**In-vitro** Plasma Protein Binding of Marbofloxacin in Healthy and Disease Condition of Buffalo Calves

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**ABSTRACT**

Marbofloxacin is a broad-spectrum fluoroquinolone antibiotic developed for use in veterinary medicine for the treatment of skin and soft tissue infections in dogs and cats. Plasma protein binding plays a vital role in distribution, elimination and therapeutic effectiveness of drugs. In the present study we evaluated the plasma protein binding of marbofloxacin in healthy and liver dysfunctioned buffalo calves. In vitro binding of marbofloxacin to plasma proteins was determined by employing the equilibrium dialysis technique and further analyzed by High Performance Liquid Chromatography assay. The plasma protein binding for healthy calves ranges between 25.3±0.34% to 30.4±0.40% with an overall binding of 28.66 ± 0.421%. Kinetic constants ($\beta_i$) and ($K_{\beta}$) was 2.6±0.12×10^-8 mole/g and 1.9±0.08×10^-7 mole, respectively. The percentage of plasma protein binding for liver dysfunctioned buffalo calves extended from 24.5 - 30.3% with an overall mean of 28.59 ± 0.693%. The binding capacity of the drug to plasma proteins ($\beta_i$) and dissociation rate constant of protein drug complex ($K_{\beta}$) were 2.53±0.13 10^-5 mole/g and 1.94±0.09×10^-6 mole respectively. There was no significant change observed in plasma protein binding and the kinetic constant of liver dysfunctioned buffalo calves when compared to the healthy group.

**Keywords:** Marbofloxacin, liver dysfunction, protein binding, buffalo calves

Marbofloxacin (MBX) is a veterinary fluoroquinolone antibiotic used mainly in companion animals. However, their pharmacological profiles in large animals have not been carefully evaluated previously (Mahmood, 2013). Marbofloxacin was developed for use in veterinary medicine, and is approved in the USA for the treatment of skin and soft tissue infections in dogs and cats and urinary tract infections (i.e. cystitis) in dogs. This fluoroquinolone is a synthetic, broad spectrum antibacterial agent which impairs the bacterial DNA gyrase, thus causing rapid bactericidal activity (Plumb’s, 2011). In mammals, almost 100% of the oral dose of marbofloxacin is eliminated unchanged, with approximately 40% excreted in the urine and 60% via bile in the feces (Hunter et al., 2007). The liver plays a central role in the drug metabolism and pharmacokinetics of the majority of drugs. Liver dysfunction may not only reduce the blood/plasma clearance of drugs eliminated by hepatic metabolism or biliary excretion, it can also affect plasma protein binding, which in turn could influence the processes of distribution and elimination (Roger, 2008). Drug binding to plasma proteins is a determinant of drug disposition. A change in drug binding causes an alteration of drug distribution and elimination (Yacobi et al., 1979). Plasma protein binding plays a vital role in distribution, elimination and therapeutic effectiveness of drugs. The binding properties of plasma proteins and their concentrations may vary depending on gender, age/or disease state of patients. The drug is primarily metabolized in the kidneys; only 10 to 15% is metabolized in the liver. In humans, laboratory animals and in pigs marbofloxacin was weakly bound to plasma proteins (<10%) binding was higher in cattle (around 30%), it was excreted mostly in the urine (EMEA, 1996). In vitro plasma protein binding of marbofloxacin in
dogs was 9.1% and in cats was 7.3% (Zeniquin®, 2013). As per our best knowledge and information gathered, there is paucity of information regarding plasma protein binding of marbofloxacin in buffalo species. In the present study, we determined the changes in the plasma protein binding in liver dysfunctioned buffalo calves with respect of healthy calves.

MATERIALS AND METHODS

Experimental animals

The experiment was conducted on four male buffalo calves (6 months to 1 year) procured from institutional livestock farm complex, College of veterinary science, GADVASU. The study was approved by Institutional Animal Ethics Committee (Order No. VMC/14/1046-73 dated 7.04.2013). After procurement animals were placed to acclimatize in the animal shed of the department under standard ambient conditions. Animals were given standard quality concentrate on body weight basis, green fodder as per the availability of the season and clean water were provided ad libitum throughout the experiment.

