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Topical application of gentamicin or cephalothin in the healing of surgical wounds in dogs

Aplicação tópica de gentamicina ou cefalotina na cicatrização de feridas cirúrgicas em cães

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ABSTRACT: The present study aimed to evaluate the influence of local administration of gentamicin or cephalothin on the healing of surgical wounds in dogs. Thirty healthy dogs were submitted to surgical castration. They were divided into 2 groups of 15 animals. After the surgical procedure, two 0.6 cm circular cutaneous wounds were made in the abdominal region. Group 1 received 0.1 ml of 0.9% saline in one wound and 0.1 ml of gentamicin (40 mg / ml) in the other wound; and group 2 received 0.1 ml of 0.9% saline and 0.1 ml of cephalothin (200 mg / ml); respectively. All wounds were sutured and assessed macroscopically 1, 3 and 10 days after the operation. On day 10, an incisional biopsy was performed for histopathological evaluation. All analyzes of macroscopic variables did not show significant differences between groups (P <0.05). There were no significant differences between groups for microscopic evaluation of collagenization, vascularization, edema and inflammatory cells (P <0.05). Thus, the topical use of the tested antibiotics does not influence skin healing in dogs.

KEY WORDS: Antibiotics; dog; healing; wound.

RESUMO: O presente estudo teve como objetivo avaliar a influência da administração local de gentamicina ou cefalotina na cicatrização de feridas cirúrgicas em cães. Trinta cães saudáveis foram submetidos à cirurgia de castração. Eles foram divididos em 2 grupos de 15 animais. Após o procedimento cirúrgico, foram feitas duas feridas cutâneas circulares de 0,6 cm na região abdominal. O grupo 1 recebeu 0,1 ml de soro fisiológico 0,9% em uma ferida e 0,1 ml de gentamicina (40 mg/ml) na outra ferida; e o grupo 2 recebeu 0,1 ml de soro fisiológico 0,9% e 0,1 ml de cefalotina (200 mg/ml); respectivamente. Todas as feridas foram suturadas e avaliadas macroscopicamente 1, 3 e 10 dias após a operação. No dia 10, foi realizada biópsia incisional para avaliação histopatológica. Todas as análises das variáveis macroscópicas não mostraram diferenças significativas entre os grupos (P<0,05). Não houve diferenças significativas entre os grupos para avaliação microscópica da colagenização, vascularização, edema e células inflamatórias (P<0,05). Assim, o uso tópico dos antibióticos testados não influencia na cicatrização da pele em cães.

PALAVRAS-CHAVE: Antibióticos; cão; cicatrização; ferida.

INTRODUCTION

Intact skin represents the most important protective barrier from external aggressors. Damaging it, allows potential injurious agents to invade internal tissues, causing inflammation and local or systemic infection (GUREL et al., 2015; DEVRIENDT et al., 2017; ÖHNSTEDT et al., 2019; THIEMAN MANKIN et al., 2020). Antiseptic agents have been extensively studied and much is known about their beneficial and harmful effects on the healing process, which has brought clear guidelines for rational use (ATIYEH et al., 2009). According to studies carried out on open wounds, for example, it was possible to identify that there is no difference between the use of topical chlorhexidine or povidone-iodine in the development of healing contraction and reepithelization (FAHIE et al., 2007).

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Antibiotics provide a fundamental role in the prevention and treatment of wound infection, whether through systemic or local use. Some clinical scenarios represent a special challenge such as excisions of large masses, wounds from bites, car accidents and burns (SUPP et al., 2005). Infection of the surgical site in these cases can lead to a variety of morbid consequences, including prolonged wound management, revision surgery, increased treatment costs and patient mortality (ATIYEH et al., 2009). Therefore, it is common to use topical antibiotic agents during wound healing, however in an empirical and unsubstantiated way.

Despite the widespread use, topical antibiotics applied in wounds is controversial. Some authors report that the elimination or inhibition of bacteria can improve healing time. Others, showed that there is no evidence of reduced healing time after the topical use of antibiotics. Additionally, it has been demonstrated tissue toxicity, resulting in delayed healing (GERONEMUS et al., 1979; DAESCHLEIN, 2013). Considering the potential for harmful effects on contraction, granulation and epithelialization, it is necessary to consider when their benefits superimpose injurious effects on healing (FAHIE et al., 2007). Once controlled studies showing the effects of topical application of antibiotics on skin healing are scarce, the aim of this study was to evaluate the influence of topical use of gentamicin and cephalothin on the healing of surgical wounds in dogs.

