# Ocular availability of gentamicin in small animals after topical administration of a conventional eye drop solution and a novel long acting bioadhesive ophthalmic drug insert

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# Ocular Availability of Gentamicin in Small Animals After Topical Administration of a Conventional Eye Drop Solution and a Novel Long Acting Bioadhesive Ophthalmic Drug Insert

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***Purpose.*** Gentamicin eye drop solutions have a short precorneal residence time. The present study investigates the effect of gentamicin using a new long acting delivery Bioadhesive Ophthalmic Insert (BODI) in healthy dogs and rabbits and compares the results with a conventional regimen using an eye drop solution.

***Methods.*** In vivo assays were performed on animals after deposition of one BODI and instillations of an eye drop solution. Tear samples were collected over 72 hours and 60 minutes, in the case of inserts and eye drop solution respectively. The gentamicin concentration profiles in tear fluid (determined by a fluorescent polarization immunoassay technique) was individually analyzed, in each animal, in relation with the minimum inhibitory concentration observed in vitro against some bacteria. A non classical pharmacokinetic approach was used for the analysis of the topically applied drug substance, involving two parameters: the efficacy area under the curve ($AUC_{eff}$) and the efficacy time ($t_{eff}$).

***Results.*** In the case of the eye drop solution, the $AUC_{eff}$ were higher in dogs ($2.80\ 10^3 - 3.64\ 10^3$ [$\mu g\ ml^{-1}\ h$]) than in rabbits ($0.64 \cdot 10^3 - 0.95 \cdot 10^3$ [$\mu g\ ml^{-1}\ h$]); the $t_{eff}$ had a similar behavior: 6-15 [h] in dogs and 2-6 [h] in rabbits. In the case of BODIs, the $AUC_{eff}$ and the $t_{eff}$ were quite similar between dogs and rabbits: $190\ 10^3 - 205\ 10^3$ [$\mu g\ ml^{-1}\ h$] and 70-76 [h], respectively. The $AUC_{eff}$ and the $t_{eff}$ were always much higher in the case of BODIs than for the eye drop solution both in dogs and rabbits.

***Conclusions.*** This study shows that topical administration of gentamicin using BODIs can improve treatment due to the decreasing number of applications while ensuring an effective level of antibiotic in tears controlled by the device.

KEY WORDS: pharmacokinetic; ocular availability; long-acting drug delivery; ophthalmic delivery; gentamicin; insert.

## INTRODUCTION

Ocular drugs are usually applied as aqueous eye drop solutions and one of the significant problems encountered with the administration of ophthalmic drops is that the drug release is pulsed, with a short initial period of overdosing followed by a long period of underdosing [1]. This poor topical availability is mainly due to the rapid precorneal elimination, conjunctival absorption and solution drainage by gravity induced lacrimal flow, blinking and normal turnover [2,3]. Thus current therapeutic regimens for severe external infections require repeated instillations of an antibiotic to maintain the drug concentration in the tear film at a satisfactory concentration [4]. In the case of gentamicin, a widely used antibiotic in ophthalmology because of its broad spectrum against bacteria such as *Pseudomonas* and *Staphylococcus* [5], 1 or 2 drops of eye drop solution from 3 up to 6 times per day are recommended. However, it has been shown that instillation of a high volume does not contribute to the increase of its topical availability because the rate of elimination of the drug solution is extremely rapid and proportional to the instilled volume [6,7]. Moreover multiple applications increase ocular and systemic side effects [8].

The information available suggests that solid ophthalmic dosage forms are more effective, require less frequent administration, avoid pulsed release and diminish the number of additives needed; these solid forms are usually named ophthalmic inserts [9]. We have developed a long acting soluble Bioadhesive Ophthalmic Drug Insert (BODI) which presents the considerable advantage of being entirely soluble and therefore there is no need to remove the insert from its site of application. Besides limiting manipulation to insertion only [9], the BODI concept offers other advantages [10-11]: a good tolerance, a decrease in insert expulsion, a prolonged residence time in the eye and a prolonged controlled drug release.

