Plasma pharmacokinetics and muscle disposition of marbofloxacin in chickens

Urzúa, N.[1], Errecalde, C.[1], Prieto, G.[1*], Lüders, C.[2], Picco, E.[3], Paula Tonini, M.[1]

[1]*Department of Pharmacology, Faculty of Agriculture and Veterinary, National University of Río Cuarto, Río Cuarto, Argentina*

[2]*School of Veterinary Medicine, Catholic University of Temuco, Temuco, Chile*

[3]*Department of Pharmacology, Faculty of Veterinary Sciences, National University of the Litoral, Esperanza, Argentina*

Key words:
chickens, HPLC, marbofloxacin, pharmacokinetics

Correspondence
Prieto, G.
Department of Pharmacology, Faculty of Agriculture and Veterinary, National University of Río Cuarto, Río Cuarto, Argentina
Tel: +54(0358) 4641986
Fax: +54(0358) 4680280
Email: gprieto@ayv.unrc.edu.ar

Received: 8 April 2015
Accepted: 17 June 2015

Abstract:

BACKGROUND: Reports on the pharmacokinetics of marbofloxacin in birds are scarce, even when it is a useful tool in poultry production. **OBJECTIVES:** Determining marbofloxacin kinetic parameters in plasma and muscle arrangement after intravenous and oral administrations and establishing its withdrawal period. **METHODS:** Clinical healthy chickens (1.08±0.22 kg) were used as experimental subjects, formed in groups of 5. In Group A (n= 45) birds received 2 mg/kg of marbofloxacin intravenously while group B (n= 65) was given the same oral dose after fasting for 12 hours. Blood samples in the groups and 5 g of muscle were obtained at different times, in A group up to 24 hours and in B group until 120 hours after application. Once the samples were obtained and treated, marbofloxacin was quantified by HPLC. **RESULTS:** Marbofloxacin in fasted chickens is almost completely absorbed (F= 97%), mean residence time is moderate (6.9 hours), with a high volume of distribution. Orally, mean residence time is less in plasma (4.9 hours) and the withdrawal time was calculated 2.5 days. **CONCLUSIONS:** The kinetic results are consistent with those presented by other fluoroquinolones in poultry and are in line with short production cycles in chickens.

Introduction

Marbofloxacin (MBFX) is a second generation fluoroquinolone marketed exclusively for veterinary medicine (Spreng et al., 1995; Martinez et al., 2006). Like other fluoroquinolones, it inhibits DNA gyrase (topoisomerase II) and topoisomerase IV creating complexes with the enzyme, which result in conformational changes, exerting antimicrobial activity against Gram negative bacteria, some Gram positive and Mycoplasma spp (García et al., 1999; Sidhu et al., 2010; Shan et al., 2013). MBFX is an organic acid, with good tissue penetration, high volume of distribution, and weak binding to plasma proteins (Anadón et al., 2002; Goudah and Hasabelnaby, 2010; Yang et al., 2014), being active at low concentrations (Ding et al., 2013). It differs from other fluoroquinolones, in the presence of an oxadiazine ring, conferring a long elimination half-life ($t_{½\beta}$) and high bioavailability (Goudah and Hasabelnaby, 2010; Yuan et al., 2010). These characteristics demonstrate that marbofloxacin presents a great potential for the treatment of bacterial infections in Veter-

inary Medicine (Thomas et al., 2001).

Investigations on MBFX pharmacokinetics in comestible birds are scarce, counting those of Anadón et al. (2002), Huang et al. (2003), and Ding et al. (2010) in chickens, Goudah and Hasabelnaby (2010) and Yuan et al. (2010) in ducks and Haritova et al. (2006, 2013) in turkeys and quail. Demonstrating short to moderate mean residence time in these species, ranging from 2.99 to 5.96 hours, the latter registered in chickens (Anadón et al., 2002), bioavailability ranges in chickens varies from 56.2% (Anadón et al., 2002) to 95.7% (Huang et al., 2003). Half-life elimination is fast in quails 2.01 (h) (Haritova et al., 2013), but slower in chickens being 5.6 and 6.5 hours after intravenous application, reported by Anadón et al. (2002) and Huang et al. (2003), respectively. The objective of this study was to describe the pharmacokinetic variables of MBFX in plasma and muscle disposition after intravenous and oral administrations, estimating MBFX withdrawal time in chickens.