Induction of hepatic dysfunction

Liver dysfunction was induced in buffalo calves by intramuscular administration of paracetamol. All the animals were weighed empty stomach before conducting the experiment for dose calculation on body weight basis. The dosage schedule of paracetamol was 250 mg.kg\(^{-1}\) B.W. on day 1, followed by 2 subsequent doses of 50 mg.kg\(^{-1}\) B.W. on day 3 and day 5 (Sharma and Ul-Haq, 2012). The extent of liver dysfunction was assessed by daily estimation of plasma levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma GlutamylTransferase (GGT), Total and Direct Bilirubin, Cholesterol, Albumin, Alkaline phosphatase (ALP) and Amylase.

Estimation of biochemical parameters in hepatic dysfunctioned buffalo calves

Blood samples were collected in heparinized vials from the jugular vein of hepatic dysfunctioned animals on 0, 1, 2, 3, 4, 5 and 6\(^{th}\) day from the start of paracetamol administration. Plasma was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20 °C till further analysis. Biochemical parameters including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Albumin, Alkaline phosphatase (ALP) and Amylase were estimated using Bayer Autopack kits and Gamma Glutamyl Transferase (GGT) was estimated using Gamma-GT FS, while Cholesterol was estimated using Erba diagnostic kits.

In vitro plasma protein binding

In vitro binding of marbofloxacin to plasma proteins was determined by employing the equilibrium dialysis technique (Gupta et al., 2006). The dialyzing bags (4 Å pore size), 10 cm long were washed in running tap water and soaked overnight in phosphate buffer. Marbofloxacin concentrations 2.5, 5, 10, 25, 50 and 100 µg ml\(^{-1}\) were prepared in pooled plasma separated from blood taken from healthy and hepatic dysfunctioned animals. Each dialyzing bag was knotted at one end before filling 5 ml of plasma containing known amount of drug and the other end was then securely tied. Each bag was immersed in separate tubes containing 5 ml of phosphate buffer (0.2 M; pH 7.4; disodium hydrogen phosphate 11.3 g, potassium-dihydrogen phosphate 2.7 g, added to 1000 ml of distilled water) and the tubes were incubated at 37°C for 24 h with intermittent shaking. At the end of incubation period phosphate buffers as well as contents of the dialyzing bags were separately analyzed for the concentration of marbofloxacin by High Performance Liquid Chromatography assay in triplicate. The extent of in vitro plasma protein binding of marbofloxacin was calculated by the following equation.

\[
\text{Percent of marbofloxacin bound to plasma protein} = \frac{CP' - CB}{CP} \times 100
\]

where, CP’ = Concentration of marbofloxacin in plasma after incubation.

CB = Concentration of marbofloxacin in buffer after incubation.

CP = Concentration of marbofloxacin in plasma before incubation.
Binding capacity of the plasma protein to marbofloxacin ($\beta_i$) and the dissociation rate constant of protein drug complex ($K_p$) were calculated by the method of Pilloud (1973).

**Statistical Analysis**

The differences between means based on individual observation were determined by t-test and further significance was tested by using Duncan’s multiple range test. All the statistical calculations were done by using SPSS® version 20 software package. The significance was assessed at P < 0.05 and P < 0.01 level (Singh et al., 1991).

**RESULT AND DISCUSSION**

*In vitro* plasma protein bindings in healthy buffalo calves

*In vitro* plasma protein binding and kinetic constants are given in Table 1. The overall binding (28.66 ± 0.421%) was similar as documented for cattle (30%) in a report of European Medical Evaluation Agency (EMEA, 1996). The similar findings has also reported in foals where the serum protein binding was 27.5%, indicating that a single shot of marbofloxacin once daily could be useful in the treatment of diseases caused by sensitive pathogens. However lower binding percentage in dog (9.1%) and cat (7.3%) was reported in a document of research lab (Zeniquin®, 2013). The plasma protein binding was 31.7–36.8% in dog and 19.1 ± 1.5% in buffalo calves for Difloxacin and Levofoxacin respectively, belongs to similar category of fluoroquinolones (Ismail, 2007 and Ram et al., 2008). The binding capacity of drug to plasma proteins ($\beta_i$) and dissociation rate constant of protein drug complex ($K_p$) of the drug-protein complex quantitatively describe the drug protein interaction. The ($\beta_i$) and ($K_p$) was $2.6 \pm 0.12 \times 10^{-8}$ mole/g and $1.9 \pm 0.08 \times 10^{-7}$ mole, respectively. The higher value of $\beta_i$ than $K_p$ indicated that binding of marbofloxacin to plasma proteins was relatively faster than dissociation of protein drug complex in buffalo calves.

