IN-VITRO PLASMA PROTEIN BINDING OF MARBOFLOXACIN IN SHEEP[#]

Pratishtha Sharma[1], Amita Ranjan, Ashok Gaur, Sunita Choudhary[2], Virendra Kumar[3] and A. P. Singh[4]
Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Science
Rajasthan University of Veterinary and Animal Sciences, Bikaner- 334001, Rajasthan, India

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ABSTRACT

Fluoroquinolones are important and popular antimicrobial agents used in veterinary medicine. Marbofloxacin is a broad-spectrum fluoroquinolone antimicrobial agent specially developed for use in veterinary medicine. The plasma protein binding is one of the most important factors, affecting significantly the overall pharmacokinetic and pharmacodynamic profile of a drug. Plasma protein binding (PPB) estimation is important for determination of drug's efficacy and safety during each drug-development programme. Interspecies differences in plasma protein binding can lead to a reduced or increased drug safety margin. In the present study we evaluated the plasma protein binding of marbofloxacin in *Bikaneri Chokla* sheep. *In vitro* binding of marbofloxacin to plasma proteins was determined by employing the equilibrium dialysis technique and further analyzed by microbiological assay method using *Escherichia coli* ATCC 25922 as test organism. The plasma protein binding in sheep ranges between 19.62 ± 0.85 and 22.08 ± 0.65 per cent with an overall binding of 21.11 ± 0.50 per cent.

Key words: Marbofloxacin, plasma protein binding, sheep

Introduction

Marbofloxacin is a fluoroquinolone antimicrobial drug used in many countries for the treatment various infections in cattle, pigs, dogs, horses and cats. It has approval in Europe for oral or parenteral administration to cattle, including lactating dairy cattle, for treatment of bovine respiratory disease at 2 mg/kg body weight for 3-5 days, or a single dose administered once at 8-10 mg/kg and parenteral administration to pigs for the treatment of Mastitis Metritis Agalactiae syndrome (CVMP, 1996, 1999; Papich, 2016). Marbofloxacin is approved for use in dogs and cats in the United States of America. Being a fluoroquinolone, marbofloxacin acts by inhibition of bacterial DNA gyrase and demonstrate a concentration-dependent bactericidal effect, so high initial plasma concentration is important (Baroni *et al.*, 2007). Of late, marbofloxacin is commercially available in India as a 10 percent w/v ready to use injectable formulation and as 50 mg tablet for oral administration, for veterinary use.

Plasma is a complex fluid part of blood which provides the medium of exchange between the blood vessels and the cells of the body. Next to water, plasma proteins are the most abundant of the substances dissolved or suspended in the water, and their concentration varies from 6 to 8 g/dL (Reece, 2015). It has high concentrations of proteins *viz.* albumin, alpha acid glycoprotein, lipoproteins like gamma-globulins *etc.,* and numerous drugs show high propensities to bind with one or more of these either specifically or non-specifically (Bohnert and Gan, 2013). Although there may be many components in plasma that arecapable of binding drugs, two proteins, in particular serum albumin and α_1-acid glycoprotein, are present in sufficient quantity and are capable of binding a broad variety of drugs with sufficient affinity to have a significant effect on drug action (Trainor, 2007).The plasma protein binding is one of the most important factors, affecting significantly the overall pharmacokinetic and pharmacodynamic profile. Plasma protein binding is generally believed one of the factors governing the concentration of free, therapeutically active drug in plasma as it controls the free drug concentrations in plasma and in those compartments in steady-state equilibrium with plasma, and thus, is a key factor in the translation of *in vitro* biochemical activity into *in vivo* pharmacological activity. If the binding of the drug is very high, plasma proteins act as drug reservoirs in the body, prolonging metabolism and elimination. Therefore, protein binding is one of the important determinants affecting transfer of drugs across the biological membranes and thus affecting the transfer of drug to different body tissues and fluids or the target site(s). Drugs with an excessively high affinity for plasma proteins (>95% bound) may require higher doses to achieve the effective concentrations *in vivo*, and usually have restricted distribution to sites of action. Changes in the binding affinity may result in unforeseen changes in the key parameters for setting up dosing regimen like apparent volume of distribution, clearance, half-life, and bioavailability (Zhivkova and Doytchinova, 2012). Plasma protein binding (PPB) is an important parameter for a drug's efficacy and safety that needs to be estimated during each drug-development programme (Buscher *et al.*, 2014). Interspecies differences in plasma protein binding can lead to a reduced or increased drug safety margin (Trainor, 2007).

