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 spectrum of fluoroquinolones (specially 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 against 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 quinolone has a 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 an old quinolone generation with marbofloxacin as a recent one.

MATERIAL AND METHODS

Drugs:

Two commercially available injectable formulations were used for the study. The first one is Entotryt 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 (r²) 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. Pharmacokinetic/pharmacodynamic integration for the in vivo Pharmacokinetic 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 marbofloxacin (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).

<table>
<thead>
<tr>
<th>parameters</th>
<th>Unite</th>
<th>Surrogate marker</th>
<th>Enrofloxacin</th>
<th>Marbofloxacin</th>
<th>Enrofloxacin / Marbofloxacin %</th>
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<td><strong>Pharmacokinetic:</strong></td>
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<td>Tmax</td>
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<td>1.0±0.02</td>
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<td>Cmax</td>
<td>µg/ml</td>
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<td>1.18±0.04</td>
<td>1.37±0.07</td>
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<tr>
<td>AUC0-24</td>
<td>µg/ml.h</td>
<td>-</td>
<td>5.10±0.25</td>
<td>6.90±0.38</td>
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<td><strong>Pharmacodynamic:</strong></td>
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<td>MIC (E.Coli o157:H7)</td>
<td>µg/ml</td>
<td>-</td>
<td>0.06</td>
<td>0.12</td>
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<td><strong>Pharmacokinetic</strong></td>
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<td>/pharmacodynamic integration:</td>
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<tr>
<td>Cmax/MIC</td>
<td>-</td>
<td>&gt;10</td>
<td>19.67±0.11</td>
<td>11.42±0.16</td>
<td>172.24</td>
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<tr>
<td>AUC0-24/MIC</td>
<td>-</td>
<td>&gt;125</td>
<td>85.00±3.90</td>
<td>57.50±2.18</td>
<td>152.74</td>
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</table>
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).
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 \textit{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 pharmacokinetics and antibacterial activities, several pharmacodynamic predictors of antimicrobial efficacy of fluoroquinolones 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 fluoroquinolones 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 (Fortest et al., 1993), break points for clinical efficacy of fluoroquinolones 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 \textit{E.Coli} infection in goats. The Cmax/MIC ratio was 19.76$\pm$0.11 for enrofloxacin and 11.42$\pm$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$\pm$3.90 for enrofloxacin and 57.50$\pm$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 العربي
مقارنة توافق الحركة والفاعليه الدوائيه للانروفلوكساسين مع الماربوفلوكساسين في الماعز
د. محمد محمد احمد، د. شاديه احمد رفعت ابراهيم وايمان سعودى احمد
معهد بحوث صحة الحيوان بالزقازيق
تم دراسة مدي توافق الحركة والفاعليه الدوائيه للانروفلوكساسين (5 مجم / كجم وزن حي) والماريوفلوكساسين (2 مجم / كجم وزن حي) عن طريق الحق بالعضل في الماعز.
اشارت النتائج إلى ان الانروفلوكساسين والماربوفلوكساسين تم اتصاصهما بسرعه خلال ساعه واحدة من اعتلاء الداء كما وصل اعلي تركيز للانروفلوكساسين في البلازميا الي 1.18±1.00 ميكروجرام/مل والماربوفلوكساسين الي 1.1±1.1 ميكروجرام/مل و كانت المساحه تحت المنحنى لتركيز الانروفلوكساسين في البلازميا 0.45±0.50 ميكروجرام/مل في حين كانت 0.37±0.30 ميكروجرام/مل. ساها لمستحضر الماربوفلوكساسين.
كما دلت مؤشرات التوافق الحركي والفاعليه الدوائيه الي ان اعلي تركيز للداء في البلازميا / اقل تركيز لمنع نمو بكتيريا القولون العصوي كان 19.77±11.01 للانروفلوكساسين و 16.42±12.00 للماربوفلوكساسين في حين كانت المساحه تحت منحنى تركيز الداء في البلازميا.
عن مدي 24 ساعة من اعطاء الدواء/ اقل تركيز لمنع نمو ميكروب القولون العصوي الي
3,9±85,00 للانترولوكساسين و 2,18±7,5 للماربوفلوكساسين.

أثبتت النتائج التي مستحضا الانترولوكساسين يستمع بفاعلية عالية مع
مستحضا الماربوفلوكساسين في علاج الاصابة بالقولون العصوي في الماعز.