# COMPARATIVE PHARMACOKINETIC/PHARMACODYNAMIC INTEGRATION OF ENROFLOXACIN WITH MARBOFLOXACIN IN GOATS

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## ABSTRACT

Comparative pharmacokinetic/pharmacodynamic integration of enrofloxacin (5.0 mg/kg.b.wt.) with marbofloxacin (2.0 mg/kg.b.wt.) were studied after intramuscular administration in goats.

The two drugs were rapidly absorbed within 1 hour and achieved average peak plasma concentration 1.18±0.04 μg/ml for enrofloxacin and 1.37±0.07 μg/ml for marbofloxacin, while, the area under plasma concentration-time curve (AUC0-24) was 5.10±0.25 and 6.90±0.38 μg/ml.h for enrofloxacin and marbofloxacin respectively.

Pharmacokinetic/pharmacodynamic integration parameters was expressed as maximum plasma concentration/minimum inhibitory concentration (Cmax/MIC) and the area under plasma concentration-time curve/minimum inhibitory concentration (AUC0-24/MIC) which reflect the antimicrobial activity as a surrogate markers.

The Cmax/MIC ratio was 19.67±0.11 for enrofloxacin and 11.42±0.16 for marbofloxacin (both exceed the Cmax/MIC ratio; >10), while, the AUC0-24/MIC ratio was 85.00±3.90 and 57.50±2.18 for enrofloxacin and marbofloxacin, respectively (but not exceed the AUC0-24/MIC ratio; >125).

Enrofloxacin exhibited superior pharmacokinetic/pharmacodynamic integration compared with marbofloxacin for *E.Coli* infection in goats.

## INTRODUCTION

Fluoroquinolones are one of the most used classes antimicrobials in human and animal medicine because of their broad spectrum and their physico-chemical properties. As such, their popularity is increasing in clinical situations (**Hanna et al., 1997** and **Watts et al., 1997**). The antimicrobial activity of fluoroquinolones (especially third generation as marbofloxacin) is wide and includes most Gram-negative and some Gram-positive bacteria, mycoplasmas and intracellular pathogens such as *Brucella* and Chlamydia species, but has poor activity aganist anaerobes (**Wolfson and Hooper, 1989 and Appelbaum and Hunter, 2000**).

Fluoroquinolones act directly on the bacterial DNA by penetrating the bacterium by simple diffusion and act directly on bacterial enzyme DNA gyrase (**Bousquet-Melou et al. 2002**). Particularly, the third generation quionlone has flat structure that allows its insertion between the chains of the DNA molecule and acts as concentration-dependent antibiotics for Gram-negative bacteria, whereas their action against certain Gram-positive bacteria is generally considered time-dependent (**Hooper and Wolfson, 1993**).

Several studies were conducted on enrofloxacin in calves (**Ismail, 2007**), sheep (**Elsheikh et al,2002**) and goats (**Narayan, 2009** ) and on marbofloxacin in cows (**Schneider et al, 2004**), calves (**Sidhu et al, 2005**) and goats (**Sidhu et al, 2010**) as a single pharmacokinetic evaluation but not in comparative investigation.

The aim of the present study is to compare the pharmacokinetic/pharmacodynamic integration as a surrogate marker which reflect the antibacterial activity of enrofloxacin as a old quinolone generation with marbofloxacin as a recent one.

Pharmacokinetic/pharmacodynamic integration parameters including the maximum plasma concentration/minimum inhibitory concentration (Cmax/MIC) ratio and the area under serum concentration-time curve/minimum inhibitory concentrations (AUC0-24/MIC) ratio have been proposed to predict the *in vivo* antimicrobial efficacy of fluoroquinolones (**Turnidge., 1999**).

## MATERIAL AND METHODS

### Drugs:

Two commercially available injectable formulations were used for the study. The first one is Enrotryl 10 %, ADWIA, Egypt ( enrofloxacin) and the second is Marbocyl 5 %, Vétoquinol's, France ( marbofloxacin).

### Animals:

Eight clinically healthy goats ( 2 males & 6 females, 20-30 kg body weight and 6 - 8 months age ) were used for the study. The animals were fed barseem and kept under

observation before starting and during the experiment.

**Experimental design:**

The animals were divided into two groups (each of four goats; 1 males & 3 females) and the study was performed in a parallel two way crossover design with two weeks washout period.

After intramuscular injection of the drugs (5.0 mg of enrofloxacin /kg.b.wt and 2.0 mg of marbofloxacin /kg.b.wt.), blood samples were withdrawn in sterile heparinized tubes prior to, at 15, 30 minutes, 1, 2, 4, 8, 12 and 24 hours after drug injection. Plasma were separated by centrifugation of blood samples at 3000 rpm for 15 minutes and kept at -20°C till analysis.

**Bioassay:**

Concentration of drugs was determined in plasma by microbiological assay method (**Bennett et al., 1966 and Grove & Rondall, 1955**) using *Bacillus Subtilis* ATCC 6633 (BD, USA) ) as a standard test organism. The correlation coefficient ($r^2$) of linearity of standard curve for both enrofloxacin and marbofloxacin was 0.99.

