GATIFLOXACIN'S ANTIMICROBIAL ACTIVITY ON IN VITRO CANINE PERIODONTOPATHIC BACTERIA

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Article History: Received: 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

The purpose of this study was to determine any potential antibacterial effects of gatifloxacin on Porphyromonas gulae, a canine periodontal infection. Investigated were gatifloxacin's minimum inhibitory concentrations (MIC) and its bactericidal properties. The canine periodontopathic bacteria were evaluated in broth, and gatifloxacin prevented their development. P. gulae was discovered to be effectively inhibited at a MIC of 50 nM. The adenosine triphosphate bioluminescence experiment revealed that gatifloxacin had concentration-dependent bactericidal effects on the tested microorganisms. Rat bone marrow mesenchymal stem (BMMS) cells were treated with gatifloxacin in order to determine whether it was safe to do so. The vitality of the treated BMMS cells was then measured. During a 3-day culture, about 80% of BMMS cells that were given 100 nM gatifloxacin survived. These findings suggest the possibility of using locally given gatifloxacin to prevent canine periodontal infection.

Keywords: Dog, Porphyromonas Gulae, Periodontal Disease, Antibiotics, And Veterinary Medicine

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DOI: 10.31838/ecb/2023.12.s3.136

1. Introduction

The most closely related animal to humans, dogs, have been domesticated by humans for approximately 15,000 years. According to new study, dogs may have undergone convergent evolution as a result of domestication (Holmström, 2012). Dogs are friends that live alongside people and are not merely tamed animals; they are a distinct species. The statement that dogs and human life are closely intertwined is not hyperbole. Dogs are susceptible to periodontal disease throughout their lives, just like humans are. Over the age of three, periodontal disease affects 80% of dogs (Kortegaard et al., 2008). The food of the dog and the arrangement of the cuspids inside the mouth cavity both have a significant role in the development of periodontal disease. Several factors can contribute to periodontal disease, including not cleaning your dog's teeth every day, feeding them food that tends to leave residue in their mouths, and the way your dog's teeth are arranged naturally. Dogs maintained in a home are frequently mouth-to-mouth given "table scraps" by their owners. The presence of Porphyromonas gingivalis, a major etiologic bacteria causing human periodontal disorders, in the canine oral cavity is evidence that as a result, the likelihood of dogs contracting human periodontal pathogenic bacteria rises. As in people, dogs can still get a dangerous infection called periodontitis. P. gulae, a gram-negative obligate anaerobe that is widely seen in animals other than humans, may be found in the oral cavity of dogs. The P. gulae 16S rRNA exhibits 97%–98% homology to the P. gingivalis 16S rRNA, which has been found in the human oral cavity.(Slots, 1984) P. gulae has catalase activity, but P. gingivalis does not (Allaker et al., 1997). Moreover, variations in the bacterial characteristics of the animal and human Porphyromonas species were noted.

Periodontal disease risk factors have been regularly studied in our lab. Titanium surfaces, a metal typically utilised for dental implants, have been observed to harbour significant levels of periodontopathic bacteria.(Fournier et al., 2001) In addition, the creation of a defensive mechanism against bacterial adherence on the surfaces of dental material was also a focus of our study.(Laliberté & Mayrand, 1983) Similar to periodontal disease in normal teeth, the presence of periodontopathic bacteria surrounding titanium implants is a risk factor for peri-implantitis, which is described as the inflammation that forms around an implant and is linked to bone loss, as well as a reason for implant failures (Alcantar et al., 2000). For the prevention of periodontal disease, such as peri-implantitis, the decrease of dental plaque deposition or tartar adherence to the tooth surface has been highlighted (Yoshinari et al., 2000). Canine periodontitis is also covered by this.

We are investigating the antibacterial properties of safe dietary components originating from live creatures, such as the antimicrobial peptide protamine, in order to create a defensive mechanism to avoid peri-implantitis or periodontitis. Protamine substantially suppresses P. gingivalis development, but these effects are only temporary, making it challenging to continue the therapeutic benefit (Augthun & Conrads, 1997).

Thus, safer medications with fewer adverse effects were sought after. In 2016, we investigated how gatifloxacin inhibits the development of periodontopathic bacteria(Leonhardt et al., 1999). The 4th-generation fluoroquinolone medication family member gatifloxacin, whose chemical structure is depicted in Figure 1, has been widely prescribed in the USA. Although there have been no reports of serious side effects in dogs, some fluoroquinolone prescriptions have been discontinued or limited as a result of the occurrence of serious side effects, including hypoglycemia and hepatotoxicity in humans, which are brought on by systemic administration(Sumida et al., 2002). For a variety of eye infections treated topically, gatifloxacin is typically the first line treatment.

