**Title:** Influence of infliximab therapy in bone healing post- dental extraction in rats

**Running Title:** Infliximab therapy in bone healing in rats

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**ABSTRACT**

**Objective:** TNF-α, that acts directly on osteoclastogenesis, may modify the bone turnover. Thus, The objective of this study was to evaluate the influence of infliximab in extractions sockets healing. **Material and methods:** 84 Wistar rats were randomized into two groups (infliximab EV 5mg / kg or saline EV 1ml / kg). The animals were sacrificed 1, 3, 7, 14, 21 and 28 days after surgery. The jaws were subjected to radiographic, histomorphometric, histochemistry (Picrosirius Red) and immunohistochemistry (TNF-α, RANKL and OPG) analysis. **Results:** Lower area filling with bone as well as increased amount of remaining cicatricial tissue was observed in the infliximab group at 14th days (p <0.001). Smaller scores of polymorphonuclear neutrophils were seen at 3th (p <0.01) and 7th days (p <0.001) lower mononuclear count on the 7th day (p <0.01) and osteoclasts in the 7th and 14th days (p <0.01 and p <0.001, respectively). It also observed lower TNF-α, RANKL and OPG immunoreactivity especially on 7th day (p <0.05). **Conclusion:** It is suggested that TNF-α inhibitors could also change the capacity in bone repair after tooth extraction. These findings could signal the need for further precautions in dental procedures in patients undergoing systemic therapy of these drugs.

**INTRODUCTION**

Post-exodontia bone tissue healing depends on variables such as surgical technique, local and systemic factors, general conditions of the patient and use of pharmacological agents (Joseph piecuch, 2012).

The process of bone remodeling with a goal of repair takes place in three main stages: 1) immediate inflammatory response, which leads to a recruitment of mesenchymal stem cells and subsequent differentiation into osteoblasts, with production of extracellular organic matrix or osteoid, 2) mineralization of the matrix with bone formation and 3) the bone remodeling with reabsorption of the neoformed bone to restore the anatomical structure and adequate support to the mechanical loads. (Canão et al., 2005).

Injury after trauma, such as exodontia, initiates an inflammatory response, necessary for the healing process. This response, together with the clot within the bone defect, forms a model for the formation of the bone callus, a repairing fibrous tissue where the bone matrix will be deposited. (Gerstenfeld et al., 2003).

A rapid and well regulated secretion of proinflammatory molecules after an acute injury, such as post-exodontic traumas, is fundamental to the repair (Gerstenfeld et al., 2003).

The initial proinflammatory response involves secretion of tumor necrosis factor-α (TNF-α) and interleukins such as IL-1, IL-6, IL-11 and IL-18, by macrophages, inflammatory cells and cells of origin mesenchymal (Gerstenfeld et al., 2003). These factors recruit inflammatory cells, increase extracellular matrix synthesis, and stimulate angiogenesis (Sfeir et al., 2005). TNF-α is expressed by macrophages and other inflammatory cells, acting as a chemotactic agent (Kon et al., 2001).

TNF-α, in vitro, induces osteogenic differentiation of CTMs (Cho et al., 2006). These effects are mediated by the activation of two receptors, TNFR1 and TNFR2, both present in osteoblasts and osteoclasts. (Kon et al., 2001; Balga, 2006).

TNF-α activates NF-κB activation and is involved in the signaling of intracellular mechanisms such as MEK, ERK and JNK (Zhang et al., 2001). Thus, it plays an important role in the activation of osteoclasts, stimulating bone resorption (Sandros et al., 2000).

Thus, it is stipulated that TNF inhibitors may reduce bone healing by inhibiting proliferative and remodeling inflammatory steps in this process, as well as altering osteoblastic differentiation and the RANK / RANKL / OPG axis in detriment of its anti-resorptive activities, participating in steps the initial and final stages of the bone repair process.

Infliximab is a chimeric, human-murine monoclonal antibody of the IgG1 class, which binds with high affinity to soluble and transmembrane forms of TNF-α. It acts by binding to TNF receptors (TNFRs), producing reverse signaling through the membrane-anchored ligand, which can induce cytokine suppression, and even apoptosis of the producer cell, or by blocking the interactions between transmembrane TNF-alpha (tmTNF ) with TNFRs (Eissner et al., 2000).

The use of infliximab has been indicated for the treatment of rheumatoid arthritis (RA), psoriatic arthritis, ankylosing spondylitis, moderate to severe Crohn's disease or fistulizing clinical forms and ulcerative colitis. (Gonçalves, 2014).

