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mv@econ-pharma.com

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ECON AG – BERLIN Labs, Teltow[1]
Beuth University of Applied Science, Berlin[2]

Antibiotic plasma levels in dogs with Otitis externa treated routinely with various topical preparations

Antibiotikaspiegel im Plasma von Hunden mit Otits externa nach praxisüblicher Behandlung mit verschiedenen topikal zu verabreichenden Arzneimitteln

Michael Voget[1], Martin Armbruster[1], Michael Meyer[2]

Summary

We aimed to determine whether, and at what levels, topical antibiotics applied to treat Otitis externa in dogs are absorbed systemically, leading to an increased risk of antibiotic resistance. 75 dogs brought to a veterinarian for Otitis externa were recruited for a non-interventional study. Selection criteria included diagnosis of Otitis externa and owner consent. The animals were divided into five groups of 15 dogs each. Each group received one of five commonly prescribed topical medications for up to 14 days according to the labeled instructions. Development and validation of low residue detection methods (HPLC-MS/MS) for all active substances studied was performed. Plasma concentrations were evaluated for gentamicin (Otomax®, Easotic®), marbofloxacin (Aurizon®), orbifloxacin (Posatex®) and polymyxin B (Surolan®). Low-level plasma concentrations of the topically applied antibiotics were detected after multiple administrations. In several samples, the concentrations detected were less than the limit of detection (LOD) of the corresponding analytical method. However, at the end of the treatment period, mean plasma concentrations were in the low pmol/ml range and exceeded the LOD for gentamicin, marbofloxacin and orbifloxacin. None of the plasma samples examined for polymyxin showed levels above the LOD. After routine topical antibiotic use in the treatment of Otitis externa in dogs, low systemic plasma concentrations are likely to develop. This low-level exposure may facilitate cellular changes that lead to an increased possibility for antibiotic resistance. These findings should provoke veterinary clinicians to optimise therapy for Otitis externa in light of minimising the development of antibiotic resistance.

Keywords: epithelial penetration, otics, systemic availability, aminoglycosides, fluoroquinolones, polypeptides

Zusammenfassung

Unsere Untersuchung sollte feststellen, ob und in welchen Konzentrationen zur Behandlung von Otitis externa bei Hunden topikal verabreichte Antibiotika systemisch absorbiert und damit zu einer Erhöhung des Resistenzbildungsrisikos führen können. Für eine nicht interventionelle Studie wurden 75 Hunde rekrutiert, die wegen Otitis externa einen Tierarzt aufsuchten. Neben einem abgesicherten Befund Otits externa musste zur Aufnahme in die Studie auch die Einwilligung der Tierbesitzer zur Blutentnahme vorliegen. Die Tiere wurden in fünf Gruppen mit je 15 Tiere aufgeteilt. Jede Gruppe wurde mit einer der fünf am häufigsten angewandten topikalen Medikationen/Antibiotika, entsprechend der Gebrauchsinformationen bis zu 14 Tagen, behandelt. Für die entsprechenden Wirkstoffe erfolgte vorab die Entwicklung und Validierung spurenanalytischer Nachweismethoden (HPLC-MS-MS) um die Konzentrationen von Gentamicin (Otomax®, Easotic®), Marbofloxacin (Aurizon®), Orbifloxacin (Posatex®) und Polymyxin B (Surolan®) nach topikaler Behandlung im Plasma zu bestimmen. In einigen Proben lagen die gefundenen Wirkstoffspiegel unterhalb der Bestimmungsgrenze (LOD) der analytischen Methode. Am Ende des Behandlungszeitraumes lagen die durchschnittlichen Plasmaspiegel bei Gentamicin, Marbofloxacin und Orbifloxa-

cin im unteren pmol/ml Bereich oberhalb der Bestimmungsgrenze. In den auf Polymyxin B untersuchten Plasmaproben wurden keine Befunde oberhalb der Bestimmungsgrenze ermittelt. Die praxisübliche topikale Antibiotika-Therapie der Otitis externa bei Hunden ist folglich mit einer erhöhten Wahrscheinlichkeit verbunden, sehr niedrige antibiotische Plasmaspiegel hervorzurufen. Diese können in Bakterien die Resistenzbildung fördern. Die Ergebnisse dieser Arbeit sollten den behandelnden Tierarzt veranlassen, die Therapie der Otitis externa auch im Hinblick auf Resistenzbildungsrisiken zu entscheiden.