Materials and Methods

Animals: 110 Ross line chickens from a farm near Río Cuarto city (Argentina) were used; these 30-day-old birds were clinically healthy, with a weight of 1.08±0.22 kg. The birds were transferred to the Veterinary Medicine Faculty at the National University of Río Cuarto and housed in a conditioned box for an acclimatization period of seven days before the study, at a temperature of 18°C, with food and water ad libitum

Experimental design (Broiler management): 110 chickens were randomly divided into two groups of 45 (A) and 65 (B) chickens respectively, with five replicates each one. Both were given a single dose of MBFX of 2 mg/kg, intravenously for group A and orally for B, after 12 hours of fasting. Following MBFX administration, according to the techniques approved by the Bioethics Committee of the National University of Río Cuarto, batches of five animals per time were slaughtered at different times, A (0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 hours) and B (0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120 hours). Blood samples from each bird (5 ml) were centrifuged at 2500 rpm x 10 minutes and the plasma obtained was saved in sterile vials. Furthermore, samples of 5 grams of pectoral muscle were obtained and stored at -20 °C along with plasma until processing. Determination of plasma and muscle concentrations of MBFX was taken out by HPLC, using the modified technique of Bottcher et al. (2001).

HPLC conditions: MBFX and internal standard (enrofloxacin 2.5 µg/ml) were eluted isocratically with a flow rate of 0.8 ml/min using a mobile phase consisting of deionized water, acetonitrile, and triethylamine (790:200:10 V/V/V) adjusted to pH 3 with phosphoric acid. Calibrating the wavelength of fluorescence detector at 295 nm (excitation) and 490 nm (emission), respectively.

Sample preparation: The modified method of Böttcher et al. (2001) was used for the quantification of MBFX in plasma and tissue samples. Plasma samples were prepared by placing them in an Eppendorf tube 150 µL of plasma, 600 µL of extraction solution (ES) consisting of deionized water, methanol and perchloric acid in a ratio (500: 500: 1 v/ v/ v), 40 µL of internal standard, and 150 µL of deionized water. underwent agitation by vortex for 30 seconds, and then they were/it was centrifuged at 13,500 rpm for 25 min at 4°C. The obtained supernatant was filtered on nylon membrane 0.22 µm. A similar process/procedure was used for muscle samples, taking 150 mg of muscle to which 600 µL of ES, 40 µL of internal standard, and 150 µL of deionized water were added; the samples were mechanically homogenized for 1 minute and kept in refrigeration at 4°C for 12 hours for subsequent centrifugation for 25 min at 13,500 rpm at 4°C, filtering the supernatant through 0.22

µm nylon membrane.

Calibration of the chromatographic method: For testing, plasma and muscle samples were used, added with known MBFX concentrations from 0.009 to 2.5 µg/ml for plasma and 0.004 to 1.25 µg/mg for muscle. Determining the degree of adjustment of the area values index and their respective calibration by linear regression.

Data analysis: MBFX average concentrations, obtained in plasma at different times and routes, were processed by means of PK Solution 2.0 kinetic software (Farrier, 1999), in order to obtain plasma pharmacokinetic parameters. Oral bioavailability (F) was determined using the equation F = (AUCoral) / (AUCiv) (Baggot, 2002; Bousquet-Melou and Toutain, 2004).

Withdrawal period in muscle was performed by analysis of muscle residues, using the EMEA WT 1.4 Software (Hekman and Hoogland, 1996), with a confidence interval of 95%. Maximum residue limit (MRL) was established in 150 µg/kg, according to FDA and EMEA directives for MBFX.

Results

Calibration method: The method proved to be linear in the examined interval (r2 > 0.99). Intra and interassay coefficients of variation were < 1.73%. 50 µl of plasma and muscle injection provides the quantitation limits of 0,006 µg/ml and 0.07 µg/mg, respectability. Recovery tests performed to establish the precision of the extractive method in plasma samples showed a percentage of 86%, whereas in muscle it was 87%.