There are inconspicuous data in the literature on changes in the oral microbiota of individuals treated with this medication, as well as on the increased risk of local infection in oral sites, although such changes may be suggested in order to reflect the changes at the systemic level.

CIANTAR and ADLAM (2006) described a clinical case of a patient with juvenile rheumatoid arthritis using infliximab at a dose of 5 mg / kg, who developed extensive osteomyelitis after lower third molar extraction, even after antibiotic prophylaxis, due to the difficulty of tissue healing .

However, there is a lack of experimental studies on the possible impact of the use of infliximab, and its consequent inhibition of TNF-α, on bone remodeling of the jaws. Thus, the present study aims to investigate the influence of this drug on the remodeling and bone repair of dental alveoli after extractions in rats.

**MATERIALS AND METHODS**

**Animals, groups and doses**

Eighty male Wistar rats (Rattus novergicus) weighing 180- 220g were used. The animals were maintained with water and feed ad libitum, in a light-dark cycle of 12 h, temperature of 20-25ºC. The animals were randomly divided into two groups: Infliximab (5 mg / kg) and Saline (0.9% NaCl). The groups were subdivided into another 6 subgroups of 7 animals each, according to the sacrifice of 1, 3, 7, 14, 21 and 28 days after the exodontia.

The infliximab and saline administrations occurred weekly, beginning four weeks prior to the exodontia procedure. The weekly applications were maintained after the surgical procedure of exodontia.

**Experimental Protocol and Analysis of Surgical Difficulty**

Exodontia was performed 24 hours after the fourth administration of infliximab or saline solution. The anesthesia was performed with ketamine 100 mg / kg and xylazine 100 mg / kg intraperitoneally and performed the syndesmotomy of the lower left 1st molar with the aid of an exploratory probe, dislocation using a Hollemback 3S spatula and extraction with a Lecron- Zalle in lever movement (Technique adapted from Maahs et al., 2011).

The animals were randomized according to the order that would be submitted to the surgical procedure, the surgical time was timed and the teeth were dry, counting the number of fractures and weighing in a balance of 10-5g of precision.

**Euthanasia**

After euthanasia due to overdose of barbiturates, the gingival tissue referring to the site submitted to surgery was excised and stored in a freezer -80ºC. The hemimandibules were stored in neutral formol at 10% and then decalcified in 10% EDTA solution for 30 days.

**Descriptive analysis of post-exodontic sites**

The healing of the dental alveoli was analyzed by an experienced pathologist blindly, following the following parameters: presence or absence of acute, mixed and chronic inflammatory infiltrate, granulation tissue, recent or late hemorrhage, areas suggestive of bone sequestration, bone tissue neoformed, osteoclast and bacterial colonies.

**Counting the number of neutrophil, mononuclear and osteoclast polymorphonuclear cells**

After a descriptive analysis, the histological slides were photographed in five (5) microscopic fields of great magnification (400x) and the number of neutrophil, mononuclear and osteoclast polymorphonuclear cells was counted using ImageJ® software (Jilka et al., 2013 ). The sum of 5 random fields was used as the sampling unit.

**Analysis of the percentage of the area represented by bone and connective tissue**

Photographs of the healing region were used at 100x magnification. The alveolus area was delimited and trimmed using the ImageJ® Software, using the Freehand selections> Clear Outside command. After this step, the total area of ​​each well was measured using the Measure (Analyze> Measure) command. Afterwards, the image was inverted from the Invert command and the images were calibrated using the Color Thershold command (Image> Adjustments> Color Thershold) in the RGB function for the Red (Minimum of 0 and Maximum of 255), Green (Minimum of 35 and Maximum of 69) and Blue (Minimum of 0 and Maximum of 255). After calibration with emphasis on bone tissue, the images were converted to 8-bit (Image> Type> 8-bit), binarized (Process> Binary> Make Binary) color scale to then measure the percentage of the marked area referenced to the bone tissue (Analyze> Analyze Particles).

The area occupied by cicatricial connective tissue was obtained by subtraction of the total area of ​​the alveolus by the area filled by neoformed bone, both calculated as previously described.

**Histochemical analysis by coloration of Picrosirius Red**

The areas of exodontia were analyzed with Picrosirius Red staining to verify the amount and type of collagen deposition in the dental alveoli. Slices with 3 μm were incubated in picrosirius solution (ScyTek®), for 30 minutes, stained with Harris hematoxylin for 45 seconds and mounted with Enhtellan® .

