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Evaluation of the effect of Levofloxacin and Cefalexin derivative antibiotics on Implant Osseointegration in rat tibia

Evaluación del efecto de los antibióticos derivados de levofloxacino y cefalexina sobre la osteointegración de implantes en tibia de rata

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ABSTRACT

Osseointegration refers to the process where an implant firmly and functionally bonds with the bone, establishing a stable union capable of bearing loads without any relative movement between the implant and the surrounding bone tissue. Post-surgery infections that may develop in the surgical area can negatively affect osseointegration, putting the success of the implant at risk. The objective of this research was to evaluate the impact of antibiotics derived from levofloxacin and cephalexin on the osseointegration of implants in the tibias of rats. A total of 21 female Sprague Dawley rats were utilized, randomly divided into three equal groups of seven rats each. Titanium implants measuring 2.5 mm in diameter and 4 mm in length were inserted into cavities created in the corticocancellous bone of the metaphyseal region of the right tibias of all animals. In the implant control group (n=7), no additional procedures were performed throughout the two-week experimental period. Subjects in the implant levofloxacin group (n=7) received subcutaneous injections of levofloxacin at a dose of 25 mg/kg, administered three times weekly for two weeks. Similarly, rats in the implant cephalexin group (n=7) were given subcutaneous injections of cephalexin at a dosage of 20 mg/kg, three times per week for two weeks. Two weeks after the operation, the implant samples in the tibias of the rats were subjected to biomechanical analysis using a digital torque device in order to evaluate the osseointegration process. As a result, it was observed that levofloxacin and cephalexin-derived antibiotics had a negative effect on implant osseointegration in the tibias of rats.

Key words: Levofloxacin; Cephalexin; osseointegration, rat

RESUMEN

La osteointegracion es el proceso mediante el cual, un implante se integra estructural y funcionalmente con el hueso, preparándose para soportar cargas y formando una conexión sólida sin movimiento entre el implante y el hueso. Las infecciones posquirúrgicas que pueden desarrollarse en la zona quirúrgica pueden afectar negativamente este proceso, poniendo en riesgo el éxito del implante. Este estudio tuvo como objetivo investigar el efecto de la levofloxacina y los antibióticos derivados de la cefalexina en la osteointegración de implantes en tibias de rata. Se utilizaron 21 ratas hembra Sprague Dawley, divididas en tres grupos de siete ratas cada uno. Se colocaron implantes de titanio de 2,5 mm de diámetro y 4 mm de longitud en las cavidades abiertas en el hueso corticoesponjoso de la porción metafisaria de la tibia derecha de todos los sujetos. No se realizó ninguna otra intervención durante el período experimental de dos semanas en el grupo control con implantes (n=7). Los sujetos del grupo con implante de levofloxacino (n=7) recibieron una inyección subcutánea de 25 mg/kg de levofloxacino tres veces por semana durante dos semanas. Las ratas del grupo con implante de cefalexina (n=7) recibieron una inyección subcutánea de 20 mg/kg de cefalexina tres veces por semana durante dos semanas. Dos semanas después de la operación, las muestras de implantes en las tibias de las ratas se sometieron a un análisis biomecánico mediante un dispositivo de torsión digital para evaluar el proceso de osteointegración. Como resultado, se observó que el levofloxacino y los antibióticos derivados de la cefalexina tuvieron un efecto negativo en la osteointegración de los implantes en las tibias de las ratas.

Palabras clave: Levofloxacino; Cefalexina; osteointegración; rata

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INTRODUCTION

Dental implants are synthetic tooth roots inserted into the jawbone to provide support and stabilization for dental prosthetics such as crowns, bridges, or dentures. Today, patient satisfaction has increased significantly thanks to the predictability, stability, comfort, aesthetic results and functionality of implants. Therefore, the areas of use of dental implants have expanded considerably. In clinical applications, they are preferred in various cases such as patients who are partially or completely edentulous, those who have suffered trauma, congenital anomalies or defects in the maxillofacial region. However, the success of implants depends on comprehensive preoperative planning and appropriate patient selection, where factors such as the patient's bone quality, oral hygiene and systemic health status are carefully evaluated. While dental implants present numerous benefits, they may not be appropriate for all individuals, particularly those with medical conditions or lifestyle factors that could elevate the risk of implant failure [1 , 2 , 3].