Schlüsselwörter: Epitheliale Penetration, (Otitis-Präparate), systemische Verfügbarkeit, Aminoglycoside, Fluoroquinolone, Polypeptide

Introduction

All species possess the capability to evolve. Therefore, the development of bacterial antibiotic resistance is, to a certain extent, a natural phenomenon. However, increasing reports of drug resistant pathogens suggest that the routine use of antibiotics may provoke this resistance (GERMAP, 2011). This upsurge of bacterial resistance makes it important to focus on the routine practises of veterinarians, which may potentially promote the development of resistance.

Low-level antibiotic concentrations due to partial curing of an infection, failed treatment compliance and poor permeability of body tissues and compartments may contribute to the emergence of resistance (Goldstein, 2007). Recent publications have increased understanding of the radical induced mutagenesis effects of antibiotic exposure below bactericidal concentrations (Kohanski et al., 2010). Active substances like quinolones and aminoglycosides provoke DNA damage by release of reactive oxygen species. In consequence, cell nuclear repair processes are escalated and may create other capabilities of the bacterium to react to its environment, leading to the acquisition of drug resistance (Dwyer et al., 2007, 2009; Kohanski, 2007, 2008).

Otitis externa is a disease frequently occurring in dogs (Lehner, 2009). Several topical products are approved and marketed for its treatment. These contain various antibiotic substances, namely aminoglycosides, quinolones and polypeptides. Even if the topically applied antibiotic dose exceeds the mutant prevention concentration (MPC) (Zhao and Drlika, 2001) at the site of administration, the concentration in adjacent tissues may fall below the MPC. Levels of antibiotics below the MPC are suspected to lead to bacterial resistance (van der Horst, 2011).

Plasma levels of topically applied drugs, if reported, are from studies performed only in healthy dogs (Summary of Product Characteristics: Aurizon®, Easotic®, Posatex®). There are no known reports describing plasma uptake of these drugs in diseased dogs. This study aimed to elucidate if, and at what rate, appropriate use of commonly prescribed otic topical drugs leads to detectable plasma concentrations of the antibiotics included in such medications. Two commonly prescribed products contain the aminoglycoside gentamicin, Otomax® (Intervet Deutschland GmbH, DE) and Easotic® (Virbac S.A., FR). Aurizon® (Vétoquinol GmbH, DE) and Posatex® (Schering Plough Ltd., UK)contain the quinolones marbofloxacin and orbifloxacin, respectively. Only Surolan® (Janssen-Cilag GmbH, DE) contains the polypeptide antibiotic polymyxin B.

Material and Methods

Study Design

The non-interventional study was conducted between October 2009 and September 2011. It focused on five medications authorised for use in dogs suffering from Otitis externa (Otomax®, Easotic®, Aurizon®, Posatex®, and Surolan®). Treatment was in accordance with label recommendations for each product and is described in Table 1.

Animals

Subjects were randomly recruited from patients under veterinary supervision because of Otitis externa acuta and without preference for breed, age, sex and bodyweight. Four companion animal practices were involved in the study. 15 dogs were included for each of the products, for a total of 75 dogs. Enrolment criteria included a diagnosis of Otitis extern aacuta and consent of the pet's owner to take the required blood samples. According to the protocol instructions only cases of acute otitis or recurrent cases (in acute phase) were included. Within this study Otitis externa was classifiedas suggested by the external clinical monitor (Sterchi, 1989):

- Otitis externa erythematosa
- Otitis externa ceruminosa
- Otitis externa squamosa
- Otitis externa ulcerosa

Treatment

According to national animal welfare laws, approval by an ethics committee was not required in this case, as all measures were part of the routine veterinary treatment. The consulted veterinarian administered the first treatment and instructed the pet owners to apply the medication according to the approved dosing instructions. Some of the products are registered with a standard dose, others with body weight depending doses (Tab. 1).

All test products were commercially available batches of the authorised medications concerned. Details including batch numbers and the recommendations for use given by the marketing authorisation holder are listed in Table 1.

Sampling

A batch design was chosen for plasma sampling, i. e. samples were taken more than once from each animal, but not at all time points. Out of the diagnosed patients 15 dogs were selected per single medical product and three subgroups with five animals each were built. Between these three groups blood sampling points were shifted from earlier to later to extend the time window

for each product (as outlined in Tab. 2). In detail, blood samples were taken from alternating subgroups between 1 and 72 h post dosing whereas a complete sampling was performed on the final visit. Serum was separated out and stored frozen at ≤ –18°C until chemical analysis. Sample preparation and analysis of all samples obtained from dogs within one product group were completed in one session.