For digital evaluation of collagen, six fields were photographed in a 200x magnification (Leica microscope) and evaluated by ImageJ® software. Photos were calibrated by the Color Thershold command (Image> Adjust> Color Thershold) in the RGB function for Red (Minimum of 71 and Maximum of 255), Green (Minimum of 0 and Maximum of 69) and Blue (Minimum of 0 and Maximum of 92). After the calibration the images were converted to 8-bit color scale (Image> Type> 8-bit), binarized (Process> Binary> Make Binary) and measured the percentage of collagen area marked in red (Analyze> Analyze Particles ). After light polarization, the same protocol was performed, however, changing the colors in the RGB function to: Red (Minimum of 0 and Maximum of 255), Green (Minimum of 0 and Maximum of 255) and Blue (Minimum of 0 and maximum of 32). After adjustment, the images were converted to 8-bit color scale, binarized and measured the percentage of collagen area marked in reddish yellow relative to the representative area of type I collagen. A whitish-green area, indicative of type III collagen was also obtained by a process similar to that previously described with changes in the RGB parameters (adapted from Andrade et al., 2011 and Vieira et al.,2015).

**Mioleperoxidase Assay and Analysis (MPO)**

The gingival tissue samples from the extraction region were macerated and homogenized in hexadecyltrimethylammonium bromide (HTBA) buffer (50 mg tissue / ml) and the homogenate was centrifuged using a refrigerated centrifuge at 2000 rpm for 15 minutes at 4 ° C. The MPO activity of the supernatant was measured by measuring the absorbance changes at 450 nm using a reading solution (5 mg of O-dianizidine, 15 μl of 1% H2 O2, 3 ml of phosphate buffer, 27 ml H2 O) of ELISA LT-450 (LabTech®). Absorbance changes were plotted on a standard curve for standardization of neutrophil density and expressed as MPO / mg tissue (Lima Júnior et al., 2012).

**Radiographic Analysis**

The hemimandibula of all groups were radiographed in a conventional X-ray machine (DabiAtlante®; 63Kvp, 8mA) coupled to Digora® digital image capture system. The hemimandibula were positioned parallel to the radiographic film, the focus-film distance was 20 cm, with exposure time of 0.18 seconds.

The radiographs were analyzed randomly and in triplicate by ImageJ® software (Freehand selections), and the arithmetic mean of the three measurements was considered as the sampling unit.

**Immunohistochemical processing and evaluation**

3 μm sections were prepared on silanized slides for immunohistochemistry using the streptavidin-biotinylated technique (adapted from Hsu et al., 1981) for TNF-α, RANKL and OPG. After dewaxing and rehydration, antigenic recovery was performed by the microwave heating technique, where the slides were packed in polypropylene containers containing 10 mM citrate buffer solution (pH = 6.0) and submitted to three sequential 5-minute warm-ups in maximum power. Endogenous peroxidase was then blocked with hydrogen peroxide, washed with phosphate buffered saline (PBS) and incubated with anti-TNF-α, RANKL and OPG primary antibodies (1: 500 [Shaker et al., 2013]), for 12 hours.

After washing with PBS, incubation with biotinylated secondary antibody (HistofineÂ®) for 60 minutes and development with 3,3'-Diamino-benzidine (DAB) for 5 minutes (DakoÂ®) was performed. The slides were counterstained with Harris Hematoxylin for 10 seconds and mounted with Enthellan®.

As a positive control, an intensely inflamed ulcer in rat jugal mucosa (TNF-α) and healthy bone from rat was used. The negative control was performed by suppressing the secondary antibody of the reaction in one of the cuts.

For microscopic evaluation, 5 fields were photographed at a magnification of 400x (Shaker et al., 2013) and counted the cells that exhibit cytoplasmic and membrane positivity, being considered the sum as unit sample.

**Statistical analysis**

The quantitative data were submitted to the Kolmogorov-Smirnov normality test and analyzed by means of the ANOVA, unifatorial or multifactorial, paired or unpaired, associated to the Bonferroni post-test (Mean ± SEM) or Student's t-test.

Dichotomous data (absence or presence) were expressed as absolute and percentage frequency and analyzed using the Fisher Exact test. The area under the curve of the Myeloperoxidase assay was calculated.

All analyzes were performed using the GraphPad Prism 5.0® statistical software, considering a significance level of 95% (p <0.05).