The purpose of this study was: (i) to measure the concentrations in tears after deposition of a BODI and administration of a commercially available eye drop solution releasing gentamicin in dogs' and rabbits' eyes, (ii) to compare the two release profiles, (iii) to evaluate the efficacy area under the curve of lacrimal fluid concentrations versus time ($AUC_{eff}$) after deposition of one BODI and 9 drops of solution (corresponding to an often used dosage schedule in veterinary medicine which is 3 instillations per day during 3 days), (iv) to evaluate the time of efficacy ($t_{eff}$) from BODI and eye drop solution and (v) to establish possible correlation between the two animal species for BODIs and eye drop solution.

## MATERIALS AND METHODS

*Materials.* The following materials were used as received: hydroxypropylcellulose (HPC, Klucel® HXF NF, Aqualon™, Wilmington, USA), ethylcellulose (EC, Ethocel® N-50 NF, Hercules™, Wilmington, USA), carbomer (CP, Carbopol® 934 P, Goodrich™, Cleveland, USA), a solid dispersion composed of cellulose acetate phthalate (CAP, Fluka™, Buchs, Switzerland) and gentamicin sulfate (GS, Medimpex™, Paris, France). The eye drop solution is a commercial eye drop solution having a drug content of 0.3% (Garamycin®, Essex™ Chemie, Lucerne, Switzerland).

*Preparation of the BODIs.* As previously reported [11], the BODIs were obtained by extruding an optimized dried powder mixture containing: HPC (40.2%), EC (18.0%), CP

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(1.8%) and a solid dispersion composed of CAP (15.0%) and GS (25.0%).

*Animals*. New Zealand white rabbits (3-4 kg) and beagles (14-18 kg) of either sex are used for these studies concerning the evolution of gentamicin concentrations. All in vivo experiments were conformed to the ARVO Resolution on the Use of Animals in Research and were approved by the local Ethics Committees for animal experimentation.

*Sampling of Tears*. Tear samples were collected from rabbits (2 µl) and dogs (4 µl) without local anesthesia using micro-capillaries (microcaps Drummond®, Thomas Scientific™, Swedesboro, USA) as previously described [11].

*Experimental Procedure*. All the in vivo assays were performed, on unanesthetized animals, after insertion of a single insert in the inferior lateral sulcus or instillation of one drop of solution. In the case of BODIs, the evolution of gentamicin concentration was monitored over 72 hours with tear samples being taken every 6 hours. In the case of the eye drop solution, the time-concentration profile was followed over 60 minutes with tear sampling at 5, 10, 15, 20, 30, 45 and 60 minutes after instillation.

*Dosage of Gentamicin*. The analyses of gentamicin in tears were performed using an apparatus (TDx® System Analyzer Abbott™ Laboratories, Dallas, USA) applying fluorescent polarization immunoassay (FPIA) [12-14] as described by Gurtler et al. [11].

*Data Analysis*. The concentrations-time curves were established individually for each assay. The minimum inhibitory concentrations MIC 90% ranging from 3.12-100 [µg/ml] were used for the pharmokinetic analysis. These MICs 90% correspond to those observed in vitro against common groups of pathogenic bacteria [15].

The following two pharmacokinetic parameters were used for the analysis of the topically applied drug substance: the efficacy area under the curve ($AUC_{eff}$), where $AUC_{eff}$ corresponds to the surface between a given MIC 90% and the in vivo gentamicin time concentration profile and the time of efficacy ($t_{eff}$), where $t_{eff}$ corresponds to the time span above the considered MIC 90%.

The $AUC_{eff}$ and $t_{eff}$ were calculated individually, after deposition of one BODI or after instillation of 9 drops of solution simulating the conventional dosage regimen of 3 instillations of one drop over 3 days. Calculation was performed using a spreadsheet (Excel® 5.0, Microsoft™ Corporation, Seattle, USA) where $AUC_{eff}$ was estimated by the trapezoidal rule. The initial part of the curve was considered by extrapolation from the first data point to time zero, where concentration was supposed to be zero. The final part of the curve was extrapolated according to the segment between the last two data points, only if the corresponding slope was negative, i.e. a decrease with time was observed. For the test of the concentration versus time profile, interpolation was performed if measured concentrations fell below MIC 90% values.