For humans and dogs, various drug doses are frequently supplied per kilogramme of body weight. It is important to keep this distinction in mind while giving dogs an antibacterial medication. This study sought to determine the effectiveness of rat cells in avoiding periodontal infection by local injection as well as the antibacterial activity of gatifloxacin on periodontal pathogens that infect canines.

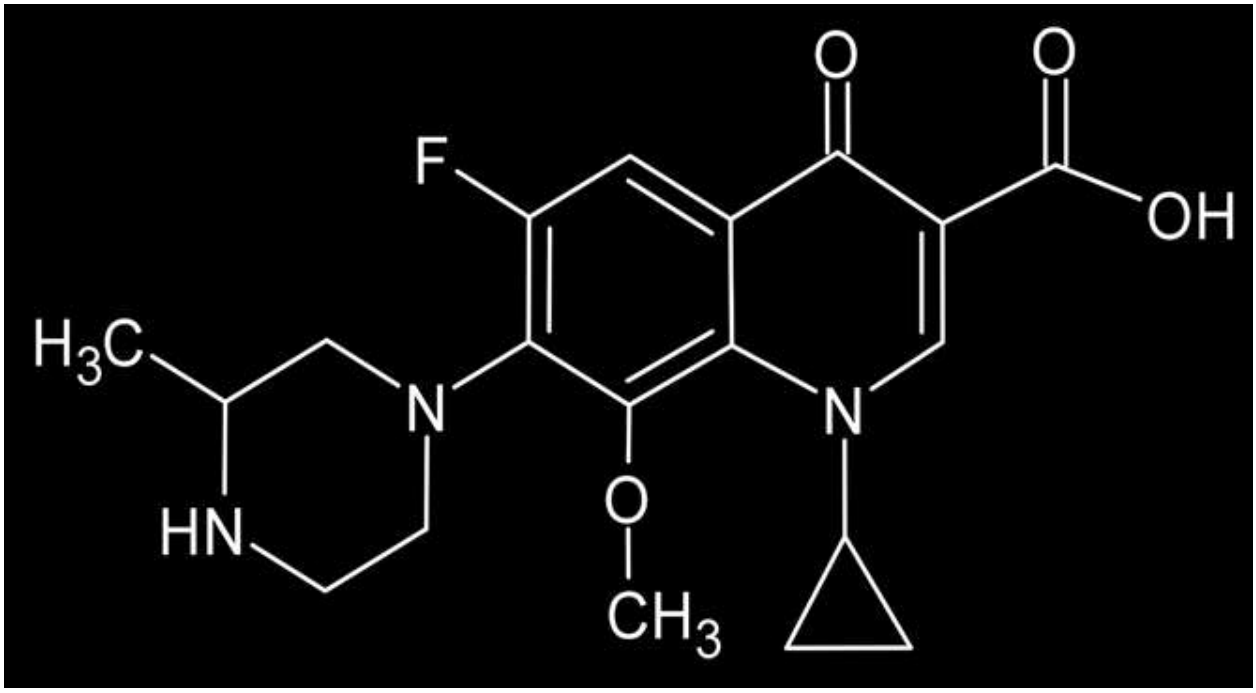

Figure 1: The composition of gatifloxacin

2. Proposed Model and materials

2.1. Materials

LKT Laboratories, Inc. provided the gatifloxacin (St. Paul, MN). Gatifloxacin was solubilized in Dulbecco's modified essential medium (Gibco, Grand Island, NY) for the cell proliferation experiment and utilised in the culture media outlined below. Gatifloxacin was solubilized in the broth as indicated below for the liquid culture of bacteria in order to measure the minimum inhibitory concentration (MIC).

2.2. Conditions for Cultivation and Bacteria

P. gulae ATCC 51700 was collected from the American Type Culture Collection in Manassas, Virginia, and cultured on plates with tryptic soy agar (40 g) for plate culture.

