Comparative Pharmacokinetics of Orbifloxacin Following a Single Intravenous or Oral Administration to Healthy and Diabetic Rats

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Summary

The single-dose disposition kinetics of orbifloxacin was determined in clinically healthy and diabetic rats after intravenous or oral administration of 5 mg/kg body weight. Orbifloxacin concentrations were determined by HPLC with fluorescence detection. The HPLC method was sensitive, specific and repeatable. A systemic bioavailability of 99.1% and 108 %, and a C_{max} of 6.55 ± 1.09 μg /mL and 8.63 ± 1.09 μg /mL were observed in healthy and diabetic rats, respectively. The terminal half-life after intravenous and oral administration was 4.17 ± 0.38 h and 4.03 ± 0.41 h for healthy and 2.31 ± 0.34 h and 3.03 ± 0.28 h for diabetic rats. Orbifloxacin was cleared more rapidly in diabetic rats (0.15 ± 0.01 L/kg.h) than healthy group (0.11 ± 0.01 L/kg.h), with longer mean resident time (MRT) values observed in the latter. Other kinetic parameters were almost the same between the healthy and diabetic groups. This investigation revealed that a dose of 5 mg/kg orbifloxacin can be safely and effectively used to combat infections in rats of either group associated with susceptible bacteria.

Introduction

Although the popularity of rodents and rabbits as pets has increased in recent years, and they make up over 90% of the animals used in research, there is no uniform source of information on rational use of antibiotics in these species comparable to that available for the common companion and domesticated species (*Flecknell, 1998; Morris, 1995*). Bacterial infections in rodents are quite common and often require antimicrobial treatment. Respiratory

diseases due to *Mycoplasma pulmonis* and Cilia-associated Respiratory (CAR) Bacillus, Tyzzer's Disease (gastrointestinal disorder) due to *Clostridium piliforme,* and ulcerative dermatitis due to *S. aureus* and *S. epidermidis* are among the most important bacterial infections in rats in which antibiotic intervention is indicated (*Emily, 2007; Michelle, 2008*). Antibiotics licensed for small mammals and exotics are still lacking in most countries, such that in general, products for dogs and cats have to be used off-label. However, antimicrobial therapy in rodents and rabbits entails greater risk than in most other species because inappropriate therapy can result in death of the patient due to enterotoxaemia (*Morris, 1995; Ramirez, 2007*).

Fluoroquinolones are among effective and safe antibiotics frequently used in small mammals such as rabbits, mice, rats, and exotic species against skin

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and visceral infections (*Papich and Riviere, 2001; Ramirez, 2007)*. Bacterial overgrowth of pathogenic opportunistic bacteria, especially clostridium organisms, and its associated gastrointestinal disturbances and enteritis in rodents has not been a problem with fluoroquinolones as it has been with other drugs like penicillins and macrolides. It is due to the fact that fluoroquinolones are not active against anaerobic bacteria that compete with clostridium organisms (*Papich and Riviere, 2001; Ramirez, 2007)*. Of the available drugs, enrofloxacin has been the most extensively studied: the pharmacokinetics, efficacy and dosage regimen of enrofloxacin were reported in small mammals, including rabbits, rats and mice (*Carpenter, 2005; Papich and Riviere, 2001; Ramirez, 2007)*. It is recommended as the drug of choice in rodents at a flexible subcutaneous/oral dose of 5-20 mg/kg body weight (*Mitchell, 2006; Papich, 2003*).

Orbifloxacin is a third-generation fluoroquinolone developed exclusively for use in veterinary medicine *(Iherke and Papich, 1999; Marin, et al., 2007; Matsumotoet al., 1999)*. It is labeled in USA for the treatment of skin, soft tissue and urinary tract infections in dogs, and skin and soft tissue infections in cats *(Davis, et al., 2006)*. In Japan, it has been licensed since 1993 for use in cattle and pigs, mainly to combat respiratory and gastrointestinal infections *(Engberg et al., 2001; Nakamura, 1995)*. The pharmacokinetics of orbifloxacin has been investigated in several species, including goats, horses, pigs, dogs and cats *(Davis, et al., 2006; Heinen, 2002 ; Lee et al., 2007; Marin et al., 2007; Matsumoto et al., 1998a,1999),* and more recently in rabbits, cattle, camels, and sheep (*Elias et al., 2008; Goudah et al., 2008 a, b; Marin et al., 2007b)*. However, the disposition kinetics, antibacterial activity and safety of orbifloxacin in rodents and other exotic species have not been determined.