Chemical analysis

Protein precipitation was performed with buffered organic solvents followed by centrifugation. Supernatants were isolated for high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) analysis. Chromatographic separations occurred at ambient temperature using a RP18 Phenomenex separation column with two buffer systems, buffer A (water and formic acid) and buffer B (acetonitrile and formic acid).

MS/MS Detector settings were ESI, positive polarity ionization, and desolvation temperature above 350°C. Mass spectra were recorded for each drug to determine the specific detection conditions of each molecule.

Stock solutions with marbofloxacin, orbifloxacin, gentamicin or polymyxin B were prepared in appropriate solvents and diluted to obtain working standard solutions. The solutions were refrigerated and protected from light. Stability tests of the stock solutions were performed regularly in parallel to the investigations of the plasma samples. Mean regression coefficient (r^2) was obtained above 0.998 for all calibration curves. Multiple assays were performed on aliquots of different concentrations. Mean recoveries were greater than 60%. Stability studies proved stock solution stability at auto-sampler conditions (15°C) over the relevant time span.

For all plasma samples gained from dogs in the Otomax® and Easotic® (gentamicin) as well as in the Surolan® (polymyxin B) groupsamikacin was used as internal standard. Norfloxacin was added as internal standard to all plasma samples gained from dogs in the Aurizon® (marbofloxacin) and the Posatex® (orbifloxacin) groups.

Calibration standards were prepared from diluted standard solutions and blank plasma with each analytical batch (i. e. on each validation day).

With each calibration curve, one blank sample ("blank") was analysed. The blank was not included in the calculation of the calibration curve.

Standards were analysed in the same way as samples. The results obtained for linear regression (r),

TABLE 1: *Summary of product characteristics*

Otomax®	
Active Ingredient (Name INN)	Gentamicin – Betamethasone valerate – Clotrimazole
Batch No.; Expiry date	APNA9186; 7/11
Marketing Authorisation Holder	Intervet Deutschland GmbH
Recommendations for use	Dogs < 15 kg 4 drops twice a day into each ear affected Dogs > 15 kg 8 drops twice a day into each ear affected Therapy to be continued for 7 days
Easotic®	
Active Ingredient (Name INN)	Gentamicinsulfate – MiconazolNitrate – Hydrocortisone aceponate
Batch No.; Expiry date	2USH; 2/12
Marketing Authorisation Holder	VIRBAC S.A.
Recommendations for use	1 ml per day into each ear affected Therapy to be continued for 5 days
Aurizon®	
Active Ingredient (Name INN)	Marbofloxacin – Clotrimazole – Dexamethasone acetate
Batch No.; Expiry date	9A1159B; 7/11
Marketing Authorisation Holder	Vétoquinol AG
Recommendations for use	10 drops once a day into each ear affected Therapy to be continued for 7 up to 14 days
Posatex®	
Active Ingredient (Name INN)	Orbifloxacin – Mometasonefuroate – Posaconazole
Batch No.; Expiry date	0530202; 31.01.2012
Marketing Authorisation Holder	Schering-Plough Animal Health Division of Schering Plough Ltd.
Recommendations for use	Dogs < 2 kg 2 drops once a day into each ear affected Dogs > 2 kg < 15 kg 4 drops once a day into each ear affected Dogs > 15 kg 8 drops once a day into each ear affected Therapy to be continued for 7 days
Surolan®	
Active Ingredient (Name INN)	Prednisolon acetate – Polymyxin-B-sulfate – Miconazole nitrate
Batch No.; Expiry date	Batch No. AGB0P00; date 6/12
Marketing Authorisation Holder	Janssen-Cilag GmbH
Recommendations for use	3 to 5 drops twice a day into each ear affected Therapy to be continued without interruption up to a few days after clinical symptoms have abated

TABLE 2: *Blood sampling points for each product*

Group	Trade name	Otomax®	Easotic®	Aurizon®	Posatex®	Surolan®
	Active substance	Gentamicin		Marbofloxacin	Orbifloxacin	Polymyxin B
I		1 h, 24 h, 7 d	1 h, 24 h, 5 d	1 h, 24 h, 7–14 d	1 h, 24h, 7–14 d*	1 h, 24 h, 7 d
II		2 h, 48 h, 7 d	2 h, 48 h, 5 d	2 h, 48 h, 7–14 d	2 h, 48 h, 7–14 d*	4 h, 48 h, 7 d
III		18 h, 72 h, 7 d	18 h, 72 h, 5 d	18 h, 72 h, 7–14 d	18 h, 72 h, 7–14 d*	8 h, 72 h, 7 d

* End of treatment.

limit of quantification (LOQ), limit of detection and recovery rates are summarised in Table 3.