- 10% defibrinated horse blood (Japan Bio serum Corp., Tokyo, Japan), hemin (5 g), and L-1; Becton, Dickinson and Company, Franklin Lakes, NJ) supplements
- L1; Sigma-Aldrich Corporation, St. Louis, MO) and menadione (0.5 g).
- Sigma-Aldrich Corporation, L-1.
- Preculture was carried out at 37°C in an anaerobic chamber with the following air ratios: N2, H2, and CO2. The bacteria were cultivated in trypticase soy broth (30 g) for liquid culture.
- 5 g of supplement (L1; Becton, Dickinson and Company)
- Hemin L1 and 0.5 g
- L1 of menadione and incubated at 37 °C in anaerobic conditions. In the same manner as indicated for the plate culture, the precultured colony was injected into a liquid broth and incubated for 2 to 4 days.

2.3. A Minimal Inhibitory Concentration Assessment

MIC was calculated using gatifloxacin-containing broths. For P. gulae cultures, eleven serial gatifloxacin concentrations ranging from 0 M to 1 M were predetermined. The strain was added to the broth and then incubated for two to three days at 37 degrees Celsius in an anaerobic environment. The smallest amount of gatifloxacin needed to prevent bacteria from growing visibly after incubation is known as the MIC. The trials were carried out four times to ensure the accuracy of the results.

2.4. Gatifloxacin's antibacterial activity against canine periodontopathic bacteria

In the broth mentioned above, the P. gulae strain was cultivated anaerobically at 37 °C until it reached the early stationary phase. After being inoculated in a freshly made aseptic broth with adequate amounts of gatifloxacin at 37°C, the collected cells were tested for bacterial cell viability after two to three days. Using the BacTiter-Glo Microbial Cell Viability Assay kit, the adenosine triphosphate bioluminescence (ATP) test was used to measure the vitality of bacterial cells (Promega, Madison, USA). Each bacterial solution received an identical volume of BacTiter-Glo reagent, which was then added and quickly mixed. The Gene Light Model GL-210A luminometer was then used to measure the solution's luminescence (Microtec Co., Ltd.,

Funabashi, Japan). As a ratio to the value at the beginning of incubation, the value acquired was stated. The mean and standard deviation of the three experiments were used to express the results.

2.5. Test of Mammalian Cell Viability

The study employed rat BMMS cells from Sigma-Aldrich in Tokyo, Japan. The culture media was a modified version of Minimum Essential Medium (MEM) from Thermo Fisher Scientific in Waltham, Massachusetts, along with antibiotics and 10% foetal bovine serum (FBS) of French provenance from Biowest in Nuaillé, France. At a concentration of 5.0 104 cells per well, cells from a subculture were suspended in MEM and incubated for 24 hours at 37 °C in a humidified environment with 5% CO2. The original medium was changed out with one that included gatifloxacin at concentrations that were controlled by progressive dilution. Every two days, the culture medium was updated. Cell viability was assessed three days after the culture was finished using a Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan). A 10-L aliquot of the CCK-8 solution was swiftly added to every well. 90 minutes were then spent incubating the cells. Using a microplate reader, absorbance was measured at 450 nm (iMark Microplate Reader, Bio-Rad, CA, USA). The tests were carried out three times. The information was presented as survival rates.

2.6. Analytical Statistics

SPSS Statistics version 25 was used to conduct the statistical analysis (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) was used to assess the data, and probability (p) values 0.05 were regarded as statistically significant after the Tukey's multiple comparison test.

3. Results and Discussion

3.1. Gatifloxacin's Minimum Inducible Concentration for Canine Periodontopathic Bacteria

According to Table 1, gatifloxacin significantly slowed the development of the tested canine periodontopathic bacteria strain. P. gulae's MIC was 50 nM. These results imply that gatifloxacin inhibits the development of canine periodontopathic bacteria specifically. Although it might be difficult to tell whether a cell is alive or dead, the turbidity of the bacterial fluid is used to determine the drug's MIC value. Discussions based on statistics are not acceptable in this situation. Our results were compared to those of a previous investigation on the inhibitory effects of the antibiotic against P. gingivalis, a genetically related human periodontopathic bacterium, and it was found that the MICs obtained in both studies were comparable.

3.2. Gatifloxacin's inhibitory effect on Porphyromonas gulae

Its bactericidal activity was evaluated to further study gatifloxacin's ability to reduce the growth of periodontopathic bacteria. According to Figure 2, gatifloxacin demonstrated concentration-dependent bactericidal efficacy against the tested microorganisms. The fluoroquinolone's interaction with the reagent caused the apparent increase from 0.25 nM to 5 nM, and the turbidity, as indicated in Table 1, was the same as the control (0 nM). The fluoroquinolone medication was thought to have no bactericidal activity within this concentration range. A very significant difference in the inhibitory activity was found between cultures with high drug concentrations and the control culture without the drug using ANOVA and Tukey's multiple comparison test. When the bacteria were exposed to gatifloxacin doses more than 100 nM, the number of viable bacterial cells drastically reduced (p 0.01). This information suggests that gatifloxacin exposure may decrease bacterial growth during incubation, suggesting that the medication may limit the growth of the canine periodontopathogen P. gulae. According to reports, this medication works by preventing bacterial DNA gyrase and topoisomerase IV from functioning. This medication is thought to affect bacterial growth negatively against the strains examined in this study through a similar method.