Furthermore, pharmacokinetic experiments are usually performed in healthy animals, and specific studies using sick animals are relatively rare. However, the kinetics of many drugs can be modified by different diseases, notably by hepatic and renal disorders (*O'Connor, 1987; Papich, 2003)*. These alterations may reach to an extent that requires dosage adjustment. In humans, the renal clearance of a number of antibiotics can increase during diabetes (*Gwilt et al., 1991; O'Connor, 1987*). Diabetes-induced kidney hyperfiltration in rats can cause a significant increase in renal clearance of some drugs (*Bae et al., 2006*). As the rate of elimination of a drug is usually an important determinant of the duration of pharmacological effect, investigation of factors that potentially modify this rate may help maximize therapeutic efficacy.

This study was designed to address two main objectives:

a) to determine the disposition kinetics of orbifloxacin following a single intravenous (IV) or oral administration to rats

b) to assess whether pathophysiological changes during diabetes have significant effect on the kinetics of the drug in rats

Materials and Methods

Chemicals

Streptozotocin, sodium citrate and citric acid monohydrate were purchased from Sigma–Aldrich Corporation (Steinheim, Germany). Orbifloxacin (Orbax®) solution and pure standard for high performance liquid chromatography (HPLC) analysis were supplied by Samyang Anipharm Company (Seoul, Korea). Acetonitrile and all other chemicals used were HPLC or reagent grade.

Animals

Male Sprague–Dawley rats, 9-10 weeks old and weighing 320–350 g, were purchased from Orient Co. (Seoul, South Korea). The rats were randomly assigned into four groups; (1) healthy rats for oral administration, (2) healthy rats for IV administration, (3) diabetic rats for oral administration and (4) diabetic rats for IV administration of orbifloxacin. Animals were provided with feed (Samyang Company,Pyeongteak, Korea) and water ad libitum and maintained in a clean room at a temperature of $22 \pm 2\ ^0C$ with 12 h light and dark cycle, and $50 \pm 5\%$

relative humidity. The animal protocol was approved by the Bioethical Committee of the College of Veterinary Medicine, Kyungpook National University.

Induction of diabetes mellitus
Freshly prepared streptozotocin (dissolved in 0.1 M citrate buffer, pH 4.5) was administered intraperitoneally, at a dose of 60 mg/kg body weight (total injection volume, 1 mL) once to overnight fasted rats. On day 7 after administration, blood glucose levels were measured using Medisense Optium Kit (Abbott Laboratories, Bedford, MA) and rats with blood glucose level higher than 250 mg/dL were considered diabetic. Body weight and 24-h urine output of rats in each group were also measured one day prior to drug administration.

Drug administration and sampling
The jugular vein (for drug administration in the IV study) and the carotid artery (for blood sampling) were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ) while each rat was under light diethyl ether anesthesia (*Bae et al., 2006; Lee et al., 2007*). Heparinized normal saline (15 units/ml), 0.25 ml, was used to flush the cannulae to prevent blood clotting. Each rat was housed individually in a metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from anesthesia for 4–5 h before the commencement of the experiment. Orbifloxacin solution at a dose of 5 mg/kg was infused over 1 min via the jugular vein of both healthy (n=6) and diabetic (n=6) rats used for the IV study. For the oral study, the same amount of drug was administered orally (total volume of approximately 0.5 mL) using a feeding tube in each healthy (n=6) and diabetic (n=6) group. Approximately 200 μL of blood sample was collected from each animal via the carotid artery before and at 0.1, 0.25, 0.5, 1, 2, 4, 6, 8, and 12 h after drug administration. Blood samples were centrifuged immediately and a 50 μL aliquot of each plasma sample was stored at -70^{0}C until analysis by HPLC. After the last sample, rats were sacrificed by decapitation, the whole kidney and liver of each rat were excised, rinsed with a 0.9%-NaCl –injectable solution, blot-dried with tissue paper, and weighed.

