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## Pharmacokinetic of Sulfisoxazole in Aqueous Humor after Topical and Subconjunctival Application in Dogs

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**Abstract:** This study was undertaken to evaluate the ocular pharmacokinetic of sulfisoxazole. Male, 2-3 years old 10 mix breed dogs weighing 12-15 kg were used. A 2 mg dose/eye of sulfisoxazole was administered to the animals by either subconjunctivally or by topically. Samples of aqueous humor were collected after 0.083, 0.25, 0.5, 1, 2, 4, 6, 24 and 72 h and level of sulfisoxazole was determined. Pharmacokinetic parameters including absorption rate constant ( $k_a$ ), slope factor ( $\beta$ ), absorption half-life ( $t_{1/2a}$ ), half-life of elimination in aqueous humor ( $t_{1/2\beta}$ ), maximal concentration in aqueous humor ( $C_{max}$ ), time to reach  $C_{max}$  ( $t_{max}$ ), mean residence time in aqueous humor (MRT) and area under the concentration time curve from zero up to  $\infty$  ( $AUC_{0-\infty}$ ) were calculated. Compared to topical application, value of  $k_a$  in subconjunctival application increased while values of  $t_{1/2a}$  and  $t_{max}$  decreased and the value of  $t_{1/2\beta}$  prolonged ( $p < 0.05$ ). There was no significant difference between groups regarding other parameters ( $p > 0.05$ ). These results indicate that sulfisoxazole may not be potent enough to treat intraocular infections caused by bacteria when applied either subconjunctivally or topically at a dose of 2 mg/eye. Furthermore, subconjunctival application of sulfisoxazole could be more efficient for treatment of intraocular infections due to higher absorption of drug and longer remaining time in the eye compared to topical application.

**Key words:** Sulfisoxazole, topical, subconjunctival, ocular, pharmacokinetic, dog

### INTRODUCTION

Sulfonamides are the first effective chemotherapeutic antimicrobial drug that was used to treat bacterial infections (Perez-Trallero and Iglesias, 2003). They are bacteriostatics and act by antagonizing with dihydrofolate reductase activity (Richards *et al.*, 1996; Kaya, 2000). Sulfisoxazole was applied in ocular disease (Nema *et al.*, 1965). Their spectrum of activity includes genus of *Staphylococcus*, *Pseudomonas* and *Escherichia* (Anonymous, 2004; Eloff, 1998). The eye flora of dogs contains most of bacteria from *Staphylococcus*, *Streptococcus* genusses and *Pseudomonaceae* and *Enterobacteria* families rather than the others. These bacteria may cause infection under some conditions (Baeyens *et al.*, 2002; Gerding *et al.*, 1988; Murphy *et al.*, 1978; Whitley, 2000). The present study was designed to evaluate the ocular pharmacokinetic of sulfisoxazole at specified dose.

### MATERIALS AND METHODS

**Animals:** In the present study, a total of 10 male, 2-3 years old mix breed dogs weighing 12-15 kg were used. The animals were divided into two groups (5 animals each groups). The dose of sulfisoxazole was 2 mg/eye (Gansol 4%<sup>®</sup>) per animal. The dose was administered by topically and subconjunctivally in the volume of 50  $\mu$ L. Following the administration of the sulfisoxazole, aqueous humor was collected at the hours of 0.083, 0.25, 0.5, 1, 2, 4, 6, 24 and 72 with an amount of 20  $\mu$ L. Prior to this each collection, local anesthetic gout in the dose of 15  $\mu$ L/eye (Alcaine 0.5%<sup>®</sup>) with no adrenaline and antibiotic were used to right eye. Aqueous humor was collected from the anterior chamber of the eye with a slight insertion of needle in the application hours mentioned previously. In the both groups, right eye was chosen for the drug application and collection of the samples. The samples were kept in the -20°C. At the end of the study, each of

the dogs in groups was treated by the antibiotic with the maximal care of not causing ocular damage and seconder infection. Dogs were feed with dog pellet food and water and feed were given *ad libitum*.