The process of an implant directly and functionally integrating with the bone, becoming ready to bear loads and creating a solid connection between the implant and the bone without any movement is called "osseointegration" [4 , 5]. This process is critical for the long-term success of the implant. However, infections that may develop in the surgical site after surgery can negatively affect osseointegration and jeopardize the success of the implant. Infections may cause significant complications, beginning with localized symptoms like pain and swelling, progressing to bone loss, and potentially resulting in the total failure of the surgical procedure. During the infection process, increased levels of pro-inflammatory cytokines and chemokines can prevent new bone formation [6 , 7].

In addition, increased biofilm accumulation can cause more serious infection conditions such as osteomyelitis [6]. Therefore, the use of antimicrobial treatments is of great importance to prevent postoperative wound infections. Such treatments support the osseointegration process by reducing the risk of infection and increase the chances of implant success [7].

Bone healing is a multifaceted biological process that occurs in three stages: inflammation, repair, and late remodeling. The inflammatory stage typically concludes within one week, while the remodeling phase commences around the third week. These stages are governed by intricate molecular-level mechanisms [8 , 9].

Bone tissue healing and successful osseointegration of implants are critical issues in modern orthopedic and dental surgery. Osseointegration refers to the formation of a functional and structural bond between the implant surface and the adjacent bone tissue, a process that is heavily affected by bone metabolism, cellular dynamics, and various environmental factors. Factors affecting implant success include surgical technique, implant surface properties, patient factors, and systemic drug use. In particular, the effects of systemically administered drugs on bone metabolism and implant osseointegration have become a focus of increasing interest in recent years [10].

Several approaches, including the administration of growth factors and pharmaceuticals as well as the use of electrical stimulation, have been explored for their potential to enhance and expedite bone healing. [10]. Studies have shown that certain drugs, especially some types of antibiotics, can slow down or negatively affect the bone healing process. If such drugs are detected and not used, bone healing can be expected to occur completely [11].

The selected antibiotic should effectively target the bacteria most commonly responsible for causing infections. In most cases, postoperative infections are caused by microorganisms that originate from the surgical site. For example, infections following oral surgery are usually caused by bacteria found in the body's natural flora, such as aerobic Gram-positive cocci (such as streptococci), anaerobic Gram-positive cocci (such as peptococci), and anaerobic Gram-negative bacilli (such as bacteroids). Although oral infections are polymicrobial, with anaerobic bacteria outnumbering aerobic bacteria by roughly 2:1, studies have shown that the growth of anaerobic bacteria depends on the presence of aerobic bacteria. In the initial phases of oral infections, aerobic streptococci are typically found, which facilitate the development of an environment conducive to the proliferation of anaerobic bacteria. As a result, an optimal antibiotic should be effective against both aerobic and anaerobic microorganisms [12].

Antibiotics are widely used to reduce the risk of postoperative infection. However, there are concerns that some antibiotics may have adverse effects on bone tissue. It has been reported that fluoroquinolone antibiotics (such as Levofloxacin) in particular may delay bone healing by suppressing the proliferation and differentiation of bone cells and may negatively affect implant osseointegration. In contrast, cephalosporin antibiotics (such as Cephalexin) are thought to have less adverse effects on bone tissue. Therefore, comparing the effects of antibiotics used for postoperative infection prophylaxis on bone healing and implant success is an important clinical question [13].

Levofloxacin is a third-generation fluoroquinolone antibiotic with a broad spectrum of activity, effective against both Gram-positive and Gram-negative aerobic bacteria, as well as certain anaerobic bacteria. It exerts its bactericidal action by blocking bacterial DNA replication. When administered orally, it is quickly absorbed and has a half-life of about 6 to 8 hours. It is mainly eliminated through urine, effectively reaches bone tissue, and is commonly chosen for the treatment of conditions such as osteomyelitis, acute sinusitis, urinary tract infections, and acute bronchitis [12 , 13].

Cephalexin is a first-generation cephalosporin antibiotic that is widely used in the treatment of various bacterial infections. It is effective against both Gram-positive and Gram-negative bacteria, working by inhibiting bacterial cell wall synthesis. This antibiotic is commonly prescribed, particularly following maxillofacial surgical procedures [11 , 14 , 15].

The aim of this study was to compare the effects of systemically administered levofloxacin and cephalexin antibiotics on the osseointegration of titanium implants placed in the tibia bone of rats.

MATERIALS AND METHODS

Animal and study design

This research was carried out at the Firat University Experimental Research Center, with approval granted by the Firat University Animal Experimentation Local Ethics Committee (Approval Number: 21839, Date: 02.02.2024). The principles outlined in the Declaration of Helsinki were rigorously adhered to throughout the experimental procedures. For the standardization of this animal study, 21 female Spraque Dawley rats that were determined to be in the same estrus period after vaginal swabs were included in the study due to the possibility that female hormones could affect bone healing.