Analysis of Orbifloxacin
Orbifloxacin concentrations in plasma samples were measured by HPLC using a Hewlett Packard 1100 system comprising an HPLC pump, HP ODS Hypersil column (200 × 4.6mm, 5μm), autoinjector and HP 1046A fluorescence detector. We followed our previously reported method in cow's serum (*Elias et al., 2008*), with slight modification. A 50 μL aliquot of rat plasma was deproteinated by adding 100 μL of acetonitrile. After vortex-mixing and centrifugation at 16,000 × g for 1 min, a 20 μL aliquot of the supernatant was injected directly onto the HPLC column. The mobile phase (15% acetonitrile and 85%, 50 mmol Potassium phosphate buffer, pH =3) was run at a flow rate of 1 mL/min. The excitation and emission wavelengths of the fluorescence detector were 287 nm and 470 nm, respectively. The HPLC method was validated for specificity, accuracy, repeatability and inter-day precision as described previously (*Elias et al., 2008*).

Data analysis
The pharmacokinetic parameters of orbifloxacin following both routes of administration to all rats were analyzed by non-compartmental methods using Winnonlin professional program Version 2.1(Pharsight Corporation, Cary, NC, USA).The total area under the plasma concentration–time curve from time zero to time infinity ($AUC_{0-\infty}$) was calculated using the trapezoidal rule–extrapolation method. The extent of absolute oral bioavailability (F%) was estimated by dividing the $AUC_{0-\infty}$ value after an oral administration by the $AUC_{0-\infty}$ value after an IV administration.

Descriptive statistical parameters as mean, standard deviation, and coefficient of variation were calculated, and the student's t-test was applied to test parameters for significant difference between healthy and diabetic groups. A p-value of less than 0.05 was considered significant.

Results
Body weight, blood glucose levels, 24-h urine output, and relative liver and kidney weights in both diabetic and healthy groups are listed in Table 1.

noted during the study. The relevant pharmacokinetic parameters derived from non-compartmental analysis of the oral and IV data are summarized in Tables 2 and 3. Mean plasma concentrations are

Table 1. The mean (± standard deviation) body weight, bloodglucose level, 24-h urine output, and relative organ weights in healthy and streptozotocin-induced diabetic rats.

	Healthy (n=12)	Diabetic (n=12)
Body weight(g)		
Initial	331.1±8.57	335.8±6.03
Final	354.6±5.61	328.8±10.3[a]
Blood glucose (mg/dL)	107.16±7.77	311.8±27.5[a]
Urine output (24-h/kg)	31.8±2.73	85.8±5.67[a]
Liver weight (% of body weight)	3.05±0.31	3.27±0.19
Kidney weight (% of body weight)	0.79±0.05	1.02±0.09[a]

[a] Significantly different ($P< 0.05$) from healthy group.

There was no statistical difference in body weight at the beginning of the study between animal groups. Diabetic rats had significantly lower weight gain, while blood glucose level, 24-h urine output and relative weight of the kidneys were significantly higher compared to the healthy group. Out of 14 rats administered with streptozotocin solution only one rat showed blood glucose level less than 250 mg/dL, probably due to improper dosing. No significant difference was observed in the relative weights of liver between the two groups. The HPLC method was sensitive, specific and repeatable. The retention time of orbifloxacin was approximately 4 min. No interfering peaks in all blank samples were noted in the elution position of orbifloxacin. A linear relationship existed in the calibration curve in a concentration range between 0.03-50 μg /mL. Orbifloxacin recovery from plasma ranged from 97.9 % to 101.9 % for different concentration levels. The repeatability and within-run precision (percent coefficient of variation, %CV) across the range of tested concentrations were 3.5-6.1% and 5.2-8.1%, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.02 and 0.03 μg /mL.
No adverse effects from drug administration were