**RESULTS**

**Analysis of Surgical Difficulty of 1st molar extraction**

In relation to the mass of the first lower molar removed (mg), the means found were 18,788 ± 0,435 mg for the saline group and 18,718 ± 0,310 mg for the infliximab group, and no statistically significant difference was observed between the saline and infliximab groups when analyzed (p = 0.8955 / Student's t-test). Regarding the number of fractures, there was no statistical difference between the groups in each group (p = 0.9077 / t test Student) as well the time elapsed between the saline group and infliximab (p = 0.8389 / Student's t-test) did not differ in any of the days analyzed.

**Digital Radiographic Analysis of Jaws**

The radiolucent area, suggestive of alveolus in the healing process, measured from digital radiographs of the mandibles, in pixels, progressively decreased between day 1 and day 28 in a similar manner for both groups, with significant differences observed from day 7 (p <0.0001 for both groups / 1 way ANOVA / Bonferroni).

However, between the saline and infliximab groups, there were no significant differences in the radiolucent areas at the post-extraction site on any of the evaluated days (p = 0.0646 / 2-way ANOVA / Bonferroni) (Supplementary material).

**Descriptive Histological Analysis of Post-Exodontic Sites**

The histological analysis revealed in the alveoli of both groups sacrificed on the 1st day after the surgical procedure, filling of the post-exodontic site by dense blood clot in the middle of numerous erythrocytes and leukocytes concentrated mainly in the uppermost part (Figure 1, A and B).

On the third day, the presence of scarce blood clot, fibrin, granulation tissue and mixed inflammatory infiltrate was observed in both groups, mainly located in the upper portion. However, in the infliximab group, an inflammatory infiltrate less prominent than the saline group was observed (Figure 1, C and D).

On the seventh day after the exodontia, granulation tissue, deposition of neoformed bone tissue in the depth and osteoclasts of the permeate were visualized. The alveoli for the animals treated with infliximab showed the granulation tissue still permeated by a moderate mixed leukocyte inflammatory infiltrate and a discrete deposition of neoformed bone tissue (Figure 1, E and F).

On day 14, characteristics similar to those described for day 7 were observed. However, an extensive amount of neoformed bone tissue was visualized inside the alveoli. Subjectively, the infliximab group had less bone formation than the saline group. (Figure 1, G and H)

On the 21st and 28th day of healing, extensive deposition of neoformed bone tissue was observed, interspersed by small remaining areas of fibrous tissue in both groups. Scarce osteoclasts were seen in the periphery of the newly formed bone, as well as rare mononuclear inflammatory cells. The animals treated with infliximab showed a greater amount of fibrous tissue remaining as well as osteoclasts when compared to the saline group (Figure 1, I, J, K and L).

**Counting the number of neutrophil, mononuclear and osteoclast polymorphonuclear cells**

Neutrophil polymorphonuclear cell counts showed lower values ​​in the infliximab group compared to saline. However, statistically significant differences were observed only on days 03 and 07 (p <0.01 and p <0.001, respectively / 2-way-ANOVA / Bonferroni) (Table 1)

There was also a lower number of mononuclear cells in the infliximab group, with significant differences on day 7 (p <0.01 / 2-way-ANOVA / Bonferroni) (Table 1)

The number of osteoclasts was lower in the infiliximab group, presenting statistically significant differences on days 07 and 14 (p <0.01 and p <0.001, respectively / 2-way-ANOVA / Bonferroni) (Table 1).

**Analysis of the percentage of filling by bone and connective tissue**

In the analysis of the percentage of area of ​​the alveolus filled by bone tissue a significant gradual filling of the post-exodontic site was visualized from day 07 in both groups.

Only on day 14, the infliximab group had significantly less bone tissue with greater persistence of connective tissue (p <0.001 / 2-way-ANOVA / Bonferroni) (Table 1).

**Color analysis of Picrossirius Red**

In the analysis of the total area represented by collagen, a gradual filling of the post-exodontic site with peaks on days 7 and 14 without differences between the infliximab and saline groups (p = 0.8682 / 2-way-ANOVA / Bonferroni) was visualized (Table 1).

After light polarization, the area represented by type I collagen the infliximab group was lower in relation to saline on days 7 and 14. (p <0.05 / 2-way-ANOVA / Bonferroni), and presente no diferences in the are of tipe III collagen (p = 0.6986 2-way-ANOVA / Bonferroni)

In addition, on days 7 and 21, the type I: III collagen ratio was lower in the infliximab group than in the saline group (p = 0.003 / 2-way-ANOVA / Bonferroni) (Table 1).