Comparison between dogs and rabbits was performed for the lowest reported MIC 90% (3.12 [µg/ml]) using the non parametric Wilcoxon-Mann-Whitney test. This test allows to evaluate two independent groups (dogs and rabbits), after deposition of one BODI or instillations of 9 drops of solution.

## RESULTS

The mean gentamicin concentrations measured by the FPIA method, as function of time after instillation of one drop of solution and deposition of one BODI are shown in Figure 1.

The release profiles obtained after deposition of one BODI are quite similar in dogs and rabbits. Both profiles exhibit two distinct periods: an initial period lasting 48

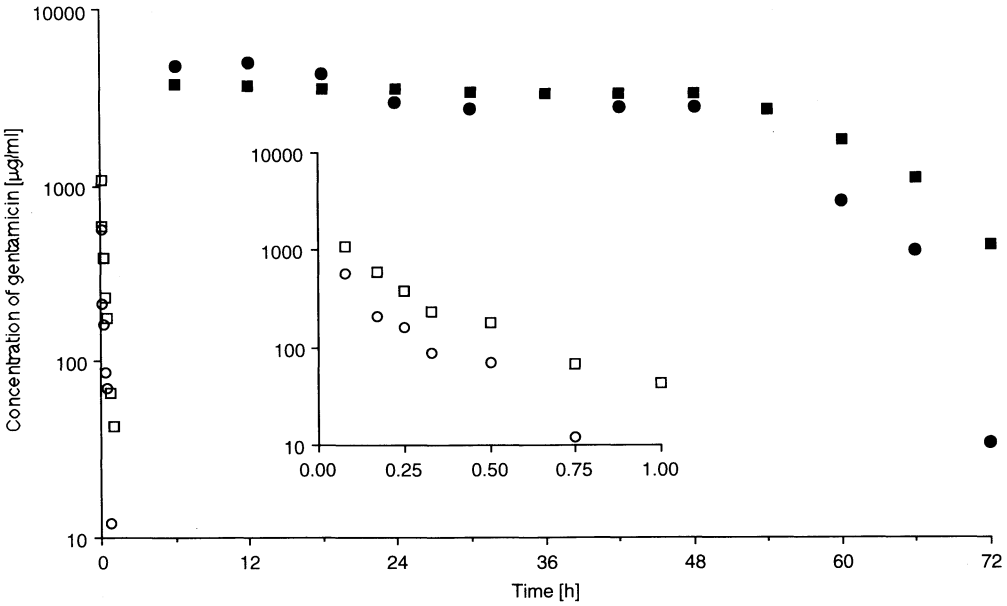


Fig. 1. Mean tear concentration of gentamicin in beagles (squares) and New Zealand white rabbits (circles) as function of time after instillation of one drop of eye drop solution (open squares and open circles) and after deposition of one BODI (solid squares and solid circles). For the BODIs: n = 8 and n = 12 and for the eye drop solution, n = 6-12 and n = 8-16 for dogs and rabbits respectively.

Table I. Efficacy Area Under the Curve ($AUC_{eff}$ ± Standard Deviation S.D.) in Tears of Healthy Beagles (n = 6–12) and New Zealand White Rabbits (n = 8–16) in Relation with Minimum Inhibitory Concentrations (MIC) after Instillation of One Drop of Collyrium Three Times Per Day During Three Days (9 Instillations) and After Deposition of Single Insert

| | Instillation of 9 drops collyrium | | Deposition of one insert | |
|---|---|---|---|---|
| | Dogs | Rabbits | Dogs | Rabbits |
| MIC [μg/ml] | $AUC_{eff} \cdot 10^3$ ± S.D. [μg · ml$^{-1}$ · h] | $AUC_{eff} \cdot 10^3$ ± S.D. [μg · ml$^{-1}$ · h] | $AUC_{eff} \cdot 10^3$ ± S.D. [μg · ml$^{-1}$ · h] | $AUC_{eff} \cdot 10^3$ ± S.D. [μg · ml$^{-1}$ · h] |
| 3.12 | 3.64 ± 2.72 | 0.95 ± 1.38 | 197 ± 10 | 205 ± 26 |
| 6.25 | 3.59 ± 2.72 | 0.93 ± 1.38 | 197 ± 10 | 205 ± 26 |
| 12.5 | 3.51 ± 2.73 | 0.90 ± 1.37 | 197 ± 10 | 205 ± 26 |
| 25.00 | 3.38 ± 2.71 | 0.84 ± 1.36 | 196 ± 10 | 204 ± 26 |
| 50.00 | 3.16 ± 2.64 | 0.76 ± 1.33 | 194 ± 10 | 202 ± 26 |
| 100.00 | 2.80 ± 2.51 | 0.64 ± 1.25 | 190 ± 10 | 198 ± 26 |