TABLE 3: *Calibration data for marbofloxacin, orbifloxacin, gentamicin, polymyxin B*

	Marbofloxacin	Orbifloxacin	Gentamicin	Polymyxin B
Linear Regression (r)	1.0	0.99	1.0	0.99
Limit of quantification (LOQ)	1 ng/ml 2.76 pmol/ml	2.5 ng/ml 6.32 pmol/ml	200 ng/ml 420 pmol/ml	20.0 ng/ml 0.017 pmol/ml
Limit of detection (LOD)	0.01 ng/ml 0.0276 pmol/ml	0.1 ng/ml 0.253 pmol/ml	0.25 ng/ml 0.523 pmol/ml	5.0 ng/ml 4.15 pmol/ml
Recovery	60–95% for all APIs			

API = Active Pharmaceutical Ingredient.

Statistical methods

Plasma concentrations at the time points specified in Table 2 were summarised by number of samples (N), number of samples below the limit of detection ($N_{<LOD}$), maximum, median, mean and standard deviation (SD). For graphical representations and summary statistics, plasma concentrations below LOD were substituted by zero for all samples taken between 1 and 72 h. This conservative assumption was used to avoid a bias of the mean towards higher concentrations. A sensitivity analysis was performed to estimate the influence of this assumption. The larger sets of samples taken on the final visit (F) were analyzed using the Kaplan-Meier estimator for left-censored data in order to determine sample estimates without any substitution. In addition to the median plasma concentration, this non-parametric method enables the calculation of the mean plasma concentration with 95% confidence intervals (95%CI) from the cumulative distribution function derived from the censored samples. These calculations were performed using the library NADA (Helsel, 2005) for the R-package for statistical computing (R-Development Core Team, 2010). Taking into account the batch design of the experiment, areas under the curve (AUC) were calculated from the measured plasma concentrations using the library PK (Jaki and Wolfsegger, 2009, 2010). All confidence intervals of the mean AUC are based on the t-distribution taking into account the small sample size

TABLE 4: *Summary of baseline data*

Product-Group	Sub-Group	Weight-range (kg)	Type of Otitis externa (n)			
			ulcerosa	squamosa	ceruminosa	erytehmatosa
OTOMAX®	I	28–38	-	2	3	–
	II	16–35	2	1	2	–
	III	14–32	2	2	1	–
EASOTIC®	I	11–31	1	3	1	–
	II	10–35	2	1	2	–
	III	11–25	-	3	1	1
AURIZON®	I	12–32	1	1	2	–
	II	18–50	2	2	1	–
	III	8–18	2	2	1	–
POSATEX®	I	10–23	2	2	1	–
	II	12–40	2	1	2	–
	III	8–23	2	1	2	–
SUROLAN®	I	8–28	2	2	1	–
	II	7–31	2	2	1	–
	III	8–30	2	1	2	–

Results

Animals

In general, the classifications of Otitis externa were evenly distributed among treatment groups. Subject details are summarised within Table 4.

Plasmaconcentrations

Data and blood specimens for Dog 3 in the Aurizon® group was lost by the veterinary practise. Due to technical reason samples from Dog 14 and Dog 15 could not be analysed. Blood samples from Dog 1 in the Otomax® group were not correctly processed into plasma and chemical analysis failed.

The findings of this study show that there was no influence between type of otitis classified and levels of residues found in plasma (Tab. 4). The results from the descriptive statistics for all active substance/final product combinations (i. e. gentamicin in Otomax®, gentamicin in Easotic®, marbofloxacin in Aurizon®, orbifloxacin in Posatex® and polymyxin B in Surolan®) are listed in Table 5.