In the area analysis under the collagen deposition curve, the saline treated group presented a collagen deposition plateau on days 7 and 14 while the infliximab treated group was only seen peak on day 7. The moment of inversion of the proportion of type III collagen for I occurred between days 3 and 7 in the saline treated group and between days 14 and 21 in the infliximab treated group (Table 1).

**Myeloperoxidase assay and dosage (MPO)**

This assay revealed a lower amount of MPO in the gingival tissue of the animals of the inflimime group on all days compared to saline animals (p = 0.051 / 2-way ANOVA / Bonferroni).

The area on the calculated curve for the saline group was 9.014 while for the infliximab group was 1.719. However, statistically significant differences were observed only on days 01 and 03 (p <0.05) (Table 2).

**Immunohistochemical evaluation**

The immunoexpression of TNF-α in the post-exodontic alveoli was lower in the infliximab group than in the saline group, and a statistically significant difference was found on days 07, 14, 21 and 28 (p <0.01, p <0.05, p < 0.05 and p <0.01 respectively / 2-way ANOVA - Bonferroni) (Table 2) (Table 2)

The score for the RANKL marker, the group treated with infliximab showed lower expression with a significant difference on the 7th healing day (p = 0.003). While for the OPG marker, lower expression was verified by the infliximab group also on the seventh day (p = 0.019). The same result was found in the relation between the expression RANKL / OPG (p <0.001 / 2-way ANOVA - Bonferroni) (Table 2)

**DISCUSSION**

Infliximab is characterized by being a potent inhibitor of TNF-α, an important proinflammatory cytokine involved in several physiological and pathological processes, which for this capacity has been widely used in the treatment of inflammatory diseases. The present study aimed to investigate whether the inhibition of TNF-α caused by this drug could interfere in the healing of dental alveoli after the exodontia procedure.

The experimental model did not show differences between the groups of animals that received infliximab or saline in relation to the time of extraction, tooth mass and number of root fractures. These parameters are reported in the literature as related to the surgical difficulty and consequently proportional to the increase of postoperative pain and inflammation (Bello et al., 2011; Ribeiro et al., 2000). Thus, it can be inferred that the surgical procedure, which was performed in a randomized manner and by a previously trained operator who was unaware of the experimental groups, minimized the influence of traumatic factors (resulting from surgical difficulty) that could generate additional tissue damage and inflammation able to interfere in the obtained results.

Regarding the radiographic findings, no significant differences were observed between the radiolucent areas post-exodontia of the infliximab and saline groups. These findings corroborate with those described by Nakachi et al. (2012), who also did not observe radiographic changes in computerized micro-tomograms (μCT) in alveolar healing model between animals of the control group and treated animals CIAM (TNF-α action blocker). These data suggest that inhibition of TNF-α does not seem to alter the bone repair under radiographic point of view.

Histological findings, however, revealed less deposition of bone tissue and greater amount of connective tissue in the group treated with infliximab. These findings corroborate with the results of Gerstenfeld et al. (2003), who described a healing deficit, with reduced bone tissue formation and persistence of fibrous scar tissue in femoral fractures of TNF receptor knockout mice (TNFR-) evaluated on days 7, 14, 21 and 28.

On the neutrophils, previous in vitro studies demonstrate that this drug has the capacity to reduce the chemotaxis and production of reactive oxygen species, mainly due to the blockade of the action of mononuclear cells on polymorphonuclear cells (Pay et al, 2005). This action was attributed to decreased levels and action of chemotactic agents such as IL-8 and neutrophil-activating epithelial peptide -78 (or CXCL5) (Dominical et al., 2011), suggesting a decrease in the number of these cells, as well as its action.

Also discussed in the literature is the effect of this drug on mononuclear cells, mainly macrophages. It is assumed that treatment with infliximab is capable of causing apoptosis of monocytes and macrophages in peripheral blood and at specific sites such as synovial fluid (Catrina et al., 2005).

It has been reported that infliximab causes increased monocyte apoptosis in patients with Crohn's disease through a caspase-3 dependent mechanism (Lugering et al., 2001) and ex-vivo studies with samples from patients treated with infiliximab demonstrated an increase in the number of apoptotic CD3 + lymphocytes (Van Der Brande et al., 2003).