hours, characterized by a plateau (3550 ± 680 [μg ml$^{-1}$]), followed by a second period, in which the decrease of the release rate certainly corresponds to the exhaustion of the drug in the BODI. In the case of eye drop solution, a rapid loss of drug is noted after the instillation.

The coefficients of variation (CV) reflect the variability in drug concentration in tear samples. These CV values are always higher in rabbits than in dogs. The most important difference lies in the calculated CV values between instillation of one drop of solution and deposition of BODIs. For dogs and rabbits and in the case of the solution, over a 60 minutes period, the CV ranged from 63% to 96% and from 96% to 287%, respectively whereas, for BODIs, over a 48 hours period, the CV values ranged from 2% to 10% and 5% to 46%, respectively.

The efficacy areas under the curve ($AUC_{eff}$) calculated after instillation of 9 drops of solution and after deposition of one BODI are given in the Table I. The $AUC_{eff}$ obtained in dogs are always higher than those obtained in rabbits: the calculated ratios $AUC_{eff\ dogs}/AUC_{eff\ rabbits}$ range from 3.3 to 4.4, thus showing a difference between dogs and rabbits after instillation the same eye drop solution.

Concerning the $AUC_{eff}$ calculated after deposition of one BODI, this ratio is close to 1, suggesting that topical availability may be similar in dogs and rabbits.

The important differences of $AUC_{eff}$ (Table I) and $t_{eff}$ (Figure 2) between the two formulations show that: (i) the topical availability achieved with BODI is much higher than that measured with the eye drop solution, (ii) the BODIs ensure a time of efficacy of about 3 days for all of the MICs 90% considered while (iii) the eye drop solution provides a short $t_{eff}$ which rapidly diminishes with increasing MIC 90% values.

The nonparametric Wilcoxon-Mann-Whitney test has been used to determine if the release of gentamicin from BODIs is influenced by the animal species or if the release profile is an inherent characteristic of the BODIs. The results given in Table II show that there is no statistical difference in the case of BODIs. Conversely, the efficacy profile of the eye drop solution significantly differs between dogs and rabbits.

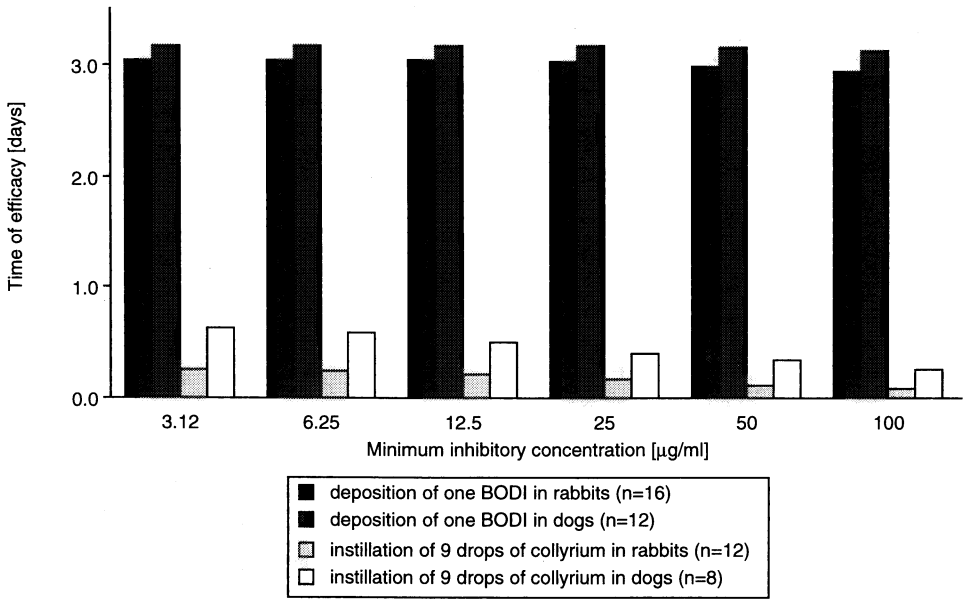


Fig. 2. Time of efficacy ($t_{eff}$) in dogs and rabbits after instillation of 9 drops of eye drop solution and after deposition of one BODI.