Intravenous and oral administrations of marbofloxacin: Figure 1 shows mean (±SD) plasma and muscle marbofloxacin concentrations versus time, whereas in Table 1 mean (±SD) values for intravenous and oral plasma pharmacokinetics parameters are shown. After a 2 mg/kg single oral dose, marbofloxacin was

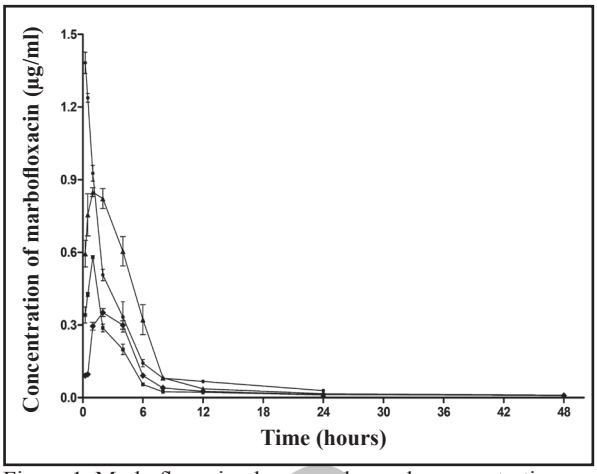

Figure 1. Marbofloxacin plasma and muscle concentration vs. time profiles, after intravenous and oral 2 mg/kg single dose in chickens (data as mean ± SD). i.v plasma —■— oral plusma —●— i.v muscle —◆— oral plusma —▲—

Table 1. Mean ± SD marbofloxacina pharmacokinetics parameters, after intravenous and oral 2 mg/kg single dose in chickens. Abbreviations: Cmax, maximum plasma concentration; Tmáx time to peak concentration; t½abs, absorption half-life; t½α distribution half-life; t½α elimination half-life; AUC, area under the curve; F (%), bioavailability; Vd area, volume of distribution; MRT, mean residence time and Cl, plasma clearance.

Pharmacokinetic parameters	IV	Oral
Cmax(µg/ml)	-	0.8
T*max* (h)	-	1.0
t½abs (h)	-	0.12
t½α (h)	0.9	2.88
t½α (h)	6.45	4.71
AUC (µg-h/ml)	4.8	4.7
F%	-	98
Vdarea L/kg	3.88	2.83
MRT (h)	6.9	4.9
Clt (L/h/kg)	0.416	0.417

quickly absorbed, resulting in 0.8 µg/mlCmax, evidenced by Tmax and t½abs values of 1 and 0.126 hours respectively in plasma. It was also rapidly and widely distributed after intravenous administration, reflected by a 0.9 hour t½α and a 3.88 L/kg Vdárea, suggesting good tissue penetration; removal was slow (t½β 6.45 hours) and the plasma clearance (Clt) was 6.94 ml/min/kg.

Marbofloxacin analysis in muscle: Orally, MBFX residues were detected in muscle

up to 48 hours after administration, reaching a maximum concentration of 0.351 µg/mg 2 hours post-administration, while intravenously, the maximum concentration was 0.342 µg/mg, and residues were detected until 24 hours post-application (Fig. 1).

Withdrawal time for MBFX in muscle was estimated to be 2.5 days.

Discussion

Plasma kinetic parameters indicate that marbofloxacin oral administration has rapid absorption, reflected by a Cmax of 0.8 µg/ml, 1 hour post-administration, similar to 1.05 µg/ml obtained by Anadón et al. (2002), but less than 1.84 µg/ml obtained by Huang et al. (2003) at higher doses (2.5 mg/kg). Yuan et al., 2011 showed a Cmax of 1.13 µg/ml in ducks with a of 2.5 mg/g dose, meanwhile Haritova et al. (2003) reported 0.67 µg/ml in turkeys with a dose of 2 mg/kg, reaching Tmax 1 hour post-administration, similar to Anadón et al., 2002, Huang et al., 2003, and Ding et al., 2013 in chickens. The volume of distribution indicates a high tissue passage after intravenous application, greater than that obtained by Anadón et al. (2002) and Huang et al. (2003).