Plasma concentrations of polymixin B were below LOD in all samples. For the other antibiotics, the proportion of samples $N_{<LOQ}/N$ roughly tends to decrease with increasing treatment time up to 72 h. Thus, we focused on the more accurate sample estimates at the end of the treatment periods and give 95% confidence intervals only for the end of the treatment (F). To take into account the different molecular weights and to achieve better comparability between all results from chemical analysis, the concentrations are expressed pmol/ml instead of ng/ml.

Otomax®

Individual and mean plasma concentrations of gentamicin following topical administration of Otomax® are graphically presented in Figure 1. The first plasma concentrations exceeding LOD were found in blood samples taken 24 h after the first dose. At the final visit seven days after the first dose, a mean plasma concentration of 2.93 pmol/ml (1.42 ng/ml; 95%CI: 1.65 to 4.20 pmol/ml) was found. The median concentration was 1.36 pmol/ml and $AUC_{0,72h}$ was found to be 146 h* pmol/ml (95%CI: 80 to 213).

Easotic®
Individual plasma concentrations of gentamicin following topical administration of Easotic® are presented graphically in Figure 2. The first plasma concentrations exceeding LOD were found in samples taken 24 h after the first dose. At the final visit five days after the first dose, a mean plasma concentration of 1.86 pmol/ml (0.89 ng/ml; 95% CI: 0.95 to 2.78 pmol/ml) was found. The median concentration was 1.19 pmol/ml and $AUC_{0,\ 72\ h}$ was 123 h* pmol/ml (95%CI: 28 to 217).

Aurizon®
Individual and mean plasma concentrations of marbofloxacin after administrations of Aurizon® are shown in Figure 3. The first concentrations exceeding LOD were found in samples taken 24 h after the first dose in four of four animals. In group II, however, no plasma concentration exceeding LOD was found 48 h after dosing in analyzed samples of three of three dogs. In these animals, LOD was only exceeded at the end of treatment. At the final visit 7–14 days after the first dose, a mean plasma concentration of 3.51 pmol/ml (1.27 ng/ml; 95%CI: 1.82 to 5.20 pmol/ml) was found. The median concentration was 2.48 pmol/ml and $AUC_{0\ ,72\ h}$ was found to be 108 h* pmol/ml (95%CI: 56 to 160).

Posatex®
Individual plasma concentrations of orbifloxacin after multiple administration of Posatex® are depicted in Figure 4. A single plasma concentration exceeding LOD was found in samples taken 1, 2 and 18 h after the first dosing in each of the three groups. At the final visit 7–14 days after the first dose, a mean plasma concentration of 2.69 pmol/ml (1.06 ng/ml; 95%CI: 1.57 to 3.81 pmol/ml) was found. The median concentration was 2.53 pmol/ml and $AUC_{0,\ 72\ h}$ was 78 h* pmol/ml (95%CI: –4 to 61).

Surolan®
All polymyxin B plasma concentrations in samples taken 1 h and 7 d after the first dose were below LOD of 4.15 pmol/ml (5.0 ng/ml).

TABLE 5: *Descriptive statistics of antibiotic plasma levels after topical administration in dogs with Otitis externa*

Gentamicin/Otomax®								
time (h)		1	4	18	24	48	72	F
$N_{<LOD}/N$		4/4	5/5	5/5	3/4	0/5	0/5	3/14
max	(pmol/ml)	< LOD	< LOD	< LOD	6.62	5.57	6.49	7.27
median		< LOD	< LOD	< LOD	< LOD	3.89	2.93	1.36
mean		–	–	–	–	–	–	2.93
SD		–	–	–	–	–	–	2.44
95% CI		–	–	–	–	–	–	1.65–4.20
Gentamicin/Easotic®								
time (h)		1	4	18	24	48	72	F
$N_{<LOD}/N$		5/5	5/5	5/5	2/5	1/5	3/5	5/15
max	(pmol/ml)	<LOD	<LOD	<LOD	6.07	7.75	3.29	7.33
median		<LOD	<LOD	<LOD	1.11	2.91	<LOD	1.19
mean		–	–	–	–	–	–	1.86
SD		–	–	–	–	–	–	1.81
95% CI		–	–	–	–	–	–	0.95–2.78
Marbofloxacin/Aurizon®								
time (h)		1	4	18	24	48	72	F
$N_{<LOD}/N$		4/4	3/3	5/5	0/4	3/3	3/5	4/12
max	(pmol/ml)	<LOD	<LOD	<LOD	8.28	<LOD	6.07	9.80
median		<LOD	<LOD	<LOD	5.81	<LOD	<LOD	2.48
mean		–	–	–	–	–	–	3.51
SD		–	–	–	–	–	–	2.99
95% CI		–	–	–	–	–	–	1.82–5.20
Orbifloxacin/Posatex®								
time (h)		1	2	18	24	48	72	F
$N_{<LOD}/N$		4/5	4/5	4/5	3/5	2/5	2/5	5/15
max	(pmol/ml)	1.19	<LOD	4.55	1.82	6.47	3.49	6.85
median		<LOD	<LOD	<LOD	<LOD	0.33	1.37	2.53
mean		–	–	–	–	–	–	2.69
SD		–	–	–	–	–	–	2.38
95% CI		–	–	–	–	–	–	1.57–3.81
Polymyxin B/Surolan®								
time (h)		1	2	18	24	48	72	F
$N_{<LOD}/N$		5/5	3/3	1/1	5/5	2/2	1/1	8/8
max	(pmol/m)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