shown in Figures 1 and 2. Oral orbifloxacin was well absorbed in both healthy and diabetic rats with an absolute bioavailability of 99.1% and 108 %, and a C_{max} of 6.55 ± 1.09 μg /mL and 8.63 ± 1.09 μg /mL. The terminal half-life of orbifloxacin after IV and oral administration was 4.17 ± 0.38 h and 4.03 ± 0.41 h (Harmonic means, 3.93 h and 3.82 h) for healthy and 2.31 ± 0.34 h and 3.03 ± 0.28 h (Harmonic means, 2.1 h and 3 h) for diabetic rats. Statistical comparison between the two treatment groups revealed significant differences in the terminal half-life, clearance and MRT values.

Discussion
In recent years the interest in rodents as pets has dramatically increased. This has led to a rapid expansion in rodent medicine (*Flecknell,1998*). On top of the potential usefulness of fluoroquinolones as orally active bactericidal agents with little cross-resistance developed to other classes of antibiotics, the relative lack of incidence of induced enterocolitis at clinical dose rates makes them an attractive option for the treatment of infections in rodents (*Morris, 1995*). Here, we report for the first time the disposition kinetics of orbifloxacin in rats after a single

Table 2. Pharmacokinetic parameters of orbifloxacin (Mean ± SD) after a single oral administration to healthy or diabetic rats (n=6).

Parameters	Healthy		Diabetic	
	Mean	SD	Mean	SD
T_{max} (h)	2.33	0.55	1.25	0.25
C_{max} (µg/mL)	6.55	1.09	8.63	1.09
$AUC_{0-12 h}$ (µg .h/mL)	38.51	3.53	36.44	3.81
$AUC_{0-\infty}$ (µg.h/mL)	44.55	3.25	39.12	4.39
λz (1 /h)	0.17	0.01	0.23[a]	0.02
$t_{1/2}\lambda z$ (h)	4.03	0.41	3.03	0.28
MRT_{0-24h} (h)	4.54	0.21	3.47	0.19
$MRT_{0-\infty}$ (h)	6.38	0.63	4.31[a]	0.47
F (%)	99.1		108	

[a] Significant at p<0.01; Tmax, time of maximum observed concentrat-ion; Cmax, maximum observed concentration; AUC, area under concentration-time curve; λz,first-order rate constant associated with the terminal portion of the curve; t1/2 λz, terminalhalf life; MRT, mean resident time; F, bioavailability.

Table 3. Pharmacokinetic parameters of orbifloxacin (Mean ± SD) after a single intravenous injection to healthy or diabetic rats (n=6).

Parameters	Healthy		Diabetic	
	Mean	SD	Mean	SD
AUC_{0-12h} (µg .h /mL)	40.78	3.18	34.53	2.82
λz (1/h)	0.17	0.02	0.33[b]	0.04
$t_{1/2}\lambda z$ (h)	4.17	0.38	2.31[b]	0.34
$AUC_{0-\infty}$ (µg.h/mL)	44.96	4.31	36.15	2.89
$V\lambda z$ (L/Kg)	0.69	0.09	0.51	0.09
Cl (L/h.Kg)	0.11	0.01	0.15[a]	0.01
MRT_{0-24h} (h)	4.17	0.18	3.67	0.15
$MRT_{0-\infty}$ (h)	6.14	0.45	4.24[b]	0.34
Vss (L/Kg)	0.68	0.09	0.65	0.08

[a] Significant at p< 0.01; [b] Significant at p< 0.05; AUC,area under concentration-time curve; λz,first-order rate constant associated with the terminal portion of the curve; $t_{1/2}$ λz, terminal half life V λz ,apparent volume of distribution; Cl, total body clearance; MRT, mean resident time; Vss, volume of distribution at steady state.