*In vitro* plasma protein binding of marbofloxacin in liver dysfunctioned buffalo calves

The extent of *in vitro* plasma protein binding of marbofloxacin in liver dysfunctioned buffalo calves is represented in Table 2. The percentage of plasma protein

| Table 1: *In vitro* plasma protein binding and kinetic constant of marbofloxacin in healthy buffalo calves |
| Expt. No. | Marbofloxacin concentration (µg/ml) | Protein binding (%) |
|-----------|-------------------------------------|---------------------|
|           | 2.5 | 5 | 10 | 25 | 50 | 100 |
| 1         | 25.3 | 28.7 | 27.5 | 29.8 | 29.2 | 30.1 |
| 2         | 24.7 | 29.1 | 28.4 | 30.1 | 31.1 | 31.2 |
| 3         | 25.9 | 27.8 | 29.1 | 29.4 | 28.7 | 29.9 |
| Mean±SE   | 25.3±0.34 | 28.5±0.38 | 28.3±0.46 | 29.7±0.20 | 29.6±0.73 | 30.4±0.40 |

$\beta_i = 2.6 \pm 0.12 \times 10^{-8}$, $K_p = 1.9 \pm 0.08 \times 10^{-7}$; $\beta_i$= Association rate constant (mol/kg) and $K_p$= Dissociation rate constant (mol); Overall (Mean ± SEM) binding = 28.66 ± 0.421%

| Table 2: *In vitro* plasma protein binding of marbofloxacin in Liver dysfunction buffalo calves |
| Expt. No. | Marbofloxacin concentration (µg/ml) | Protein binding (%) |
|-----------|-------------------------------------|---------------------|
|           | 2.5 | 5 | 10 | 25 | 50 | 100 |
| 1         | 24.3 | 27.5 | 28.1 | 30.8 | 29.3 | 31.7 |
| 2         | 25.8 | 29.5 | 28.6 | 27.8 | 30.6 | 28.6 |
| 3         | 23.5 | 27.9 | 29.7 | 28.9 | 31.4 | 30.7 |
| Mean±SE   | 24.5±0.67 | 28.3±0.61 | 28.8±0.47 | 29.1±0.87 | 30.4±0.61 | 30.3±0.91 |

$\beta_i = 2.53 \pm 0.13 \times 10^{-5}$, $K_p = 1.94 \pm 0.09\times 10^{-6}$; Overall (Mean ± SEM) binding = 28.59 ± 0.693%. 

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binding extended from 24.5 - 30.3% with an overall mean of 28.59 ± 0.693%. There was no significant change observed in the plasma protein binding of marbofloxacin in liver dysfunctioned buffalo calves as compared to healthy group. Similar to this study the liner marbofloxacin kinetics over a dose ranging from 1.25 to 10mg/kg of body weight was observed in a neutropenic infected mice and the protein binding in the plasma was 29.77% (Qu et al., 2015). The binding capacity of drug to plasma proteins (βi) and dissociation rate constant of protein drug complex (Kβ) were 2.53±0.13 10^{-5} mole/g and 1.94±0.09×10^{-6} mole respectively. There was no significant change observed in plasma protein binding and kinetic constant of liver dysfunctioned buffalo calves when compared to healthy group. As per my best knowledge no experimental data is available for plasma protein binding of marbofloxacin in liver dysfunctioned buffalo calves.

CONCLUSION

In the present study in vitro plasma protein and kinetic constant of marbofloxacin did not show any significant change in healthy and liver dysfunctioned buffalo calves, hence no need of alteration in recommended dose. Although as per my best knowledge no experimental data is available for plasma protein binding of marbofloxacin in liver dysfunctioned buffalo calves but further study is recommended.

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