LOD: Limit of detection; N: number of samples; $N_{<LOD}$ number of samples with concentrations below LOD;
SD: standard deviation; F: final visit.

Discussion

Reported knowledge is limited regarding trace analytical methods to measure residual concentrations of aminoglycosides, quinolones and polypeptide antibiotics in dog plasma. Only a few recent publications have been identified concerning the determination of active antibiotic substances in plasma (Baietto, 2010; Cheng et al.,

2010). The chemical analysis of the plasma obtained from the dogs treated with products containing marbofloxacin, orbifloxacin, gentamicin and polymyxin B was conducted by application of an HPLC-MS/MS method developed and validated according to VICH guidelines especially for this study (ECON, 2010). The specificity, accuracy and reproducibility were approved according to regulatory standards.

We confirmed that topical administration according to the recommended dosing of Otomax®, Easotic®, Aurizon® and Posatex® can provoke low systemic plasma concentrations of antibiotics in dogs with Otitis externa acuta. However, in several samples, concentrations were found to be below the LODs achieved by the specially developed residue methods. Assumptions had to be made for data analysis for samples taken between 1 and 72 h when the sample size was small. For the purpose of summary statistics and calculation of areas under the curve, these concentrations were set to zero to prevent a bias towards high concentrations. To evaluate the influence of this assumption, we used LOD/2, another widely used assumption, as a substitution rule for those concentrations below LOD. This sensitivity of analysis led to a minor increase of the mean plasma concentrations and the AUC increased up to 8%. For the plasma samples taken at the end of the study, we used methods of survival analysis developed explicitly for left truncated data that are superior to commonly used substitution rules (Helsel, 2005; Gillespie, 2010). Mean plasma concentrations based on this approach are similar to the median values. Overall, the results appear to be fairly stable and the finding of low systemic plasma levels is widely independent of our assumptions.

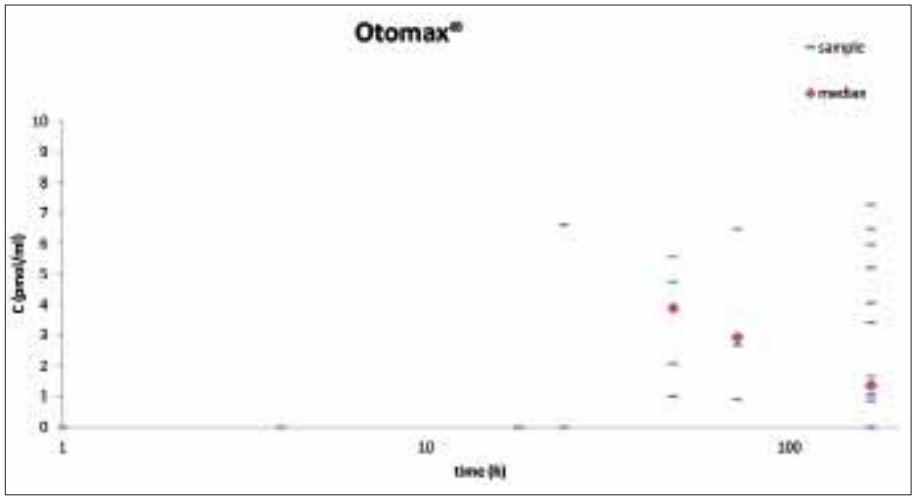

FIGURE 1: *Individual and median gentamicin plasma concentrations after multiple administrations of Otomax®. Experimental plasma concentrations below LOD were set to 0.*