intravenous and oral administration. After oral administration of orbifloxacin at 5 mg/kg, a rapid and nearly complete absorption with absolute bioavailability of 99.1% (healthy) and 108% (diabetic) rats was observed. These values are comparable to intramuscular bioavailability in goats (105.01 %), rabbits (109.87%), camels (97.47%), Korean Hanwoo cows (101.4%), and sheep (114%) (*Elias et al., 2008; Goudah et al., 2008 a, b; Marin et al., 2007a, b*). However, the bioavailability of orbifloxacin in rats was higher than the oral bioavailability of orbifloxacin reported in horses (68.35%) (*Davis et al.,*
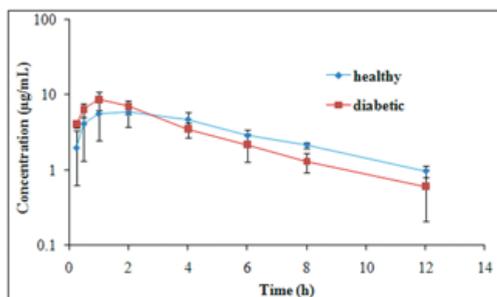
Figure 1. Plasma concentrations (mean ± SD, n=6) of orbifloxacin following intravenous (i.v.) administration at a dose rate of 5 mg/kg to clinically healthy and diabetic rats.
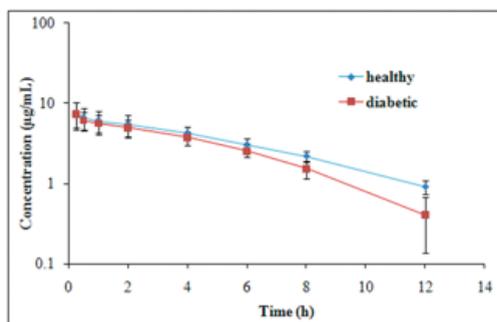

Figure 2. Plasma concentrations (mean ± SD, n=6) of orbifloxacin following oral administration at a dose rate of 5 mg/kg to clinically healthy and diabetic rats.

2006). The terminal half-lives of orbifloxacin after IV and oral dosing to healthy rats were comparable (harmonic mean: 3.92 and 3.82 h), suggesting that absorption does not seem to affect elimination. The IV terminal half-life of orbifloxacin in rats was longer than in goats (1.84 h), rabbits (2.5 h) and cows (3.2 h) (*Elias et al., 2008; Marin et al., 2007a, b*), and shorter than in horses (5.08 h) and camels (5.74 h) (*Davis et al., 2006*; *Goudah et al., 2008 a*). A comparable half-life of orbifloxacin (4.19 h) was observed in beagle dogs (*Elias et al., 2009*).

The effect of disease and altered physiological states on the pharmacokinetics of drugs has been reported in animals and humans. When assessing changes in drug disposition, the relevant pharmacokinetic parameters to use are body (systemic) clearance, volume of distribution, half-life and possibly mean residence time (*Boxenbaum, 1984*). Statistical comparison of the kinetic parameters between the healthy and diabetic rats revealed significant differences in such parameters as body clearance, terminal half-life and MRT values. As observed in the concentration-time plots of both IV and oral data, the plasma level of orbifloxacin was higher in healthy than in diabetic rats after distribution or absorption (oral administration) period. Following IV administration, the terminal half-life of orbifloxacin in diabetic rats (2.31 ± 0.31 h) was significantly shorter than that of healthy rats (4.17± 0.38 h). Although it did not reach a significant level, the terminal half-life after oral dosing to diabetic rats was also shorter by 1 h compared to healthy rats (Table 3). As we hypothesized, diabetes has significant effect on the clearance of orbifloxacin; accelerating it. And longer MRT values were observed in the healthy rats. Orbifloxacin, like most other fluoroquinolones, is primarily excreted via the kidneys, and a major portion of an oral dose is excreted in urine unchanged within 24 hours of administration (*Matsumoto et al.,1998 a, b,1999*). The rapid clearance and shorter elimination half-life of orbifloxacin observed in diabetic rats may be due to increased renal hyperfiltration as evidenced by higher 24-h urine output and heavier kidneys in these animals, which suggest somewhat impaired renal function. In favor of this hypothesis, other groups of streptozotocin-induced diabetic rats we used for different experiment during the same study period demonstrated a higher glomerular filtration rate (GFR) as estimated by creatinine clearance, together with increased kidney weight, although no significant changes were observed in the kidneys and livers between diabetic and control groups based on histology.