Under the rapid dissolution in intestinal media of fluoroquinolones (Kim & Nightingale, 2000; Scholar, 2002), marbofloxacin plasma concentrations could be measured from 15 minutes to 48 hours after oral administration, and these data were consistent with other studies conducted with these drugs in chickens (Anadón et al., 2002; Huang et al., 2003).

The AUCárea obtained in the study, 4.8 mg-h/ml, is lower than in chickens. Anadón et al. (2002) observed values of 10.5 mg-h/ml, giving 2 mg/kg repeated for three days, whereas Ding et al. (2013) obtained 9.8 g-h/ml, in infected animals at 5 mg/kg in repeated doses. Since fluoroquinolones are dose-dependent antimicrobials, treatments with high and repeated doses may increase the AUC.

Oral bioavailability (F= 98%) is similar to 95% reported by Huang et al. (2003), but higher than 56.8% cited by Anadón et al. (2002); this difference is attributable to the 12 hour fasting.

The high dissolution in the intestinal environment and the amphoteric character of marbofloxacin must be considered.

Plasma t½β was 6.4 hours, similar to that presented in other studies with MBFX in chickens by Anadón et al. (2002), Huang et al. (2003), and Ding et al. (2013), in which the time periods were 5.26, 6.48, and 6.8 hours, respectively. An oxadiazinic ring in MBFX structure determinates the pharmacokinetic characteristics associated with elimination half-life and bioavailability (Haritova et al., 2006), showing a moderate plasma permanence reflected by t½β and TMR. These data are consistent with the history of fluoroquinolones in poultry (Anadón et al., 2002; Ding et al., 2013) with greater tissue retention.

Marbofloxacin kinetic profile after oral and intravenous administration is consistent with that described by Anadón et al. (2002), Huang et al. (2003), Haritova et al. (2006), Yuan et al. (2011), and Ding et al. (2013) for this drug in birds.

After oral administration, muscle disposition occurs quickly detecting levels at 15 minutes and after 72 hours post-application (Anadón et al., 2002; Huang et al., 2003; Ding et al., 2013). The disposition curve versus time in muscle shows the most significant levels between 1 and 4 hours; considerable tissue levels are attributable to reduced plasma proteins binding, lipid solubility, and amphoteric characteristics determining poor ionization of the drug in blood thus facilitating passage through membranes, and slower elimination compared to the plasma (Thomas et al., 2000; Anadón et al., 2002; Baggot, 2002; Guiguére et al., 2013.)

The withdrawal period of 2.5 days agrees with that calculated by Anadón et al. (2002), but is less than that presented by Yang et al.

(2014), estimated in four days but not calculated with the software used here. The withdrawal period of this experience, which influences when choosing an antimicrobial in poultry, is reasonable and consistent with the brevity of broiler production cycles.