The rats were obtained from the Firat University Experimental Research Center. The rats were housed in plastic cages, with the room temperature consistently maintained at 22 °C each day. Throughout the experimental period, the rats were maintained on a 12-hour light and 12-hour dark cycle. They were given free access to food and water.

Cavities were created in the corticocancellous bone of the metaphyseal region of the right tibias of the rats. Titanium implants, measuring 2.5 mm in diameter and 4 mm in length, were inserted into the cavities. The animals were allocated into three equal groups, with each group consisting of seven rats. Implant control group (n=7): In this group, 2.5 mm in diameter and 4 mm in length bone cavities were created in the corticocancellous bone of the metaphyseal area of the right tibias of the subjects under serum cooling.

Titanium implants of the same dimensions (2.5 mm diameter and 4 mm length) were then inserted into these cavities. No other intervention was performed during the two-week experimental period. Implant levofloxacin group (n=7): In this group, 2.5 mm diameter and 4 mm long bone cavities were opened in the corticocancellous bone tissue of the metaphyseal part of the right tibia bones of the subjects under serum cooling, and titanium implants of the same size (2.5 mm diameter and 4 mm length) were placed in these cavities.

During the two-week experimental time, 25 mg/kg levofloxacin was injected subcutaneously three times a week, including the day of surgical procedures. Implant cephalexin group (n=7): In this group, bone cavities of 2.5 mm in diameter and 4 mm in length were opened in the corticocancellous bone tissue of the metaphyseal part of the right tibia bones of the subjects under serum cooling and titanium implants of the same dimensions (2.5 mm in diameter and 4 mm in length) were placed in these cavities. During the two-week experimental time, 2 mg/kg cephalexin was injected subcutaneously three times a week, including the day of surgical procedures. All the rats were sacrificed at the end of the experimental setup.

Surgical procedure

General anesthesia was provided by intraperitoneal injection of 10 mg/kg Xylazine and 50 mg/kg Ketamine [1]. Subsequently, 0.5 mL of articaine with 1:200,000 epinephrine was administered to the surgical site for local anesthesia and to control bleeding. After anesthesia and shaving, the relevant legs of the rats were disinfected with povidone-iodine (FIG. 1).

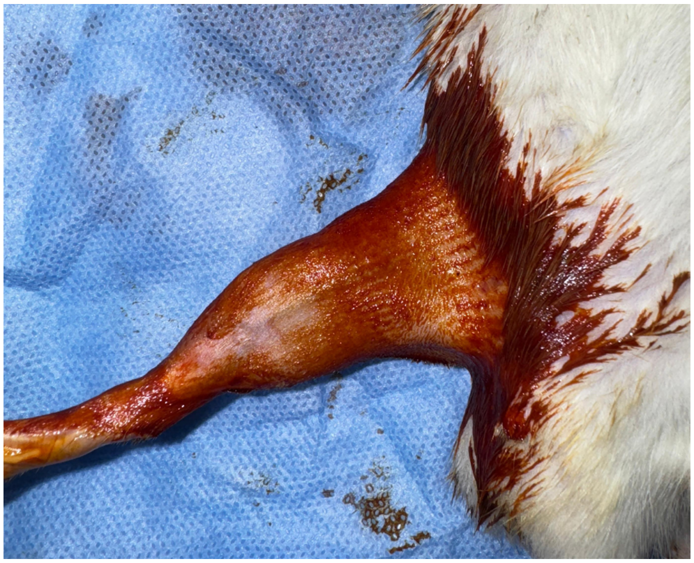

FIGURE 1. Preoperative preparation of the surgical site in a rat for titanium implant placement in the tibia bone

An incision about 2 cm long was made using a number 22 scalpel, maintaining contact with the crestal bone of the tibia. The soft tissues and periosteum were removed using a periosteal elevator and the tibia bone was reached (FIG. 2).

FIGURE 2. Cortico cancellous region of the tibia where the implant will be applied after cutting the skin and the subcutaneous connective tissue

Bone cavities measuring 2.5 mm in diameter and 4 mm in length were created under serum cooling, and titanium implants of identical dimensions (2.5 mm diameter and 4 mm length) were inserted into these cavities (FIG. 3).

Once the implants were positioned, the surgical site was closed using 5-0 absorbable sutures. All subjects who underwent surgery received 1 mg/kg of tramadol hydrochloride intramuscularly for pain management on the day of the operation and for the subsequent three days. Following euthanasia, the implants along with the surrounding bone tissues were dissected from the soft tissues and preserved in formaldehyde. All surgical interventions were carried out by the same surgeon.