We hypothesized that the topical application of antibiotics would not influence negatively the healing process of surgical wounds.

MATERIAL AND METHODS

This study was approved by the Ethics Committee on Animal Use of Sao Paulo State University - Unesp (Campus of Jaboticabal - SP), under protocol number 022733. All procedures were performed at the Veterinary Teaching Hospital. Patients were recruited after owner's agreement.

Thirty healthy, sexually intact, of both sexes, adult dogs were used. They should not been receiving or have received any local or systemic medication from the last 4 weeks at the time for the experiment to start. All animals were undergoing complete physical evaluation to check for the presence of dermatological changes. Dermatological alterations or any condition that could interfere in the treatment and evaluation of the variables made a candidate to be excluded from the study. The animals were divided into two groups: Group 1 (15 dogs) treated with gentamicin and Group 2 (15 dogs) with cephalothin.

The animals were premedicated with morphine hydrochloride (0.5 mg / kg intramuscularly) and induced with propofol (5 mg / kg intravenously). Anesthetic maintenance was performed with isoflurane trough endotracheal tube. The dogs were properly prepared for an aseptic procedure and submitted to previous surgical area antisepsis with 2% chlorhexidine followed by 0.5% alcoholic chlorhexidine.

They were submitted to ovariohisterectomy (OH) or orchiectomy (OC). Additionally, two circular cutaneous surgical wounds of 6.0 mm of diameter were created at each side of the ventral abdominal region using a disposable biopsy punch.

After randomized selection, dogs in group 1 (15 animals) received 0.1ml of 0.9% saline solution in the first wound, and the same volume of gentamicin (40 mg / ml) in the second. In group 2 (15 animals), the first wound received 0.9% saline solution and the second one, the same volume of cephalothin (200mg / ml). Surgical wounds were sutured with 3-0 monofilament nylon thread, with two separate stitches. At the end of the surgery, all wounds were cleaned with gauze soaked in sterile 0.9% saline solution, protected with gauze pad and tape Micropore®.

Surgical wounds were daily cleaned with 0.9% saline solution. The animals remained with an Elizabethan collar during recovery and evaluation period. Meloxicam was administered subcutaneously (0.1 mg / kg) for 2 days. Patients were identified by numbers given at the moment of their inclusion in the study. They were kept in individual kenne ls for 10 days, in which they were fed with specific dog food and water *ad libitum*.

The sutured wounds were evaluated and scored to check for inflammatory response as described by Paim[11] as: presence of edema, erythema or pain / discomfort, vocalization to manipulation, presence of pruritus and amount of secretion.

The parameters were classified as absent, discreet, moderate and intense, with values from 0 to 3, respectively. Presence of secretion and evolution of the healing process were evaluated as absent, serous, erythematous-ceruminous, purulent and mucoid (0 to 4), and good, regular and bad (1 to 3), respectively.

These values were observed and analyzed on days 1, 3 and 10 postoperatively, in a double blind system. Photographic images of the wounds were taken and analyzed on the following days. After the observation period, the animals were anesthetized (using the aforementioned protocol) and submitted to an excisional biopsy of the wounds (scars formed), which were sutured as previously described. All samples were fixed in 10% formalin for 24 hours, transferred to 70% alcohol after that period and, subsequently, sent for processing and analysis.

Collagenization analysis was performed with Masson's Trichrome coloring technique and the other variables with Hematoxylin-eosin. An optical microscope was used to evaluate histological changes, designated as the healing process: collagenization, vascularization, edema, degree of inflammatory cells and degree of chronic inflammatory cells; which were scored from 0 to 3, as absent, discreet, moderate and intense, respectively, as described by Garros (2006).

Macroscopic comparison analyzes between the control and treated groups, was performed using the simple chi-square test. Wilcoxon test was used to test the data for each variable of the microscopic analysis. For all statistical tests, macroscopic and microscopic variables were submitted to the software R, and $P < 0.05$ was considered a significant value.

RESULTS

Macroscopic evaluations of the inflammatory process showed no significant difference between the groups (*P> 0,05)*.

According to the photographic records of surgical wounds at the proposed times, specific statistical analyzes were performed for each variable. The difference between treated wound and control wound of Group 1, and between treated wound and control wound of Group 2, was evaluated. For all statistical tests performed with macroscopic variables, there was no statistically significant difference (*P*<0,05).

There were no significant differences between the treated and control groups in the microscopic evaluation (p <0.05). Collagenization between the groups was analyzed the value of p = 0.2346 ; Vascularization the value of p = 1; Edema the value of p = 0.736; Acute inflammation the value of p = 0.2837; and in chronic inflammation, the value of p = 0.6888.