Most fluoroquinolones, including orbifloxacin, exhibit concentration-dependent killing against many gram-negative bacteria, and clinical response is usually predicted by using the C_{max}/MIC ratio as

well as the AUC_{24h}/MIC values relative to the target organism (*Grosset , 2004;Mckellar et al., 2004*). Against gram positive-organisms, such as *S. intermedius*, however, the killing activity of orbifloxacin has been reported to be time-dependent (*Ganièreet al., 2004*), such that therapeutic efficacy is dependent on the maintenance in target tissues of antibiotic concentrations above the MIC for the pathogen for a large portion of the dosage interval. The faster clearance of orbifloxacin in diabetic animals may have significance with respect to the therapeutic time. This observation would have more clinical relevance if diabetes has similar effect on the kinetics of the drug in species like dogs and cats.

Despite the above significant differences between healthy and diabetic rats, other kinetic parameters such as volume of distribution and area under plasma concentration- time curve (AUC) were similar in both groups, and a slightly higher C_{max} value of 8.63 µg/mL in diabetic rats vs. 6.55 µg/mL in healthy rats was achieved relatively earlier. These may indicate that streptozotocin-induced diabetes in rats has little or no effect on oral absorption of orbifloxacin as it has on the elimination rate.

Sprague–Dawley rats were used in our study. The clinical significance and relevance of antibacterial treatment to the Sprague-Dawley strain rats may not be justified due to public health reasons. However, the paucity of published pharmacokinetic data in rodents using such antibacterials, the similarity between the experimental strains and rats used as pets, and the possible extrapolation with a reasonable accuracy of the present data to other rodents that have similar biology with rats would make our findings useful.

Streptozotocin injection in rats induces primarily type 1 diabetes by selective destruction of the pancreatic β-cells, while type 2 diabetes can also be induced in rats by intravenous or intraperitoneal treatment of neonatal rats with 100 mg/kg b.w. streptozotocin (*Szkudelski, 2001*).The form of diabetes in companion animals varies with species. Type 1 diabetes, previously called insulin-dependent diabetes, is most common in dogs, whereas, type 2, previously called non-insulin dependent or adult-onset diabetes, appears to be the most common form of diabetes in cats (*Rand et al., 2006*). In this regard, the model of streptozotocin-induced diabetes in the experimental rats may not reflect the latter form of diabetes in cats and dogs. Furthermore, disorders of glucose homeostasis have been reported in association with fluoroquinolone therapy in diabetes mellitus of humans and animals, in particular with gatifloxacin and levofloxacin (*Vallurupalli et al., 2008*). This necessitates further investigations on the comparative disposition of orbifloxacin to the pancreas, liver and kidney of diabetic and healthy animals.

In conclusion, the present study evaluated for the first time the single-dose disposition kinetics of orbifloxacin in clinically healthy and in diabetic rats .We applied the experimental dose of 5 mg/kg based on the minimum recommended dose of enrofloxacin in rats. After administration of orbifloxacin to healthy rats, the PK was characterized by a rapid and nearly complete absorption, good distribution into body fluids, and prolonged elimination half-life. Based on the $AUC_{0-12 h}$ and $AUC_{0-\infty}$ values obtained in healthy animals and applying the equation: Dose=AUC× CL, orbifloxacin at 5 mg/kg dose would be worthy of consideration to treat infection in rats associated with susceptible bacteria.

