Effect of the Ingestion of Low-molecular-weight Anticoagulant on the Progression of Induced Periodontitis in Rats

Alex Semenoff Segundo, Álvaro Henrique Borges, Alessandra Nogueira Porto, Matheus Coelho Bandeca, Ana Carolina Soares Diniz, Ana Paula da Cunha Barbosa de Lima, Samyra Lopes Buzelle, Tereza A Delle Vedove Semenoff

Introduction

Among the anticoagulants is heparin, a chemical mediator released naturally from mast cells. It can inhibit enzymes linked to clotting process (the clotting factors II, IX, and X). The therapeutic use of low-molecular-weight heparin (LMWH) is employed in large scale for patients under various clinical conditions. In the case of presence of circulatory problems, LMWH provides blood with fluidity and prevents thrombi and clots related to serious complications often ensuring longer survival of people. Among these risks stand out deep thrombosis, treatment of acute coronary syndromes, treatment of pulmonary embolism, hemodialysis, treatment of patients undergoing major orthopedic surgeries, among others.

It is known that the tissue repair process may be classified into two distinct ways: Regeneration, in which damaged cells are replaced by others from the same source; or healing, in which damaged cells are replaced by fibrous connective tissue. In both forms of tissue repair, one may found neovascular events related to the coagulation factors interfering in the process.

Periodontal disease encompasses infectious and inflammatory features, mostly of chronic nature, in which vascular changes might interfere with the disease progression and systemic repercussions. In a condition of periodontal disease, defense cells and chemical mediators of inflammation such as interleukins (ILs), insulin-like growth factor, tumor necrosis factor (TNF), platelet-derived growth factor, and Von Willebrand proteins, among others, are released to modulate the inflammatory process. As such, one of the most important aims is to provide microcirculation with fluidity, eliminating the infectious focus, and promoting tissue repair.

Hence, the aim of this article is to assess whether the administration of LMWH is able to interfere with the progression of bone loss in experimental ligature-induced periodontitis in rats.

Materials and Methods

This study was approved by the Research Ethics Committee at the University of Cuiaba, Brazil, under the protocol #2010065.
For the present experiment, we selected 48 rats of the Rattus norvegicus species and Wistar lineage. These animals were maintained with balanced food and water ad libitum, under light/dark cycle of 12 h, controlled temperature of 23°C, and humidity about ± 50%. They underwent an adaptation process to the new environment for 7 days before the study began.

The animals were randomly divided into four groups of equal numbers (n = 12). The first group received only LMWH; the second one had periodontal disease induced (LG); the third one was the combination of heparin and induced periodontitis (HLG), and the fourth and last group was the control (CG) – nothing was done.

Heparin was used in this experiment at a concentration of 5000 IU/mL (porcine sodium heparin 5000 IU/mL; Blausiegel®, São Paulo, SP, Brazil). It was administered 600 IU/kg/day, subcutaneously, in a single dose. The administration began on the first day of the experiment for the groups HG and HLG and remained for 30 days. To achieve this dose, we undertook a pilot study before the start of the investigation herein reported.

Periodontitis was induced in groups LG and HLG 15 days after the beginning of the experiment and remained for 15 days longer. We chose the maxillary right second molar area to induce the disease. The maxillary left second molar area was not undergone to ligature, and these specimens functioned as a control. To perform such procedure, animals were anesthetized by means of intramuscular injection of 0.1 ml of ketamine hydrochloride (Dopalen, Agribrands. Animal Health, Paulinia, SP, Brazil) – 50 mg/mL – coupled with 0.05 ml of xylazine hydrochloride (Dopalen, Agribrands. Animal Health, Paulinia, SP, Brazil) – 2 g per 100 ml for each 100 g of body weight. While animals were anesthetized, a silk suture (Ethicon 4.0, Johnson and Johnson) was adapted around the maxillary right second molar, and there it remained for 15 days.

All animals were tested for clotting on the first and last days of the experiment to assess whether heparin caused changes in blood clotting. Bleeding time was determined by the capillary method (Sabrazés’s method). For this, a small puncture was made in the animals’ tails with the aid of a lancet. The blood, whose first drops were discarded, was collected in a capillary tube of 1 mm diameter and kept horizontally. Time began to be marked by a chronometer, and at each 30 s a small fragment was removed from the tube. When we observed the formation of a cylinder composed mainly of fibrin binding the broken ends of the capillary tube, the chronometer was stopped and the exact time was written down. The data were compared using statistical analysis.

On the 30th day of the experiment, we carried out the euthanasia of all animals by an excess of anesthetic.

Right and left hemi-maxillae were fixed in 10% formalin solution for 48 h. Radiographs were taken using an X-ray unit (70x Specto, Dabi Atlante, Ribeirão Preto, SP, Brazil). To take the images, samples were placed on the film (Kodak dental vision, São Paulo, SP, Brazil), and X-ray cone was set perpendicular to it. The distance between the cone and the samples, as well as the exposure time of 0.3 s, was standardized based on a previous pilot study. A trained operator processed all radiographs using the same time and temperature.