Although no significant difference was found between the control and treated groups, it is possible to visualize microscopic findings such as the presence of inflammatory cells, collagenization, hemorrhage and angiogenesis, as shown in (Figure 1.).

DISCUSSION

Historically, substances are used in wounds aiming to accelerate healing, preventing or treating infections, reducing pain or removing dead tissue (ATIYEH et al., 2009; BOLTON et al., 1994). The use of topical antibiotics in the treatment and prevention of open wound infection is widely studied, and the results are still somewhat contradictory (ÖHNSTEDT et al., 2019; LINDEN et al., 2014), however the role and effects of the use of these substances as a washing solution in surgical wounds to be sutured has been scarce. Additionally, this practice has been used frequently in human and veterinary patients, and there is no clear evidence of the benefits and potential risks for wound healing. The present study provides more objective and clear information for understanding the effects of this practice on wound healing, despite the action of antibiotics in preventing infection, which was not the scope of the study.

Some studies carried out on veterinary patients show evidence of the effectiveness of topical antibiotic agents in eliminating or inhibiting bacteria and improving the time

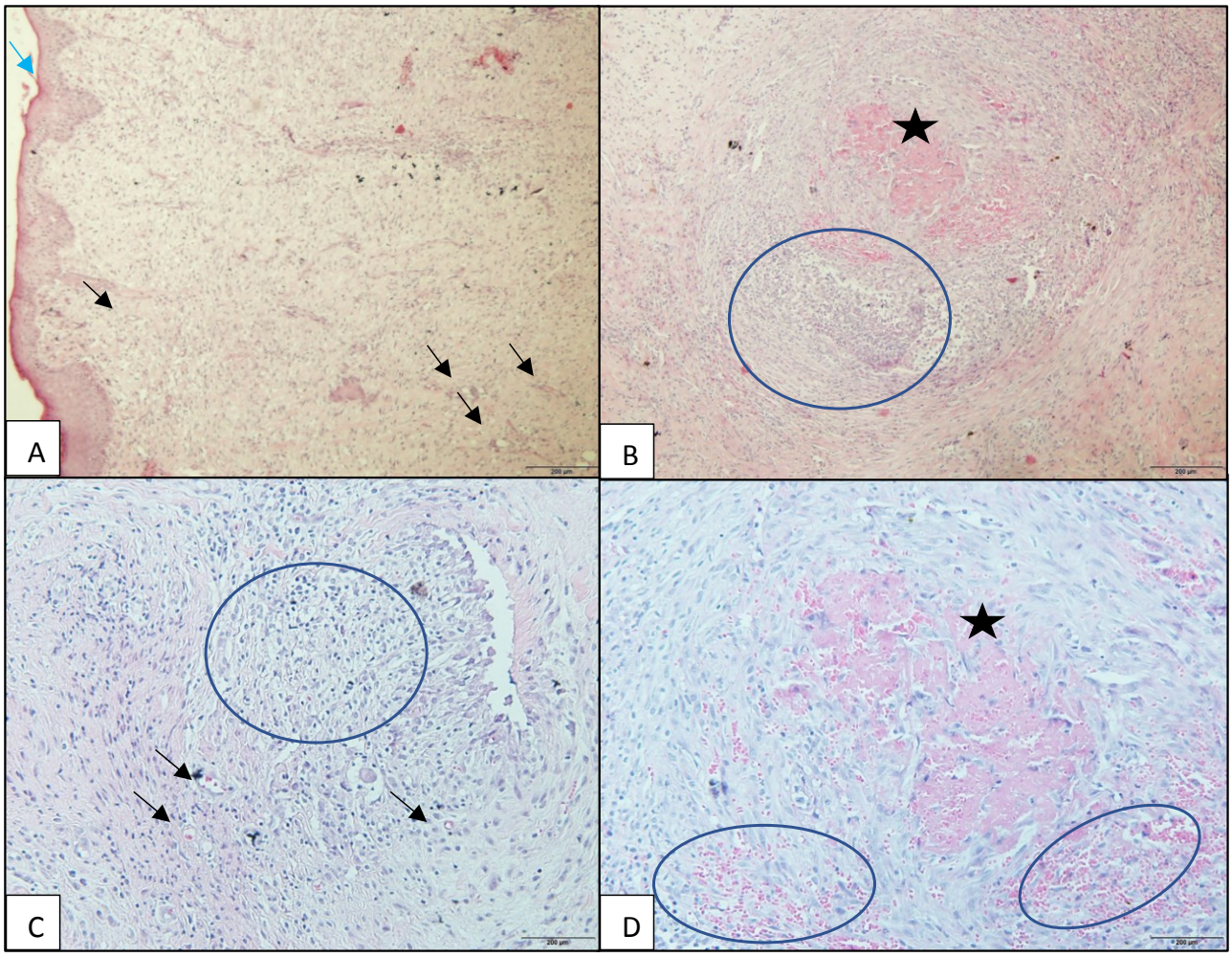

Figure 1. Photomicrograph of the dog's dermis 10 days after the surgical procedure. A) Presence of reepithelization (blue arrow), angiogenesis (black arrow). B) Collagenization area (star), inflammatory infiltrate (circumscribed region). C) Angiogenesis (black arrow), inflammatory infiltrate (circumscribed region). D) Collagenization (star), moderate hemorrhage (circumscribed region). H&E coloring, 200x.

and quality of healing, however, others have failed to present similar results considering skin healing. There are still references of possible tissue toxicity, resulting in delayed healing (GERONEMUS et al., 1979). The results of this study, in turn, did not show any negative interference on healing when using topical application of gentamicin or cephalothin, accepting our initial hypothesis.

In humans, the practice of topical use of antibiotics or antiseptic solutions has been described (ATIYEH et al., 2009). It has been used for appendectomy, some abdominal surgeries and in addition to superficial injuries. The lack of controlled studies focused on this particular objective, associated with the controversial and polemic of this practice, shows an imminent need for further studies supporting the decision-making process regarding the real benefit and adverse effects of the use of topical antibiotics in wound healing.

Despite of the results published by Fahie et al. (2007) showing unwanted reactions such as local dermatitis and other inflammatory processes when using topical gentamicin, and attributing unsatisfactory results in wound contraction and epithelialization, we did not observe the same in our study. It is acceptable that topical gentamicin might prevent or reduce microbial rate (ATIYEH et al., 2007), which hypothetically can interfere positively on wound healing, however our results do not allow us to validate it.