References

1. Anadón, A., Martinez-Larrañaga, M.R., Díaz, M.J., Martinez, M.A., Frejo, M.T., Martínez, M., Tafur, M., Castellano, V.J. (2002) Pharmacokinetic characteristics and tissue residues for marbofloxacin and its metabolite N-desmethyl marbofloxacin in broiler chickens. Am J Vet Res. 63: 927-93.
2. Baggot, J.D. (2002) En: Terapéutica Antimicrobiana Veterinanria: Principios de BiodiSponibilidad Y Disposición De Los Fármacos Antimicrobianos. Prescott J. (ed.). (3rd ed.) Buenos Aires, Argentina p. 43-76.
3. Böttcher, S., Baum, H., Hoppe-Tychy, T., Benz, C. (2001) An HPLC assay as a microbiological assay to determine levofloxacin in soft tissue, bone, bile and serum. J Pharm Biomed Anal. 25: 197-203.
4. Ding, H., Wang, L., Shen, X., G.U, X., Zeng, D., Zeng, Z. (2013) Plasma and tissue pharmacokinetics of marbofloxacin in experimentally infected chickens with Mycoplasma gallisepticum and *Escherichia coli*. J Vet Pharmacol Therap. 36: 511-515.
5. Farrier, D.S. (1999) PK Solutions 2.0, Noncompartmental Pharmacokinetics. Data Analysis. Ashland, USA.
6. García, M.A., Solansa, C., Aramayonab, J.J., Ruedab, S., Breganteb, M.A. (1999) Determination of marbofloxacin in plasma samples by high-performance liquid chromatography using fluorescence detection. J Chromatogr B. 729: 157-161.
7. Giguére, S., Prescott, J., Dowling, P., (2013) Antimicrobial Therapy in Veterinary Medicine. John Wiley and Sons. (eds.). (5th ed.) Wiley Blackwell. Iowa, USA.
8. Goudah, A., Hasabelnaby, S. (2010) The disposition of marbofloxacin after single dose intravenous, intramuscular and oral administration to Muscovy duck. J Vet Pharmacol Therap. 34: 197-201.
9. Haritova, A.M., Dimitrova, D., Dinev, T., Moutafchieva, R., Lashev, L. (2013) Comparative pharmacokinetics of enrofloxacin, danofloxacin and marbofloxacin after intravenous and oral administration in Japanese Quail (*Conturnix conturnix japónica*). J avian Med Surg. 1: 23-31.
10. Haritova, A.M., Rusenova, N.V., Parvanov, P.R., Lashev, L.D., Fink- Gremmels, J. (2006) Integration of pharmacokinetic and pharmacodynamic indices of marbofloxacin in turkeys. Antimicrob Agents Chemother. 50: 3779-3785.
11. Hekman, P. (1996) WT1.4 Withdrawal time calculation program. Freeware, distributed by the European Agency for the Evaluation of Medicinal Products (EMEA), London, UK.
12. Huang, X.H., Chen, Z.L., Zhang, S.T., Zeng, Z.L. (2003) Influence of experimentally *Pasteurella multocida* Infection on the pharmacokinetics of marbofloxacin in broiler chickens. Acta Veterinaria et Zootechnica Sinica. 34: 98-102.
13. Kim, M., Nightingale, C. (2000) The Quinolones:Pharmacokinect and pharmacodynamic of the fluoroquinolones. Andriole, V. (ed.) (3rd ed.) California, USA. p. 169-193.
14. Martinez, M., McDermott, P., Walker, R. (2006) Pharmacology of the fluoroquinolones: a perspective for the use in domestic animals. Vet J. 172: 10-28.
15. Shan, Q., Wang, J., Yang, F., Ding, H., Liang, C., Lv, Z., Li, Z., Zen, Z. (2013) Pharmacokinetic/pharmacodynamic relationship of marbofloxacin against *Pasteurella multocida* in a tissue-cage model in yellow cattle. J Vet Pharmacol Therap. 37: 222-230.
16. Sidhu, P.K., Landoni, M.F., AliAbadi F.S., Lees, P. (2010) Pharmacokinetic and phar-

macodynamic modelling of marbofloxacin administered alone and in combination with tolfenamic acid in goats. Vet J. 184: 219-229.
17. Spreng, M., Deleforge, J., Thomas, V., Boisram_e, B., Drugeon, H. (1995) Antibacterial activity of marbofloxacin. A new fluoroquinolone for veterinary use against canine and feline isolates. J Vet Pharmacol Therap. 18: 284-289.
18. Thomas, E., Caldow G.L., Borell, D., Davot J.L (2001) A field comparison of the efficacy and tolerance of marbofloxacin in the treatment of bovine respiratory disease. J Ve Pharmacol Therap. 24: 353-358.
19. Thomas, E., Grandemange, E., Pommier, P., Wessel-Robert, S., Davot, J.L (2000) Field evaluation of efficacy and tolerance of a 2% marbofloxacin injectable solution for the treatment of respiratory disease in fattening pigs. Vet Q. 22: 131-135.
20. Toutain, P., Bousquet-Mélou, A. (2004) Bioavailability and its assessment. J Vet Pharmacol Therap. 27: 455-466.
21. Yang, F., Yang, Y.R., Wang, L., Huang, X.H., Qiao, G., Zeng, Z.L. (2014) Estimating marbofloxacin withdrawal time in broiler chickens using a population physiologically based pharmacokinetics model. J Vet Pharmacol Therap. 37: 579-588.
22. Yuan, L.G., Wang, R., Sun, L.H., Luo, X.Y., Sun, J., Fang, B.H., Liu, Y.H. (2011) Pharmacokinetics of marbofloxacin in Muscovy ducks (*Carina moschata*). J Vet Pharmacol Therap. 30: 10-15.