**Drug analysis:** Collected samples were kept as frozen until analyzed. The analyses were made by spectrophotometrical method as described by Hammond (1977), which is based on Bratton and Marshall (1939). According to this method 0.2 mL deionised water was added to 0.02 mL of aqueous humor in a standard experimental tube. Afterwards 0.4 mL of trichloroacetic acid (10%) was added to this mixture and stirred for a minute. After 5 min of incubation, the mixture was centrifuged (3000 rpm) for 10 min. The supernatant (0.5 mL) was forwarded to another tube and 0.1 mL of sodium nitrite (0.1%) solution was added, stirred and incubated for 3 min. After the incubation, 0.1 mL of ammonium sulphomate solution (0.5%) was added, stirred and incubated for 2 min. Afterwards 0.3 mL of N-(1-naphthyl)-ethylenediamine dihydrochloride solution (0.1%) was added to this mixture, stirred and incubated for another 5 min in the dark. After the incubation the absorbance of the mixture was measured spectrophotometrically with a use of microcuvette in a wavelength of 545 nm.

**Pharmacokinetic analysis:** The PKCALC pharmacokinetic program that consisted of the formularies of Shumaker (1986) was used to make pharmacokinetic analysis for absorption rate constant ( $k_a$ ), slope factor ( $\beta$ ), absorption half-life ( $t_{1/2a}$ ), half-life of elimination in aqueous humor ( $t_{1/2\beta}$ ), maximal concentration in aqueous humor after topical and subconjunctival administration ( $C_{max}$ ), time needed to reach  $C_{max}$  in aqueous humor ( $t_{max}$ ), mean residence time in aqueous humor (MRT) and area under the concentration time curve from zero up to  $\infty$  ( $AUC_{0-\infty}$ ).

**Statistical analysis:** The statistical evaluations were performed by SPSS 11.0 for windows pocket programme. The data were provided as arithmetic means and standard deviations. The differences between groups were detected by Mann-Whitney U-test.

**RESULTS**

There were significant ( $p < 0.05$ ) differences in  $k_a$ ,  $t_{1/2a}$ ,  $t_{1/2\beta}$  and  $t_{max}$  between the topical and subconjunctival applications. While the values of  $k_a$  increased,  $t_{max}$  value shortened for subconjunctival applications. On the other hand, the value of  $t_{1/2a}$  diminished in subconjunctival

Table 1: Ocular pharmacokinetic parameters of sulfisoxazole (m±ds) studied in dogs with topical application (Group 1) and in dogs with subconjunctival application (Group 2)

Pharmacokinetic parameters**	Group 1 (n: 5) topical application	Group 2 (n: 5) subconjunctival application
$k_a$ ( $h^{-1}$ )	1.10±0.21	7.13±4.02*
$\beta$ ( $h^{-1}$ )	0.019±0.002	0.015±0.002
$t_{1/2a}$ (h)	0.64±0.12	0.11±0.06*
$t_{1/2\beta}$ (h)	36.43±4.92	44.47±6.84*
$C_{max}$ ( $\mu g mL^{-1}$ )	3.14±1.90	3.74±1.66
$t_{max}$ (h)	2.10±1.24	0.43±0.12*
MRT (h)	47.76±8.60	58.21±11.30
$AUC_{0-\infty}$ ( $\mu g mL^{-1} h^{-1}$ )	54.45±12.42	83.50±45.84

\* $p < 0.05$ , \*\* $k_a$ : Absorption rate constant;  $\beta$ : Slope factor;  $t_{1/2a}$ : Absorption half-life;  $t_{1/2\beta}$ : Half-life of elimination in aqueous humor;  $C_{max}$ : Maximal concentration in aqueous humor after topical and subconjunctival administration;  $t_{max}$ : Time needed to reach  $C_{max}$  in aqueous humor; MRT: Mean residence time in aqueous humor;  $AUC_{0-\infty}$ : Area under the concentration time curve from zero up to  $\infty$