Biomechanical analysis

Two weeks after the operation, implant samples from the rats' tibias were taken by removing the tibia as a block to evaluate the osseointegration process. The tibia blocks with implants were prepared for biomechanical analysis. The samples were promptly assessed to avoid dehydration and subsequently stored in a 10% buffered formalin solution. All implants were secured onto polymethylmethacrylate blocks. A rachet (Implance; Istanbul, Turkiye) was utilized to assess the torque of the implants, and a gradually increasing removal force was applied counterclockwise using a digital torque meter (Mark 10, USA). The procedure was stopped once the dental implant began to rotate within the bone socket. The maximum torque value (N/cm) was automatically recorded by the digital torque device at the initial moment of implant rotation within the socket.

Statistical analysis

Descriptive statistics such as standard deviation and median are given in the data included in the study. Kolmogorov-Smirnov and Shapiro Wilk tests were performed to determine the normality of the data. As the normality assumptions were satisfied, One-Way ANOVA was employed to compare all groups. A statistical significance level of $P<0.05$ was considered in the analyses. Statistical analyses were conducted using the SPSS 21 software (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL).

FIGURE 3. After a hole is opened in the bone using a drill, the implant is placed in this hole

RESULTS AND DISCUSSION

In the comparison of bone implant fusion values with One Way ANOVA test, a statistically significant difference was observed between the biomechanical bone implant connection (BIC) (N/cm) levels of the groups ($P<0.05$). The BIC value of Levofloxacin and Cephalexin application was found to be significantly lower than the control applications ($P<0.05$) (TABLE I).

TABLE I.
Biomechanic bone implant connection (BIC) (N/cm) levels of the groups

Groups	N	Mean (N/cm) (BIC)	Std. Deviation	P*
Control	7	12.24	2.27	<0.05
Levofloxacin^{a1}	7	8.50	1.50	
Cephalexin^{a2}	7	8.36	1.22	

*One Way Anova (P=0.001). $^{a1, a2}$: Statistically significantly different compared with control. ^{a1}: 0.002 ^{a2}: 0.001. ($^{a1, a2}$: Tukey HSD Test)

The BIC values of Levofloxacin a1 and Cephalexin a2 groups are statistically significantly different compared to the control group. Levofloxacin a1: P=0.002 and Cephalexin a2: P=0.001 indicate that these groups showed a significant difference compared to the control group. Since both P values are less than 0.05, these differences are statistically significant.

The findings show that these antibiotics significantly affect the osseointegration process. In particular, biomechanical connection strength (BIC) values revealed that antibiotic use altered the healing process at the bone-implant interface.

Numerous studies in the literature investigate the impact of antibiotics on bone metabolism and implant osseointegration. In studies conducted by Golestani *et al*. [16] and Perry *et al*. [11], it was reported that fluoroquinolones group antibiotics (including levofloxacin) inhibit osteoblast activity and negatively affected bone formation after fracture healing in rat tibias.

Holtom *et al*. [17] reported that in their study Cephalosporin group antibiotics (including Cephalexin) slowed inhibitory effects on the proliferation of osteoblasts. These findings are consistent with the low BIC values observed in the levofloxacin and cephalexin groups in this study.

In this study, BIC values were found to be significantly lower in the levofloxacin group compared to the control group (P=0.002). This situation can be explained by the hypothesis that fluoroquinolones may inhibit bone cell proliferation and matrix synthesis. A similar trend was observed in the cephalexin group, where BIC values were found to be significantly lower in this group compared to the control group (P=0.001). These results support the potential negative effects of cephalosporins on bone healing.

Biomechanical analyses showed that new bone formation at the bone-implant interface was less in the levofloxacin and cephalexin groups compared to the control group. These findings are in line with a study conducted by Zhang *et al*. [18]. In their experimental study on fractures of the rat tibia, Huddleston *et al*. [19] reported in their study that levofloxacin and ciprofloxacin inhibited chondrocyte proliferation, significantly reduced callus formation at the fracture site, and hindered complete fracture healing. The study further indicated that these antibiotics delayed osseointegration by slowing the bone remodeling process.". In addition, only two different antibiotic groups were examined in the study, and the effects of other antibiotics were not evaluated. Therefore, studies examining a wider range of antibiotics are needed.

CONCLUSION

This study shows that Levofloxacin and Cephalexin derivative antibiotics negatively affect implant osseointegration in rat tibias. These findings emphasize that caution should be exercised in the selection of antibiotics after implant surgery and that potential effects on bone healing should be considered. Future studies may reveal the mechanisms of these effects in more detail.

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Conflict of interests

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