal bone loss.

As demonstrated, LiCl protected bone tissue of animals submitted to periodontitis. This agent has been used to treat mental disorders, but considering its inhibition of GSK3b [38–41], LiCl induces activation of WNT pathway. In bone tissue, the activation of WNT signaling causes GSK3b inhibition, allowing the accumulation of beta-catenin in the cytoplasm, which then, gain accesses the nucleus and stimulates

the expression of genes such as Runx2, the main transcription factor of osteoblasts and OPG, a marker of osteoblast function [42] corroborating our findings.

Specifically, in the periodontium, previous studies have confirmed our findings regarding the osteoprotective effect of LiCl. It has been described that LiCl reduces ligature-induced bone loss in estrogen-deficient rats, improving the trabecular area with high expression of bone markers [18]. In an orthodontic tooth movement model, LiCl reduced root resorption and minimized periodontal ligament cell death [43]. Lithium accelerated healing of apical periodontitis in an animal model [40, 44] showed that LiCl caused an increase in osteogenic markers, such as Runx2 and Osterix.

Acknowledging the great effects of LiCl and Mon on bone tissue, we have decided to investigate if LiCl could rescue the monensin-potentiated bone loss in animals with periodontitis. In this study, it was seen that LiCl reversed bone loss, reducing osteoclasts counts and CTx serum levels. Meanwhile, it restored the number and activity osteoblasts in animals with periodontal bone loss potentiated by Monensin. It is noteworthy, that other types of GSK3b inhibitors, such as BIO and CHIR99021, were unable to reverse the inhibition of Wnt signaling caused by Mon [16].

In order to explain these finding, and considering that LiCL and Mon directly interact with Wnt signaling, molecular assays were performed. Dkk-1 is an antagonist of Wnt pathway, stimulated by inflammation, has been report to contribute to inflammatory bone [8]. Despite the high levels of Dkk-1 in animals with periodontitis, there was no change after the treatments, indicating the neither LiCl or Mon has Dkk-1 as a target.

Downstream the pathway it was evaluated to role of beta-catenin, and both genetic and protein expressions were restored by the use of LiCl in animals with periodontitis receiving Mon [16, 45]. It has been reported that Mon inhibits beta-catenin but LiCl can provide a greater expression of beta-catenin, in turn. LiCl directly compete with magnesium ions for the binding site of this GSK3b, promoting its inhibition [46] and also can, indirectly, through phosphorylation in the Ser9 amino acid, act as a pseudosubstrate, inactivating GSK3b [47]. Taken together both direct and indirect effect of LiCl inhibiting GSK3b may stimulate a greater beta-catenin accumulation rescuing Wnt pathway and protecting bone tissue. Moreover, Monensin, as an ionophore can bind to Na+, K + and Li+, facilitating their entry into the cell [48–50], thus we suggest that Mon favor the entry of Li + into the cell potentiating GSK3b inhibition with beta-catenin accumulation (Fig. 6). However, more studies are needed to confirm this hypothesis.

CONCLUSION

In summary, the results of this study showed that LiCl significantly mitigated bone loss potentiated by Monensin in experimental periodontitis, due to a strong inhibition of GSK3b. Therefore, LiCl can be an important pharmacological tool to restore Wnt activation in condition where this pathway has been intensively blocked.

Declarations

Acknowledgments: We are grateful to the Nucleus of Study in Microscopy and Image Processing (NEMPI) from the Faculty of Medicine of Federal University of Ceará (UFC) for all histology and digital imaging services. As well as Brazilian National Council for Scientific and Technological Development (CNPq) for financial support (process #402349/2021-0).

Funding: This study was funded by Brazilian National Council for Scientific and Technological Development (CNPq) for financial support (grant number #402349/2021-0).

Data availability: All data are available and presented on the manuscript.

Author contribution: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Anderson Chagas, Sthefane Gomes, Denis Oliveira, Khalil Viana, Jennifer Chaves, Conceição Martins, Vanessa Costa, Gisele Angelino, Sislana Azevedo. Molecular docking assays were performed by Diego Almeida, João Martins Sena. The first draft of the manuscript was written by Delane Gondim, Renata Leitão, Mirna Marques, Paula Goes and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval: The experimental protocol was approved by the Institutional Committee of Animal Care and Use of the School of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil (Permit Number: 7128020620).

Informed consent: For this type of study, formal consent is not required.

Conflict of interest: The authors declare no competing interests.

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Figures



Figure 1

Chemical structure of Monensin.



Lithium Chloride mitigates alveolar bone loss potentiated by Monensin. A) Alveolar bone loss; B) Osteoclast count/bone perimeter (N.Oc./B.Pm); C) Serum CTx levels; D) Macroscopic and Histological aspect of the periodontal tissue. (δ) indicates the statistical difference compared to the Naive (N) group; (*) indicates the statistical difference compared to the Experimental Periodontitis (EP) group; (#) indicates statistical difference compared to Monensin (Mon) group. ANOVA and Tukey tests (p<0.05). AB=alveolar bone; G=gingiva; D=dentin; PL=periodontal ligament; C=cementum; *=inflammatory infiltrate. Black arrows indicate Osteoclasts. 40X and 400x magnification, HE staining.



Lithium Chloride rescues the deleterious effect periodontitis and Monensin on osteoblasts. A) Osteoblast count/bone perimeter (N.Ob./B.Pm); B) Runx2 mRNA expression; C) OPG mRNA expression; D) Histological aspect of the periodontal tissue. (δ) indicates the statistical difference compared to the Naive (N) group; (*) indicates the statistical difference compared to the Experimental Periodontitis (EP) group; (#) indicates statistical difference compared to Monensin (Mon) group. ANOVA and Tukey tests

(p<0.05). Black arrows indicate Osteoblasts. 400x magnification, HE staining.





Lithium Chloride stimulates Wnt signaling reactivation. A) Serum DKK-1 levels; B) Beta-catenin mRNA expression; C) Immunopositive osteoblast cells count/mm²; D) Immunohistochemical aspect of hemimaxillae. ANOVA and Tukey tests. (p<0.05) (δ) indicates the statistical difference compared to the Naive (N) group; (*) indicates the statistical difference compared to the experimental periodontitis (EP) group; (#) indicates statistical difference compared to Monensin (Mon) group. ANOVA and Tukey tests (p<0.05). Black arrows indicate immunopositive staining. 400x magnification.



Molecular interaction of Monensin with Wnt pathway. A) Binding mode between β -catenin (cyan) and Monensin (pink); B) Binding mode LRP6 and Monensin



Proposed mechanism for LiCl to rescue Wnt signaling in animals receiving Monensin under an inflammatory condition