مجله طب دامی ایران، ۱۳۹۵، دوره ۱۰، شماره ۱، ۶-۱

فارماکوکینتیک ماربوفلوکساسین در پلاسما وعضله جوجه‌های گوشتی

ناتالی ارزوا[۱] کارلوس اراکالده[۱] گویلرمو پریتو[۱] کارلوس لودرس[۲] ادواردو پیکو[۳] ماریا پاوئلا تونینی[۱]

۱) گروه فارماکولوژی، دانشکده کشاورزی و دامپزشکی، دانشگاه ملی ریو کوارتو، ریوکوارتو، آرژانتین
۲) دانشکده دامپزشکی، دانشگاه کاتولیک تموکو، تموکو، شیلی
۳) گروه فارماکولوژی، دانشکده علوم دامپزشکی، دانشگاه ملی لیتورال، اسپرانزا، آرژانتین

(دریافت مقاله: ۱۹ فروردین ماه ۱۳۹۴، پذیرش نهایی: ۲۷ خرداد ماه ۱۳۹۴)

چکیده

زمینه مطالعه: گزارشات اندکی در مورد فارماکوکینتیک ماربوفلوکساسین در پرندگان وجود دارد و یک داروی مفید در پرورش طیور است. **هدف:** تعیین پارامترهای کینتیک ماربوفلوکساسین پس از تزریق داخل وریدی و تجویز خوراکی در پلاسما و عضله و تخمین دوره قطع دارو می باشد. **روش کار:** جوجه‌هایی که از نظر کلینیکی سالم بودند با محدوده وزنی ۰/۲۲kg±۱/۰۸ در دسته‌های ۵ تایی در ۲ گروه استفاده گردید. در گروه A (n=۴۵۰)، ۲mg به ازای هر کیلوگرم ماربوفلوکساسین به صورت داخل وریدی دریافت کردند در حالیکه گروه B (n=۶۵) همان دوز از دارو را پس از ۱۲ ساعت محدودیت غذایی بصورت خوراکی دریافت کردند. نمونه‌های خون و ۵g بافت عضله در زمان‌های مختلف (در گروه آ تا ۲۴ ساعت و در گروه ب تا ۱۲۰ ساعت پس از تجویز دارو) جمع آوری شدند. ماربوفلوکساسین بوسیله (اچ پی ال سی) اندازه گیری شد. **نتایج:** ماربوفلوکساسین در جوجه‌های تحت محرومیت غذایی تقریباً بطور کامل جذب گردید. (F=٪۹۸) متوسط زمان باقی ماندن آن ۶/۹ ساعت همراه با یک حجم پخش بالا بود. در تجویز خوراکی، میانگین زمان باقی ماندن در پلاسما کمتر بود (۴/۹ ساعت). زمان‌های قطع دارو تا ۲/۵ روز محاسبه شد. **نتیجه‌گیری نهایی:** ویژگی‌های فارماکوکینتیک ماربوفلوکساسین شبیه سایر فلوروکینولون‌ها در طیور بود و برای دوره‌های تولید کوتاه مناسب می‌باشد.

واژه های کلیدی: جوجه گوشتی، HPLC، ماربوفلوکساسین، فارماکوکینتیک

---

*) نویسنده مسؤول: تلفن: ۴۶۴۱۹۸۶ (۰۳۵۸)۵۴+ نمابر: ۴۶۸۰۲۸۰ (۰۳۵۸)۵۴+ Email: gprieto@ayv.unrc.edu.ar