There are potential limitations of the current study. First, we investigated the single dose disposition of the drug in rats, but orbifloxacin is repeatedly given to objective animals in clinical fields. Therefore, further study using repeated administration of the drug may be necessary. Besides, orbifloxacin is arbitrarily chosen for this investigation. However, there are many analog agents in veterinary area, including danofloxacin, difloxacin, enrofloxacin, and marbofloxacin, for which pharmacokinetic data in rodents is scarce with the exception of enrofloxacin. Further studies using the different fluoroquinolones would reveal the comparative advantage of one agent over the other, and promote the rational use of antibiotics in these species.

References

Bae, SK, JY Kim, SH Yang, JW Kim, T Kim and MG Lee. Pharmacokinetics of oltipraz in rat models of diabetes mellitus induced by alloxan or streptozotocin. Life Sci. 2006, *78*(20): 2287-94.

Boxenbaum, H. Interspecies pharmacokinetic scaling and the evolutionary-comparative paradigm. Drug Metab Rev. 1984. *15* (5-6): 1071-121

Carpenter, JW. Exotic Animal Formulary. 3rd ed. Elsevier Saunders, St. Louis, MO. 2005.

Davis, JL, MG Papich and A Weingarten. The pharmacokinetics of orbifloxacin in the horse following oral and intravenous administration. J Vet Pharmacol Ther. 2006, *29*,191-7.

Elias, G., JS Lee, MH Hwang, YS Park, KH Cho, YH Kim and SC Park. Pharmacokinetics and Pharmacokinetic/Pharmacodynamic Integration of Orbifloxacin in Korean Hanwoo Cattle. J Vet Pharmacol Ther. doi: 10.1111/j.1365-2885.2008.01027.x .

Elias, G, JS Lee, ZQ Chang, MH Hwang, H Cheng, and SC Park. Integration of Pharmacokinetic and Pharmacodynamic Indices of Orbifloxacin in Beagle Dogs after a Single Intravenous and Intramuscular Administration. Antimicrob Agents Chemother, 2009.In Press.

Emily, H. Rodent Dermatology. J Exot Pet Med. 2007, *16*(4), 238–255

Engberg, J, FM Aarestrup, E Taylor, P Gerner-Smidt and I Nachamkin. Quinolone and macrolide resistance in Campylobacter jejuni and C. coli: resistance mechanisms and trends in human isolates. Emerg Infect Dis. 2001, *7*, 24-34.

Flecknell, P. Developments in the veterinary care of rabbits and rodents. In Practice. 1998, *20*, 286-295

Ganière, JP, C Mèdaille and F Etorè. In vitro antimicrobial activity of orbifloxacin against Staphylococcus intermedius isolates from canine skin and ear infections. Res Vet Sci. 2004, *77*, 67–71.

Goudah, A and K Abo-El-Sooud. Pharmacokinetics and milk penetration of orbifloxacin after intravenous and intramuscular injections to dromedary lactating camels (Camelus dromedaries). J Vet Pharmacol Ther. 2008, *31*, 276–280.

Goudah, A, HJ Cho, HC Shin, JH Shim, NL Regmi, M Shimoda and AM Abd El-Aty. Pharmacokinetics and milk distribution characteristics of orbifloxacin following intravenous and intramuscular injection in lactating ewes. J Vet Pharmacol Therap. doi: 10.1111/j.1365-2885.2008.01046.x.

Grosset, NE. Pharmacokinetic and pharmacodynamic issues in the treatment of bacterial infectious diseases. Eur J Clin Microbiol. 2004, *23*, 271-88.

Gwilt, PR, RR Nahhas and WG Tracewell. The effects of diabetes mellitus on pharmacokinetics and pharmacodynamics in humans. *Clin Pharmacokinet*.1991, *20* (6), 477-90

Heinen, E. Comparative serum pharmacokinetics of the fluoroquinolones enrofloxacin, difloxacin, marbofloxacin, and orbifloxacin in dogs after single oral administration. J Vet Pharmacol Ther. 2002, *25*, 1-5.