Some authors (BERRÍOS-TORRES et al., 2017), on the other hand, strongly recommend not to use antibiotics in the wound for prevention of infection, but they emphasize that there is little evidence to prove its contraindication. Despite of outer limitations of topical use of antibiotics, we believe this practice is potentially safe, once harmful effects on healing were observed after local administration of cephalothin and gentamicin. It is important to note that even though our findings show neutrality, there was no evidence of improvement of skin healing.

Microscopically, the results found in this study indicates that the topical application of antibiotic to the bed of a surgical wound was not able to cause healing interference, since there was no significant difference in inflammatory cell count, collagenization, nor interference in vascularization. It has been shown that commonly used antiseptics potentially cause deleterious effects on wound repair, adversely impacting cellular activities, in humans and veterinary patients (ATIYEH et al., 2009). It has been shown that commonly used antiseptics potentially cause deleterious effects on wound repair, adversely impacting cellular activities, in humans and veterinary patients (ATIYEH et al., 2009). The action of antibiotics and antiseptics are similar in effectiveness, since antiseptics are capable of altering the cell wall and cytoplasmic membrane components, modifying their permeability. Antiseptics, as well as antimicrobials, can also cause bacterial resistance, and according to Silva et al. (2021), some microorganisms are resistant to chlorhexidine, such as Pseudomonas aeruginosa and Proteus mirabilis. Although our results have not shown that effects, it might be expected some level of interference, that needs to be better understood in the future.

It is clear that topical antibiotics can be used to reduce the incidence of clinical infections (GUREL et al., 2015), especially considering 3 to 12 % of surgical wounds infections in veterinary patients (THIEMAN MANKIN et al., 2020), however there is no evidence that it improves skin healing. Additionally, it may cause microbial resistance (ÖHNSTEDT et al., 2019; DAESCHLEIN, 2013; LINDEN et al., 2014).

The use of antibiotics in the present study proved to be feasible and safe, with no negative interference in the tissue repair process that could not justify its contraindication, but it is important to declare that we do not enter the merit of using antibiotics or not. Although our results point to safety and neutrality, it is ponderable if it is really necessary.

The main limitations of this study rest on the fact that surgically created clean wounds were used, which prevents the deleterious proteolytic effects naturally expected from the pathological environment of a wound. Additionally, although just 2 antibiotics agents were tested, it is believed that many other agents might show a similar outcome, however, further studies need to be developed to validate other antibiotics for this use.

To conclude, the use of topical gentamicin or cephalothin does not affect negatively the healing of surgical wounds, proving then to be feasible and safe.

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