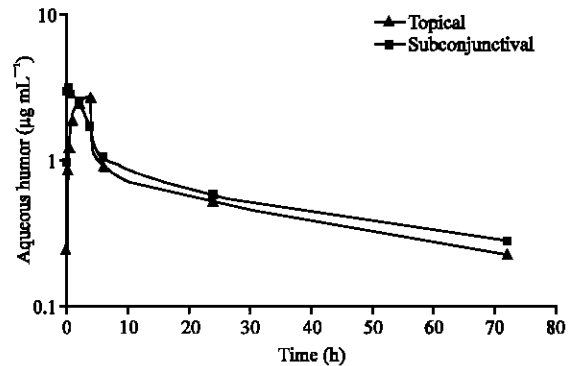


Fig. 1: Intraocular disposition kinetic of single dose sulfisoxazole following topical and subconjunctival application in dogs

application and the value of  $t_{1/2\beta}$  more prolonged (Group 2) compared to the topical application (Group 1). Although the values of MRT more prolonged  $C_{max}$  arisen higher and  $\beta$  more decrease and  $AUC_{0-\infty}$  more enlarged compared to topical application (Table 1). These differences were not significant between the two types of applications. It is possible to expect these parametrial alterations by using aqueous humor of drug-time curve (Fig. 1).

**DISCUSSION**

In the present study, sulfisoxazole evaluated whether it is effective at the specified dose (2 mg/eye) for microorganisms caused to ocular infection. During the study, the aqueous humor taken in the regular periods and their sulfisoxazole analysis were done. Consequently the pharmacokinetic calculations, done by the determination coefficient of curve showed that the drug was distributed according to two compartment model. Therefore, the pharmacokinetic calculations were based on the two compartment model. During the study,

the aqueous humor was taken in the regular periods and their sulfisoxazole analysis was performed. The aqueous humor-time plot of drug indicated that concentration of the drug rapidly reached the maximum level of  $3.74 \pm 1.66 \mu\text{g mL}^{-1}$  in  $0.43 \pm 0.12$  h when used subconjunctivally. The decrease of maximum concentration was slower which  $0.27 \mu\text{g mL}^{-1}$  in 72 h was. In topical application, the maximum concentration was  $3.14 \pm 1.90 \mu\text{g mL}^{-1}$  and reached in  $2.10 \pm 1.24$  h, subsequently, followed by a decline to  $0.22 \mu\text{g mL}^{-1}$  in 72 h. *Staphylococcus* spp. are dominant part of the ocular flora of dogs (Baeyens *et al.*, 2002; Gerding *et al.*, 1988). The MIC<sub>90</sub> value of sulfisoxazole was 24-34  $\mu\text{g mL}^{-1}$  for this bacterium (*S. aureus*, ATCC 25923) (Anonymous, 2004). The value of MIC<sub>90</sub> is 125  $\mu\text{g mL}^{-1}$  for *Pseudomonas aeruginosa* which also involves in ocular flora and not specifically sensitive to this chemotherapeutic (Eloff, 1998). However, the concentration of sulfonamide must generally be in the range of 80-100  $\mu\text{g mL}^{-1}$  in order to be effective against bacteria (Banerjee *et al.*, 1974). In other words, the sulfisoxazole could not be effective at 2 mg/eye dose for ocular infections because the highest concentration in eye obtained in subconjunctival application was  $3.74 \pm 1.66 \mu\text{g mL}^{-1}$ . This level was very low compared to the effective intraocular concentration for eliminating bacteria. On the other hand, it should be noted that in case of superficial ocular infection, the topical application achieves much higher concentration in the surface of eye rather than inside of it, therefore, it could be effective for bacteria. The results revealed that the subconjunctival application of drug is usefully. Thus, the absorption half-life of the drug was shortened in subconjunctival application compared to topical application. This argument is supported by the higher value of  $k_a$ . In addition, lower  $t_{\text{max}}$  found in subconjunctival application,  $C_{\text{max}}$  was higher. It indicates that the absorption of drug was much more rapid in subconjunctival application. However,  $t_{1/2\beta}$  and MRT of drug that were penetrated into aqueous humor is much prolonged compared to topical application. This situation proved that the intraocular duration of the drug was longer in the subconjunctival application. Since the lack of similar pharmacokinetic studies performed with similar sulfonamides the present study is not to be able to compare with the result of other studies.

Overall, there is no promising result to use sulfisoxazole in intra-ocular infection at a 2 mg/eye dose. Therefore, it should be given at higher doses, topically or subconjunctivally in the treatment of intra-ocular infections. It should be applied for the superficial infections of eye because the sulfisoxazole reaches very high concentrations in eye surface, following topical application. On the other hand, subconjunctival

application for this drug is more advantageous in the potential bacterial intraocular infections in dogs if the application dose kept in much higher.

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