**Minimum inhibitory concentration (MIC):**

Minimum inhibitory concentration (MIC) of enrofloxacin and marbofloxacin against *E.Coli* o157:H7 (BD, USA) was performed using agar plate diffusion technique (**Kolmer et al, 1951**).

**Data analysis:**

Data was expressed as mean±SE (**Snedicor and Cochran, 1987**). Pharmacokinetic analysis was performed with the plasma concentration-time profile. The Cmax, tmax and AUC0-24 was determined according to **Baggot (1977)**.

Cmax/MIC and AUC0-24/MIC ratio were calculated using Cmax, AUC0-24 of individuals and the *E.Coli* MIC value.

## RESULTS

Mean plasma concentrations of both enrofloxacin (5.0 mg/kg.b.wt.) and marbofloxacin (2.0 mg/kg.b.wt.) after intramuscular injection in goats are represented in **figures 1 and 2**, respectively. Both enrofloxacin and marbofloxacin were achieved the maximum concentration (tmax) after 1 hour of administration with maximum plasma concentration was (Cmax) 1.18±0.04 µg/ml for enrofloxacin and 1.37±0.07 µg/ml for marbofloxacin. While, the area under concentration curve (AUC0-24) was 5.10±0.25 and 6.90±0.38 µg/ml.h for enrofloxacin and marbofloxacin, respectively ( **Table 1**).

Minimum inhibitory concentration (MIC) of enrofloxacin and marbofloxacin against *E.Coli* o157:H7 was 0.06 µg/ml and 0.12 µg/ml, respectively.

Pharmacokintic/pharmacodynamic integration for the *in vivo* Pharmacokintic parameters (Cmax & AUC0-24) and the pharmacodynamic data (MIC) are represented in **table (2)**.

The intramuscular injection of enrofloxacin resulted an Cmax/MIC ratio of 19.67±0.11, while, AUC0-24/MIC ratio of 85.00±3.90 compared with 11.42±0.16 and 57.50±2.18 for marbolfoxacin (**Table 1**).

**Table (1): Pharmacokinetic/pharmacodynamic integration parameters (mean±SE) of enrofloxacin (5.0 mg/kg.b.wt.) and marbofloxacin (2.0 mg/kg.b.wt.) after single intramuscular injection in goats (n=8).**

| parameters | Unite | Surrogate marker | Enrofloxacin | Marbofloxacin | Enrofloxacin / Marbofloxacin % |
|---|---|---|---|---|---|
| **Pharmacokinetic:** | | | | | |
| Tmax | h | - | 1.0±0.02 | 1.0±0.04 | |
| Cmax | µg/ml | - | 1.18±0.04 | 1.37±0.07 | |
| AUC0-24 | µg/ml.h | - | 5.10±0.25 | 6.90±0.38 | |
| **Pharmacodynamic:** | | | | | |
| MIC (*E.Coli* o157:H7) | µg/ml | - | 0.06 | 0.12 | |
| **Pharmacokinetic /pharmacodynamic integration:** | | | | | |
| Cmax/MIC | - | >10 | 19.67±0.11 | 11.42±0.16 | 172.24 |
| AUC0-24/MIC | - | >125 | 85.00±3.90 | 57.50±2.18 | 152.74 |

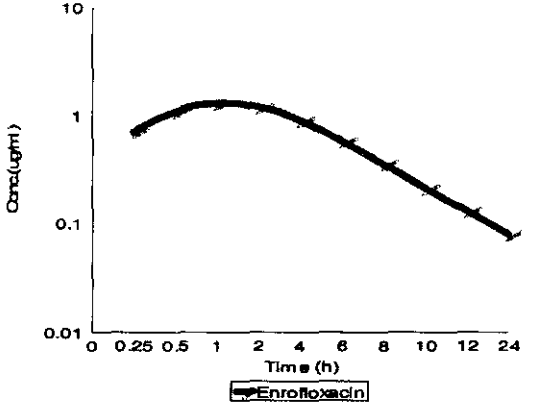


**Figure (1): Mean plasma concentrations of enrofloxacin (5.0 mg/kg.b.wt.) after single intramuscular injection in goats (n=8).**

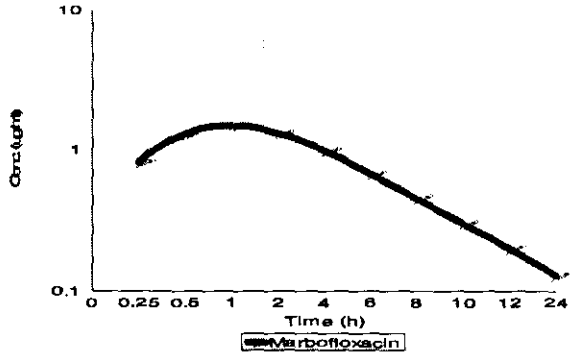


**Figure (2): Mean plasma concentrations of marbofloxacin (2.0 mg/kg.b.wt.) after single intramuscular injection in goats (n=8).**

## DISCUSSION

The aim of the present study is to compare the pharmacokinetic/ pharmacodynamic integration parameters of enrofloxacin as a one of old quinolone generations with marbofloxacin as the more recent one, where, this integration was used as a surrogate marker to evaluate the *in vivo* antimicrobial activity of antimicrobial agents.