Gunnlaugsdottir et al. (2008) reported that in vitro use of infliximab renders naive T lymphocytes unresponsive when co-stimulated by CD28. The authors suggested that inflimabbe seems to prevent the division of naive CD4 + and CD8 + T lymphocytes and consequently activate cell death induced by non-sensitization of these cells.

The TNF-α pathway appears to be related to the RANK / RANKL / OPG axis, appearing to be synergistic pathways. Kimachi et al. (2011) demonstrated that the number of mature osteoclasts is considerably higher when induced by TNF and RANKL, simultaneously, than when induced only by RANKL. Furthermore, Zhang et al., 2001 observed that the osteoclastogenic action of TNF is potentiated in cells pretreated with RANKL, and that knockout cells to the TNF receptor (TNFR-1) have lower RANK expression, thus demonstrating interdependence and cooperative action of these two regulatory avenues.

It has been described that infliximab can act by reducing the number of osteoclast precursor cells, probably due to effects of this drug in the production of bone marrow precursors (Gengebacher et al, 2008). Studies also indicate that infliximab acts negatively on the formation of osteoclasts from mononuclear precursor cells, inhibiting their growth and grouping (De Vries et al., 2015). Furthermore, it has been observed that patients undergoing TNF inhibitors may present suppression of RANK and RANK-L rather than higher levels of OPG, culminating in lower osteoclast formation (Lee et al., 2004).

On collagen deposition, in the present study, smaller percentages of filling by type I collagen in the infliximab group were visualized in relation to saline. These findings corroborate with those described in the literature by Gerstenfeld et al. (2001) who described that in femur fracture model of knouck-out mice for TNF-α there was less quantification of type I collagen.

The type I collagen is an important component of the bone matrix, assisting in the formation of apatite crystals and providing structural support, flexibility and tensile strength for the neoformed bone (Linder et al., 2015).

On TNF-α and collagen deposition, the studies of Turgut et al. (2014) described that the administration of infliximab in an experimental model could delay tissue healing by decreasing the activity and quantity of fibroblasts, as well as the expression of TGF-β, FGF-β and PDGF, which are also important for the production of collagen. In the present study, although no differences were found in the total collagen, the infliximab group showed less collagen type I at days 7 and 14 and delayed the replacement of collagen III by I.

Previous studies have demonstrated that infliximab is able to reduce serum and local levels of MPO in several experimental models, such as chronic inflammatory joint disease (Feijóo et al., 2009), intestinal ischemia (Akdogan et al., 2014) periodontal (Gonçalves et al., 2014)

TNF-α is related to neutrophil recruitment and chemotaxis (Dominical et al, 2011). It is suggested that the decrease in the number of neutrophils observed in the cell count of the present study, associated to the decrease of the MPO levels in the gingival tissues, points to the suppression of TNF-α by infliximab, culminating in less chemotaxis and activity of these cells.

Analysis of TNF-α expression in the infliximab-treated group showed a lower amount of labeled cells when compared to the saline group, with significant differences being found between the groups on days 07, 14, 21 and 28. In addition, the peak expression of this cytokine, in both groups, was the 7th day. Similarly, other studies in experimental models of exodontia have demonstrated that greater amount of TNF-α is detected in immunohistochemical reactions in the intervals between 3 and 7 days (Kim et al., 2012) with peak, generally, in the 6th day of alveolar repair (Cardoso et al., 2011). Both studies also state that on the first day of observation there is poor expression of TNF-α, as verified in the present work.

Therefore, administration of infliximab (5 mg / kg, 1x per week) was able to interfere in the bone healing process of post-exodontia alveoli in rats. This is the first study on the influence of monoclonal antibodies inhibiting TNF-α cicatrization in dental alveoli.

The need to conduct new experimental studies and clinical trials that support the indication of additional care in surgical drainage procedures in patients undergoing therapy with TNF-α inhibitors is emphasized.

**CONCLUSIONS**

Inhibitors of TNF-α (infliximab) may alter bone repair capacity in post-exodontia sites.

 The immunoexpression of TNF-α, RANKL and OPG in the post-exodontic dental alveoli, especially in the 7th day , was lower in the infliximab group than in the control group.

Negative changes in the deposition of type I collagen and bone tissue in the number of neutrophil, mononuclear and osteoclast polymorphonuclear cells and MPO dosage were observed on different days of the cicatricial process.

So, additional cautions may be need in the patients who used this class of medication after dental extraction.