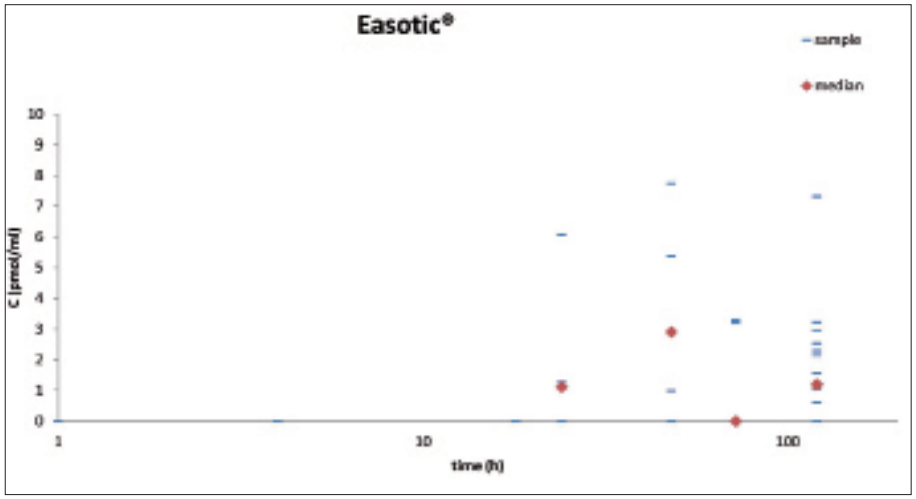

FIGURE 2: *Individual median gentamicin plasma concentrations after multiple administration of Easotic®.*

At the end of the treatment period, after five, seven or 14 days, the mean plasma concentrations of gentamicin, marbofloxacin and orbifloxacin exceeded LOD in a statistically significant way. Median plasma concentrations at the end of the treatment and areas under the curve calculated for the time range between start of treatment and 72 h support the finding of low systemic plasma levels. In general, the first plasma concentrations exceeding LOD were found 24 h after the first treatment. Orbifloxacin, however, was detected in single samples starting from 1 h after the first dose. In contrast, all plasma concentrations of polymyxin B were below LOD of 4.15 pmol/ml. It should be noted that the maximum concentrations of other antibiotics exceeded this concentration at several time points.

After multiple administrations of Otomax® and Easotic®, plasma concentrations of gentamicin were of comparable magnitude. Similarly, there was no significant difference in $AUC_{0,\ 72\ h}$ since the confidence intervals overlap. All results comply in the order of magnitude. Although it could be assumed that biofilm effect from pus (Bryant, 1974) as well as ulcers would influence the results there is no impact on the findings obtained related to the type of otitis classified.

For Surolan®, an upper limit of 4.15 pmol/ml was established for the plasma concentration of polymyxin B after seven days of treatment. Polymyxin B was not found in plasma samples, although the chemical methodology applied here represents a detection level at the lowest level technically possible. There are several reasons for polymyxin B not to penetrate. One of the reasons for this is polymyxin's high polarity. The molecule forms strong electrophilic interaction with cations in proteins. Another reason is the molecular size of polymyxin, which is approximately three times as large as the other antibiotics studied. Lesions induced by an ulcerative form of Otitis externa, polarity and molecu-

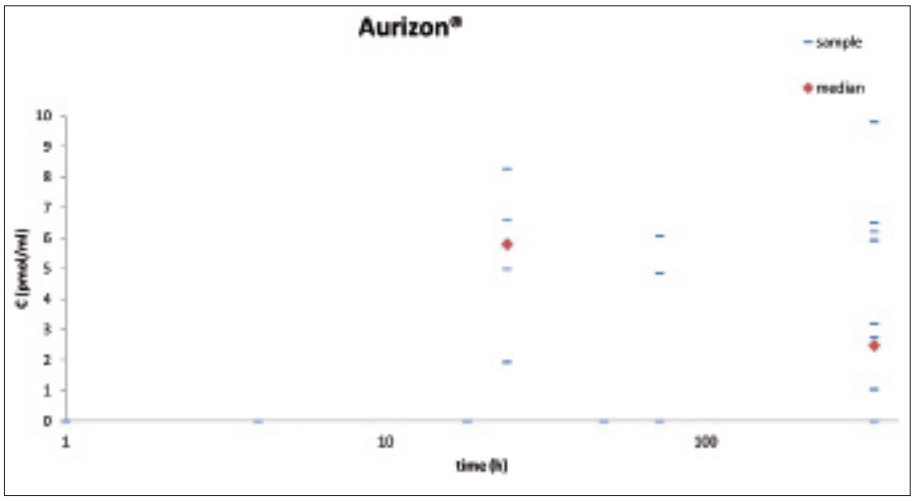

FIGURE 3: *Individual median marbofloxacin plasma concentrations after multiple administration of Aurizon®.*