Radiographs were digitized and projected onto a whiteboard by a multimedia projector (Mini LED projector Samsung SP-P410M, Brazil), which provided a magnification of 15 times. Initially, we accomplished the identification of the structures between the first and second molars, including teeth and interproximal bone crest. The measurements were made through two lines. First, a horizontal line was drawn between the tips of the cusps of both teeth. Second, given the point of contact between the molars, a vertical line perpendicular to the horizontal one was drawn. The distance between the bone crest and the horizontal line was determined with a digital caliper (Digital Caliper, Mitsu, São Paulo, SP, Brazil) and recorded for later analysis and comparison. A previously calibrated examiner who was unaware of the analyzes performed the assessments. The statistical test used was an analysis of variance with the post-hoc Tukey’s test. The significance level chosen in the study was 5%.

Results
The results show that for the clotting test (Graph 1), the groups that were subjected to administration of heparin – HG and HLG – had an average time of 152.91 and 158.75 s, respectively, with no statistical differences between groups (P > 0.05). The CG and LG had an average time of 77.91 and 88.66, respectively, and were also found to present no statistical differences between them (P > 0.05). Nevertheless, when the groups HG and HLG were compared with LG and CG, it was noticed that the clotting time was greater in the first two groups with no statistical differences compared with the last two ones (P < 0.05).

With regard to bone loss, the findings show that the left side without induction of periodontal disease (Table 1), showed the lowest radiographic bone loss with no significant differences between groups (P > 0.05). On the side where disease was induced, HLG presented the highest radiographic bone loss in relation to the other groups (P < 0.05), followed by LG (P < 0.05) and CG and GH with no differences between them (P > 0.05).

In comparisons between the right side (with induction of disease) and the left side (without induction of disease), there were differences for all comparisons (P > 0.05), excepting the one between HG and CG.
The effect of prophylactic dose of a low molecular weight heparin related to tissue repair in rats, authors demonstrated that the group subjected to the heparin use protocol had the inflammatory repair process diminished, greater fragility of the web of repaired collagen fibers, decrease of the presence of growth factors, low epithelial repair, in addition to hemorrhages troubling healing.

To validate the methodology of this study, it was accomplished a pilot study to develop the technique for subcutaneous administration of LMWH, and to standardize an adequate dosage, avoiding animals suffering, and their loss. The assessment of radiographic bone loss has been widely used in the literature, as many authors have opted for this tool due to the agility and lower costs.

In this investigation, it seems that the periodontium defense process may be altered by the LMWH. In a study using heparin-related to tissue repair in rats, authors demonstrated that the group subjected to the heparin use protocol had the inflammatory repair process diminished, greater fragility of the web of repaired collagen fibers, decrease of the presence of growth factors, low epithelial repair, in addition to hemorrhages troubling healing.

Discussion

Undoubtedly, anticoagulants have demonstrated a great help in maintaining health, considering the challenges of diseases that affect the circulatory system. The relationship between the coagulopathy’s problems and dental care seems to be present in the current days. Unfortunately, it seems that sometimes the dentist needs more care and training for supporting these patients who represent a large number of the population.

Before the challenge of periodontal disease, the human body presents systemic inflammatory markers related to microcirculation. Among them are the C-reactive protein, fibrinogen, Von Willebrand factor, soluble endothelial markers, E-selectin (s-Es), IL-1b, and TNF-alpha; all of the markers demonstrate that given the infection present in the mouth, the systemic pattern might be modified. The findings of the present study corroborate with those of the literature. That is, the ones who use anticoagulant (such as cardiopathy) have their care related to toothbrush worsened and have been found to present greater loss of periodontal structures.

In this investigation, it seems that the periodontium defense process may be altered by the LMWH. In a study using heparin-related to tissue repair in rats, authors demonstrated that the group subjected to the heparin use protocol had the inflammatory repair process diminished, greater fragility of the web of repaired collagen fibers, decrease of the presence of growth factors, low epithelial repair, in addition to hemorrhages troubling healing.

In this line of research, some difficulties may arise. Human studies do not demonstrate the exact proportions of the effect of anticoagulants on oral health. Another good alternative would be the study of patients with hematological disorders, but the small sample size comes to hamper the reliability of results. In the case of confounding factors, the employment of univariate and multivariate analyzes sometimes characterizes shortcomings with the treatment of co-variables such as heparin. In this sense, studies with animals help to understand the biology and outlining research in humans with increased knowledge, much of which would be considered impossible in humans.

In relation to animals, it becomes interesting to test hypotheses as we did herein. However, we emphasize that further studies in rats and humans are required. It is certain that before the emergence of problems of the hematopoietic system, the dentist should be alert and work in a team until discovery of further information on this matter, which is still uncertain within its indications.

Conclusion

Rats undergoing heparin therapy tests had greater bone loss after induction of ligature-induced periodontitis.

References