Protein binding of drugs is indeed species dependent and varied results are also possible within species when researchers report it working from different places in the world. It may differ in healthy and diseased states, pyrexia, in the diseases affecting protein levels in blood. In different animal species, various previous investigators have reported low to moderate protein binding values for marbofloxacin as 15-28 per cent (dogs), below 30 per cent (calves), 29 ± 2.2 per cent and 27 ± 2.6 per cent (healthy and diseased calves, respectively), 26 ± 1.3 per cent (rabbits),16.5-21.8 per cent (muscovy ducks; with an average of 18.4%) when searched

[#1]Part of Ph.D. Thesis work of first author and corresponding author email: drpratishthasharma@gmail.com; [2]Assistant Professor, Dept. of Clinical Veterinary Medicine, E & J; [3]In-charge and Asstt. Professor, Livestock Research Station (LRS), RAJUVAS, Kodamdesar, Bikaner; [4]Principal Investigator, Centre for Ethno-veterinary Practices and Alternative Medicines, RAJUVAS, Bikaner

for, in respective literatures (Aliabadi and Lees, 2002; Bidgood and Papich, 2005; Ismail and El-Kattan, 2007; Abo-El-Sooud and Goudah, 2010; Goudah and Hasabelnaby, 2010). Serum protein binding of marbofloxacin was reported as 49.4 ± 9.61 per cent (mean ± SD) in pigs and was independent of concentration (Dorey *et al*., 2017) while in foals it was reported as 27.5 per cent (Tohamy and El-Gendy, 2013). In the aim to rationalise dosage regimen of the drug, the presented study was undertaken as an independent part, with the objective to determine the *in vitro* plasma protein binding of marbofloxacin in *Bikaneri Chokla* sheep.

Materials and Methods

Blood samples were drawn in anticoagulant coated tubes, from clinically healthy sheep those were not given any drug treatment, and plasma was harvested from the blood samples. The total protein values were estimated from small part of individual blood samples and the same were found to be within normal range of 72-75 g/litre. The plasma samples were pooled and the pooled plasma was used for *in vitro* plasma protein binding studies of marbofloxacin.

In vitro plasma protein binding of marbofloxacin was determined by equilibrium dialysis technique (Kunin *et al.*, 1959) which is still regarded as most popular (Buscher *et al*., 2014). Dialyzing bags having pore size 4 Å were washed with plain water and then soaked overnight in 0.2 M phosphate buffer of pH 7.4. Pooled plasma was spiked with marbofloxacin *in vitro* to prepare known plasma concentrations of 0.625, 1.25, 2.5, 5, 10 and 20 µg/ml of the drug. Three dialyzing bags, tied at one end, were charged with 5 ml volume of each of these concentrations so as to make triplicates of each concentration. After filling, the bags were tied at the other end too and each was separately immersed in equal volume of phosphate buffer (5 ml) in separate test tubes. The tubes were incubated at 37°C for 24 h with intermittent shaking. At the end of incubation period, buffer as well as contents of the dialyzing bags were collected separately and analyzed for the concentration of marbofloxacin by microbiological assay using *E. coli* ATCC25922 as test organism (Dinodia *et al*., 2019). The extent of *in vitro* plasma protein binding of marbofloxacin was calculated with the help of the following equation:

$$\text{Per cent protein binding} = \frac{CP' - CB}{CP} \text{ (Kunin } et\ al.\text{,1959; Kunin, 1965)}$$

Where,

CP' - Concentration of marbofloxacin in plasma after incubation;

CB - Concentration of marbofloxacin in buffer after incubation; and

CP - Concentration of marbofloxacin in plasma before incubation

Results and Discussion

The result are presented in Table 1. The plasma protein binding of marbofloxacin in sheep plasma ranged between 19.62 ± 0.85 and 22.08 ± 0.65 per cent and it did not appear to significantly increase or decrease with increased *in vitro* concentrations. The overall plasma protein binding of marbofloxacin in sheep was found to be 21.11± 0.50 per cent.

The plasma protein binding of marbofloxacin sheep estimated in the present study was comparable to that in dogs (Aliabadi and Lees, 2002) while higher than that reported in Muscovy ducks (Goudah and Hasabelnaby, 2010). Higher plasma protein binding values have been reported in calves (Ismail and El-Kattan, 2007), buffalo calves (Singh *et al*., 2019), mice (Qu *et al*., 2015), and rabbits (Abo-El-Sooud and Goudah, 2010). Similarly, higher serum protein binding of marbofloxacin was reported in foals (Tohamy and El-Gendy, 2013) and pigs (Dorey *et al*., 2017).

Table 1: *In vitro* plasma protein binding of marbofloxacin in sheep.

Marbofloxacin (µg/ml)	Set I	Set II	Set III	Mean ± SE
0.625	19.46	21.17	18.24	19.62 ± 0.85
1.25	20.82	19.84	21.72	20.79 ± 0.54
2.50	21.22	20.86	22.14	21.41 ± 0.38
5.00	20.42	23.31	21.48	21.74 ± 0.84
10.00	18.96	23.12	20.94	21.04 ± 1.20
20.00	23.06	20.86	22.32	22.08 ± 0.65
Overall protein binding: 21.11 ± 0.50%				

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