**Table II.** Statistical Analysis to Test for Differences between Two Independent Groups (Dogs and Rabbits) with Respect to $AUC_{eff}$ after Deposition of One BODI and Instillations of 9 Drops of Solution for a Minimum Inhibitory Concentration 90% of 3.12 [$\mu g \cdot ml^{-1}$]

| | $AUC_{eff} \cdot [\mu g \cdot ml^{-1} \cdot h]$ | Dogs | Rabbits | Significance |
|---|---|---|---|---|
| Solution | median | 2088 | 558 | |
| | minimum | 1314 | 180 | $p < 0.001$ |
| | maximum | 7461 | 5211 | |
| BODIs | median | 200377 | 205890 | |
| | minimum | 180418 | 152425 | no statistical difference |
| | maximum | 210589 | 254678 | |

## DISCUSSION

The measurement of gentamicin concentration in the lacrimal fluid of dogs and rabbits using the highly sensitive FPIA method enables to compare the topical availability of two formulations in dogs and rabbits. The concentration-time profiles have been individually analysed and the duration of efficacy as well as the efficacy area under the curve were calculated.

In the case of the solution, the high interindividual variability is explained by the variable amount of drug present in the conjunctival sulcus. In fact, the instillation of one drop, having a volume of 50 µl ± 5 µl does not mean that the entire volume will be present in the conjunctival sac. It has been observed that a part of the instilled drop flows out of the eye because the available volume of the conjunctival sac is usually smaller than 50 µl. Moreover, the volume of the inferior fornix is variable in each animal. The deposition of one insert allows a precise dosing of the drug introduced in the inferior fornix. In the case of an eye drop solution, the release profile is characterized by a rapid decrease in gentamicin concentration both in dogs and rabbits. This is certainly the result of the short contact time between the drug and the eye surface caused by the rapid dilution of the eye drop solution by the tear fluid and its elimination through the puncta into the nasolacrimal duct.

However, an interesting difference between dogs and rabbits appears when an eye drop solution is administered: the efficacy time is approx. 2.5 higher in the case of dogs than for rabbits. Efficacy areas under the curves confirm such a difference: they are about 4 times higher in dogs than in rabbits. This indicates that the treatment using an eye drop solution may be more effective with dogs than with rabbits.

The release profiles of the inserts are quite similar for dogs and rabbits and characterized by a two-phases profile: the gentamicin concentration exhibits a plateau during the first 48 hours and declines progressively over the following 24 hours corresponding to the exhaustion of the drug in the BODI. The efficacy areas under the curves and efficacy times are quite similar in dogs and rabbits. This indicates that the gentamicin release from BODIs is not influenced by the animal species but is inherent to the design of the drug delivery system.

The comparison of efficacy area under the curves and efficacy times after deposition of one BODI and instillations of 9 drops of solution, both in dogs and rabbits, shows that these $AUC_{eff}$ and $t_{eff}$ are always higher for BODIs than for the eye drop solution. In the case of the rabbits, the ratio $AUC_{eff\ BODI}$ to $AUC_{eff\ sol}$ ranged from 215 for the lowest MIC 90% (3.12 [µg/ml]) to 309 for the highest MIC 90% (100 [µg/ml]). In the case of dogs, this same ratio ranged from 54 to 68. Similarly, the corresponding ratio for $t_{eff}$ ranged from 12 to 37 in rabbits and from 5 to 12 in dogs. These results show that after deposition of one BODI, both in dogs and rabbits, the inserts ensure a prolonged release profile with little concentration variations of gentamicin as expressed by the CV values given at all time points compared to that obtained after instillation of an eye drop solution.

The investigation shows that topical ocular administration of gentamicin using BODIs can improve treatment due to the decreasing number of applications while ensuring an effective level of antibiotic in tears.

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