Plasma samples were analyzed using a microbiological assay which does not separate the parent compound from the active metabolites. It measures the total activity which could be more useful for pharmacodynamic evaluations than high performance liquid chromatography (HPLC) methods (**Mckellar et al., 1999**) which separate the parent compound from the active metabolites.

Based on pharmackokinetics and antibacterial activities, several pharmacodynamic predictors of antimicrobial efficacy of flouroquinolones including the Cmax/MIC and AUC/MIC ratio have been investigated (**Forrest et al., 1993., Kung et al., 1993 and Madaras et al. 1996**). These relationships are highly veritable depending on the compound used, the bacterial species involved and the type of study performed. Such as clinical trial experimental infection or in vitro killing experiment flouroquinolones are considered to act in a concentration dependent manner. Hence, Cmax/MIC and AUC0-24/MIC ratio seem to be the best parameters for predicting their antimicrobial effect and comparing quinolones (**Lode et al, 1998 and Turnkge. 1999**).

Based on a neutropenic rat model (**Drusano et al., 1993**) and a clinical study including ventilated critical ill patients (**Forrest et al.,1993**), break points for clinical efficacy of fluoquinolones were determined at Cmax/MIC $\geq$10 and AUC0-24/MIC $\geq$125, respectively. Those findings were also widely discussed in veterinary medicine (**Merinen et al., 1995**). However, it seems that the proposed breakpoints are not generally valid and

that distinct AUC/MIC ratio are required for clinical cure depending on the host or pathogen (**Zhanel et al., 2001**).

The results of this work denoted that, enrofloxacin has better results of Pharmacokinetic/pharmacodynamic integration than marbofloxim for *E.Coli* infection in goats. The Cmax/MIC ratio was 19.76±0.11 for enrofloxacin and 11.42±0.16 for marbofloxacin (both exceed the Cmax/MIC ratio; >10) and enrofloxacin/marbofloxacin % was 172.24, while, the AUC0-24/MIC was 85.00±3.90 for enrofloxacin and 57.50±2.18 for marbofloxacin (but not exceed the AUC0-24/MIC ratio; 125) and enrofloxacin/marbofloxacin % was 152.74. These results was agreement with those recorded in dogs (**Henin, 2002**), where, the Cmax/MIC ratio was 23.5 for enrofloxacin and 11.8 for marbofloxacin and the AUC0-24/MIC ratio was 146 and 105 for the two drugs, respectively.

Finally, we concluded that, enrofloxacin as a old one of quinolones exhibited superior Cmax/MIC ratio and AUC0-24/MIC compared with the marbofloxacin which reflect their comparable pharmacokinetic and activity against *E.Coli* infection in goats.

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## الملخص العربى

### مقارنة توافق الحركه والفاعليه الدوائيه للانروفلوكساسين مع الماربوفلوكساسين في الماعز

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تم دراسة مدي توافق الحركه والفاعليه الدوائيه للانروفلوكساسين (5 مجم / كجم وزن حي ) والماربوفلوكساسين (2 مجم / كجم وزن حي ) عن طريق الحقن بالعضل في الماعز.

اشارت النتائج الي ان الانروفلوكساسين والماربوفلوكساسين تم امتصاصهما بسرعه خلال ساعه واحده من اعطاء الدواء كما وصل اعلي تركيز للانروفلوكساسين في البلازما الي 1.18±0.04 ميكروجرام/مل والماربوفلوكساسين الي 1.37±0.07 ميكروجرام/مل و كانت المساحه تحت المنحني لتركيز الانروفلوكساسين في البلازما 5.10±0.25 ميكروجرام.ساعه/مل في حين كانت 6.90 ±0.38 ميكروجرام/ مل. ساعه لمستحضر الماربوفلوكساسين.

كما دلت مؤشرات التوافق الحركي والفاعليه الدوائيه الى ان اعلي تركيز للدواء في البلازما / اقل تركيز لمنع نمو ميكروب القولون العصوي كان 19.67±0.11 للانروفلوكساسين و11.42±0.16 للماربوفلوكساسين في حين كانت المساحه تحت منحني تركيز الدواء في البلازما عن مدي 24 ساعه من اعطاء الدواء/ اقل تركيز لمنع نمو ميكروب القولون العصوي الي 85.00±3.90 للانروفلوكساسين و 57.50±2.18 للماربوفلوكساسين.

اثبتت النتائج الي ان مستحضر الانروفلوكساسين يتمتع بفاعليه اعلي من مستحضر الماربوفلوكساسين فى علاج الاصابه بالقولون العصوي في الماعز.