Current professional dental treatments for persistent canine periodontitis include surgical scaling and general anaesthesia. The findings of this study confirm the efficacy of gatifloxacin local injection as a nonsurgical treatment.

Table 1 shows the impact of gatifloxacin on the development of the periodontopathic bacterium P. gulae ATCC 51700 as well as the reagent's minimum inhibitory concentration (MIC). The condition of bacterial growth is represented by each mathematical symbol. ++: highly developed; +: limited growth; and : no evidence of bacterial suspension

Bacterial stain	0 M	0.25 nM	0.75 nM	1.25 nM	2.5 nM	5 nM	50 nM	100 nM –	250 nM –	500 nM –	750 nM –	1 μM	MIC

P. gulae ATCC51700	++	++	++	++	++	++	+					−	50 nM

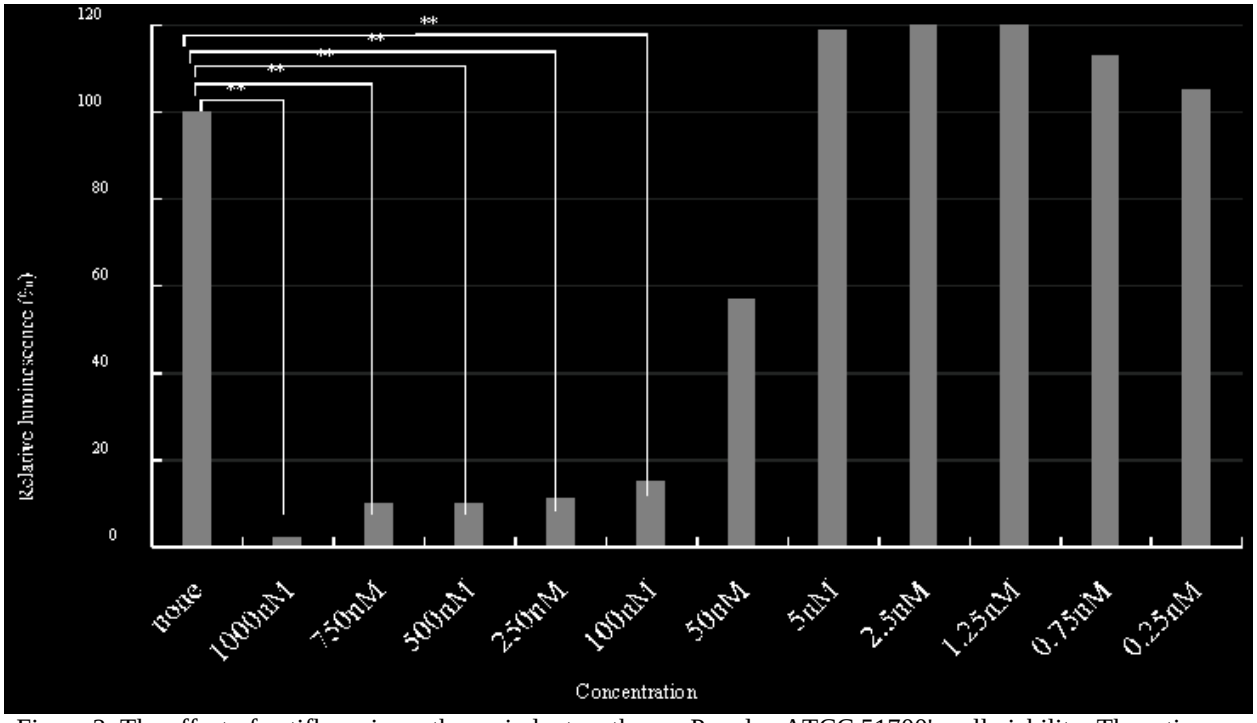

Figure 2: The effect of gatifloxacin on the periodontopathogen P. gulae ATCC 51700's cell viability. The ratio of viable cell numbers after a 2-day incubation to starting cell counts at 0 M of gatifloxacin is displayed in bars. With a bar, standard deviation is represented. A value of p 0.01, which was regarded as statistically significant, is indicated by an asterisk (**).