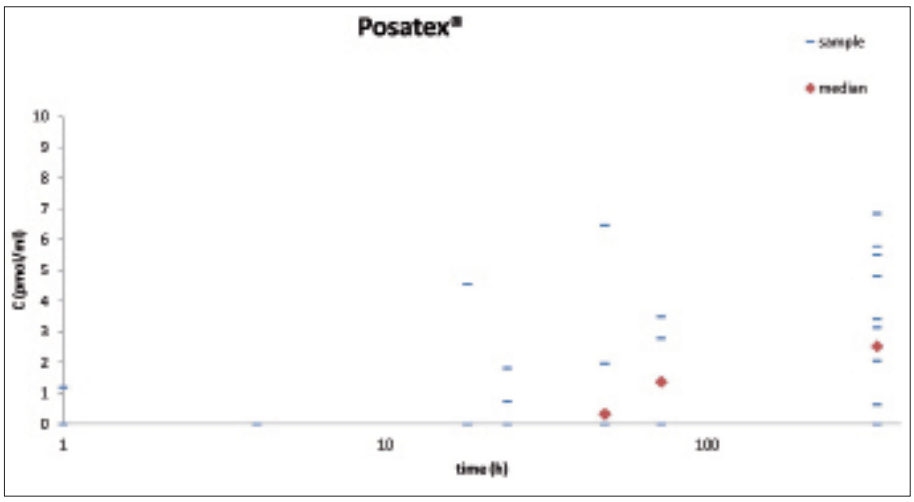

FIGURE 4: *Individualmedianorbifloxacin plasma concentrations after multiple administration of Posatex®.*

although at the highest technical standard may have influenced these findings. But there are additional arguments that just the physico-chemical properties of polymyxin B may reason the non penetrating properties observed here.

One must bear in mind that due to the extremely low concentrations involved, this does not present an acute safety issue for the animals affected. However, it is precisely the low-level concentration which may raise concern. Bacteria acquire resistance by mutation and gene exchange. If drug levels are lower than bactericidal concentrations, bacterial cells exposed to such low concentrations may develop resistance through mutation and/or gene exchange (Tenover, 2006). The bacteria gain an advantage for ongoing proliferation and cellular reproduction, as they will have less competition from bacterial cells more susceptible to the antibiotic (Gullberg et al., 2011). The probability of this process can be reduced by lowering the exposure of bacterial cells to these distinct molecules. Focus of the present study was the determination of plasma levels after topical administration of otitis products in naturally diseased animals – we did not conduct sub MIC low level resistance induction studies. However, our findings create a strong argument for exposure reduction management in the veterinary practice setting, especially in pets suffering chronically from Otitis externa.

lar size may be decisive factors for whether a substance penetrates the epithelial tissues or not.

Conclusion

The results reported here confirm a distinct probability of systemic absorption of topically administered antibiotics during the treatment of Otitis externa in dogs. Physical-chemical properties like lipophilicity or polarity, molecular size and the three-dimensional molecular structures may trigger the order of magnitude of uptake observed. Exactly these factors determined the analytical limits achieved. At first sight these differences seem considerable. But taking exactly the individual physical chemical properties into account and presenting the concentrations as molar mass instead of weight, the first sight differences clearly decrease. Under the given circumstances, polymyxin B has not been detected in plasma. The reason for this is not elucidated by the oberservational study approach. It may not be fully excluded, that the difficulties in sensitivity of the chemical method

Molecules with a known mechanism of resistance in some bacterial species should be replaced by those known to evoke less potential for resistant bacteria.

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Address for correspondence:
Dr. Michael G. Voget
ECON AG – Berlin Labs
Warthestrasse 21
14513 Teltow
Germany
mv@econ-pharma.com