Iherke, PJ, MG Papich and TC Demanuelle. The use of fluoroquinolones in veterinary dermatology. Vet Dermatol. 1999, *10*,193-204

Lee, DY, MG Lee, HS Shin and I Lee. Changes in omeprazol pharmacokinetics in rats with diabetes induced by alloxan or streptozotocin: faster clearance of omeprazol due to induction of hepatic CYP1A2 and 3A1. J Pharm Pharmaceut Sci. 2007, *10* (4),420-433.

Marin, P, E Escudero, E Fernandez-Varon and CM Carceles. Pharmacokinetics and milk penetration of orbifloxacin after intravenous, subcutaneous, and intramuscular administration to lactating goats. J Dairy Sci. 2007, *90*(42),19-25.

Marin, P, E Fernandez-Varon, E Escudero and CM Carceles. Pharmacokinetic-pharmacodynamic

integration of orbifloxacin in rabbits after in-travenous, subcutaneous and intramuscular ad-ministration. J Vet Pharmacol Ther. 2007. *31***, 77–82.

Matsumoto, S, M Nakai, M Yoshida and H Katae. A study of metabolites isolated from urine samples of pigs and calves administered orbifloxacin. J Vet Pharmacol Ther.1999, *22***, 286-9.

Matsumoto, S, M Nakai, M Yoshida and H Katae. Absorption, distribution and excretion of orbi-floxacin in swine and calves. J Japan Vet Med Ass. 1998, *51***, 13-18 (In Japanese with English abstract)

Matsumoto, S, M Takahashi, N Kitadai and H Ka-tae. A study of metabolites isolated from the urine samples of cats and dogs administered orbifloxacin. J Vet Med Sci. 1998, *60*, 1259-61.

Mckellar, QA, SF Sanchez Bruni and DG JONES. Pharmacokinetic/pharmacodynamic relation-ships of antimicrobial drugs used in veterinary medicine. J Vet Pharmacol Ther. 2004, *27*, 503–514.

Michelle, W. Pet rodents: What every vet should know. Irish Vet J. 2008, *61*(3), 176-181.

Mitchell, MA.. Enrofloxacin. J Exot Pet Med*, 2006, *15*(1), 66-69.

Morris, TH.. Antibiotic therapeutics in laboratory animals. Lab Anim, 1995,*29*, 16-36

Nakamura, S. Veterinary use of new quinolones in Japan. Drugs, *49* Suppl 2. 1995, 152-8.

O'Connor, P, J Feely. Clinical pharmacokinetics and endocrine disorders. Therapeutic implications*.* Clin Pharmacokinet. 1987, *13*(6), 345-64

Papich, MG and JE Riviere. Fluoroquinolone anti-microbial drugs. *In*: Adams, HR (ed.). Veteri-nary Pharmacology and Therapeutics, 8th ed. Iowa State Press, Ames Iowa. 2001, 898-917.

Papich, MG. Drug Therapy and Selection in Ex-otic Medicine. Supplementary Compendium on Continuing Education for the Practicing Veteri-narian. 2003, *25*(3), 15-20.

Ramirez, HE. Antimicrobial drug use in rodents, rabbits and ferrets. *In*: Giguère, S, JF Prescott, JD Baggot, RD Walker, PM Dowling (Eds.).

Antimicrobial therapy in veterinary medicine. 4th ed, Blackwell publishing, Ames Iowa. 2007,565-581.

Rand, JS, LM Fleeman, HA Farrow, DJ Appleton, and R Ledere. Canine and Feline Diabetes Mellitus: Nature or Nurture? 2006. http://www.uq.edu. au/ccah/index.html?page=43608&pid=0.

Szkudelski, T. The mechanism of alloxan and strep-tozotocin action in β- cells of the rat pancreas. Physiol Res. 2001. *50,* 536-546.

Vallurupalli, S, G Huesmann, J Gregory and MG, Jakoby. Levofloxacin-associated hypoglycae-mia complicated by pontine myelinolysis and quadriplegia*.* Diabet Med. 2008, *25***, 856–859.