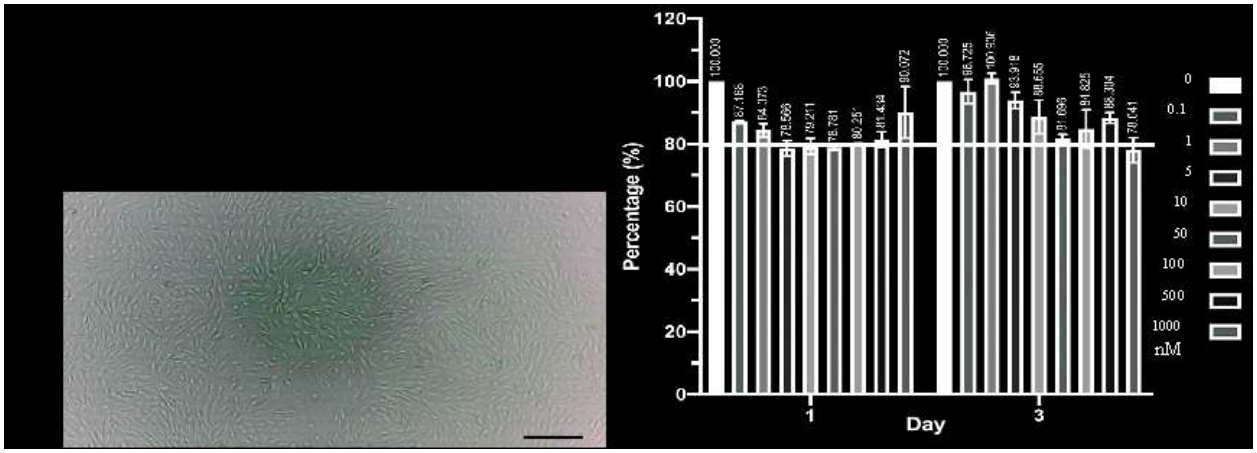

Figure 3 displays the survival rate of rat BMMS cells at each concentration of gatifloxacin after 1-day and 3-day incubations in controls (0 M), which was regarded as 100%. (a) Microscopical pictures of rat BMMS cells cultured for three days in media containing gatifloxacin. Scale bars indicate 50 m. (b) Rat BMMS cell growth following a one-day culture. (c) Rat BMMS cell growth following a 3-day culture.

3.3. Mammalian Cells: Mammalian Cell Viability Assay

Gatifloxacin had no morphological impact on the proliferation of mammalian BMMS cells after a 3-day culture, as illustrated in Figure 3(a). At 100 nM, which is a dose greater than the MIC of the bacteria studied, nearly all implanted BMMS cells survived after a 1-day culture (Figure 3(b)), and 84.8% of the cells survived following a 3-day culture. Gatifloxacin can be used on animals other than dogs since it is safe for mammalian cells after a one-day culture. It is believed to be a medication that can therapeutically inhibit the growth of drug-resistant bacteria in tests of both Pneumococcal and

Haemophilus strains (unpublished results from study of Kyorin Pharmaceutical Co. Ltd.). This is thought to be because DNA gyrase and topoisomerase IV are both being inhibited simultaneously. The results of the toxicity test utilising common dermal fibroblast cells obtained from adult human connective tissue significantly corroborated the results of the gatifloxacin cell viability experiment in rat cells.

4. Conclusion

According to the findings of the tests for antibacterial activity and cell viability, gatifloxacin had bactericidal effects on P. gulae at low doses but had no negative effects on mammalian cells. At doses over 100 nM, the medication also had bactericidal effects on P. gulae, and most of the mammalian cells survived the 3-day culture period. This shows that this medication would be risk-free at a MIC, particularly when administered locally for a brief period of time. According to the study's findings, gatifloxacin had an inhibitory impact on the bacteria that cause canine periodontal disease. Future research must assess gatifloxacin's effectiveness against other canine periodontal disease-causing bacteria. The canine symbiotic microbiota should also be examined for gatifloxacin's efficacy. However, more research is required to understand the characteristics of this locally delivered medication and to evaluate the sustained release complexes developed in our lab. We want to contribute to the treatment of severe periodontal disease in dogs and other pets by creating a defence against peri-implantitis and using dental material technology in veterinary medicine. It is anticipated that using gatifloxacin to treat canine periodontal disorders would be extremely beneficial and progress the field of canine oral hygiene.

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