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PHARMACOKINETIC AND THERAPEUTIC STUDIES ON DANOFLOXACIN AND MARBOFLOXACIN IN LACTATING COWS

(With 2 Tables and 2 Figures)

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دراسات فارماكوكينيتيكية على الدانوفلوكساسين والماربوفلوكساسين في الأبقار الحلابة

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تم حقن جرعة واحدة من عقاري الدانوفلوكساسين أو الماربوفلوكساسين بالعضل في عدد ١٠ من الأبقار الحلابة (تتراوح أعمارها من ٣,٥ إلى ٦,٥ عام) بجرعة ١,٢٥ و ٢ مليجرام/كجم على التوالي وتم جمع عينات من مصل الدم واللبن وقياس مستوى الدواءين بهما. أظهرت النتائج أن انتقال الدانوفلوكساسين من الدم إلى اللبن كان سريع ومكثف حيث كان مستواه في اللبن أعلى منه في السيرم بنسبة ٢,٤ بعد ٤ ساعات و ٤,٢ مرة بعد ٨ ساعات، في حين كانت النسبة مع الماربوفلوكساسين في نفس الوقتين كانت١,١٥ و ١,١٤ على التوالي. كما أظهرت النتائج أن تركيز الدانوفلوكساسين يظل في اللبن مساو أو أعلي من أقل تركيز مثبط لـ ٩٠% من ميكروب الإي كولاي والمايكوبلازما لمدة ٢٤ساعة وللميكروب العنقودي لمدة ١٢ساعة، بينما يظل تركيز الماربوفلوكساسين لمدة ٢٤ ساعة بالنسبة للإي كولاي وما يقرب من ٨ ساعات للميكروب العنقودي والمايكوبلازما.

SUMMARY

A single dose of danofloxacin (1.25 mg/kg b.w.) or marbofloxacin (2 mg/kg b.w.) were injected intramuscularly to ten dairy cows between 3.5 and 6.5 years old and weighed 500 to 580 kg. for evaluation its plasma and milk concentrations as well as its antibacterial characteristics against comman mastitis pathogens. Blood and milk samples were collected for determination of concentrations of two drugs by HPLC method. Results showed that, the passage of two drugs from blood into milk were rapid and extensive specially danofloxacin which at 4 and 8 hrs. its milk concentration was 2.4 and 4.2 times higher than the

plasma concentration, but this ratios with marbofloxacin was 1.15 and 1.14 at the same time respectively. Danofloxacin concentrations were maintained in the cow's milk equal to or higher than the MIC_{90} of *E.coli* (0.06 μg/ml) and *Mycoplasma* spp. (0.008-0.5 μg/ml) for 24 hrs. and of *S. aureus* (0.18 μg/ml) for 12 hrs.. The marbofloxacin concentrations were still equal to or higher than MIC_{90} of *E.coli* (0.016 μg/ml) for 24 hrs. and of *S. aureus* (0.229 μg/ml) and *Mycoplasma* spp. (0.48 μg/ml)for nearly 8 hrs.

Key words: *Pharmacokinetic, danofloxacin, marbofloxacin, cows*

INTRODUCTION

Fluoroquinolones are one of the most useful classes of antimicrobial agents used today in human and animal medicine because of their spectrum and their physico-chemical properties. As such, their popularity is increasing in clinical situations (Wolfson and Hooper, 1989, and Sárközy, 2001). The antimicrobial activity spectrum of fluoroquinolones (specially third generation as danofloxacin and 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 (Hannan *et al.,* 1989; Wolfson and Hooper, 1989; Neu, 1991; and Appelbaum and Hunter, 2000).

This fluoroquinolones acts directly on the bacterial DNA by penetrating the bacterium by simple diffusion and the target is a bacterial enzyme DNA gyrase (Bousquet-Melou *et al.* (2002). Particularly, the third generation quionlone 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 and Bousquet-Melou *et al.,* 2002).

As the pharmacokinetic parameters of antibiotics may change in lactating animals (Oukessou *et al.,* 1990; Petracca *et al.,* 1993 and Soback *et al.,* 1994) and the plasma-to-milk drug concentration ratio of these drugs is often unknown (Atkinson and Begg, 1990). So, the aim of the present study was determination of the pharmacokinetic parameters (C_{max}, maximum serum concentration; t_{max}, time of maximum concentration; $t_{1/2el}$, elimination half-life time and AUC, area under curve) and penetration of danofloxacin and marbofloxacin from blood

into milk in lactating cows following their intramuscular injections in recommended dose was calculated and expressed as $C_{max\text{-}milk}/C_{max\text{-}serum}$, AUC_{milk}/AUC_{serum}, and $t_{1/2el\text{-}milk}/t_{1/2el\text{-}serum}$.

MATERIALS and METHODS

A-Drugs:-

1- Danofloxacin: - (Advocin® 2.5% injectable solution, Pfizer Ltd). It is a synthetic fluoroquinolone 3rd generation with broad spectrum antibacterial activity. It is used in treatment of respiratory diseases in chickens, cattle and pigs (Schneider *et al.*, 1993) and has a potential use in the treatment of bovine mastitis (Shem-Tov *et al.*, 1998).

2- Marbofloxacin: - (Marbocyl®10% injectable solution, Vétoquinol). It is a synthetic fluoroquinolone 3rd generation devoloped by Vétoquinol exclusivley for veterinary use for oral and parenteral administrations to cattle; including lactating dairy cattle, and pigs for treatment of mastitis and respiratory diseases. It has a good therapeutic antibacterial activity for cattle, pigs, dogs, cats and other species (Hooper and Wolfson, 1993; Thomas *et al.*, 1998 and Thoulon *et al.*, 1999)

B- Animals:-

Ten Friesian cows between 3.5 and 6.5 years old and weighed 500 to 580 kg., fed antibiotic free ration, not received any drug for at least one month, produced 15-24 liters of milk/day, their udders secreted macroscopically normal milk of pH 6.6-6.9, with somatic cell count of <500 000/ml, were injected intramuscularly once; just after the morning milking, with danofloxacin (n = 5) at a dose of 1.25 mg/kg b.w. or marbofloxacin (n = 5) at a dose of 2 mg/kg b.w.

C- Sampling:-

Blood and milk samples (5 ml of each) were collected prior and at 1/2, 1, 2, 4, 8, 12, 24. 30, 36 hrs. after drugs injection. Collected samples (serum and milk) were stored at -20°c until estimation of drug concentrations (Shojaee-Aliabadi and Lees, 2003). Danofloxacin and marbofloxacin concentrations were mesured by HPLC with UV detector with quantitation limits of 0.05 μg/ml for danofloxacin and 0.01 μg/ml for marbofloxacin according methods described by McKellar *et al.* (1999), and Schneider *et al.* (2004).

D- Analysis:-

Statistical were carried out using "t" test according to SAS (1987), and the kinetic parameters (C_{max}, t_{max}, $t_{1/2el}$, and AUC) were calculated according to Baggot (1977) and penetration of danofloxacin

and marbofloxacin from blood into milk in lactating cows following their intramuscular injections in recommended dose was calculated and expressed as $C_{max\text{-}milk}/C_{max\text{-}serum}$, AUC_{milk}/AUC_{serum}, and $t_{1/2el\text{-}milk}/t_{1/2el\text{-}serum}$ according to Shem-Tov *et al.* (1998).

RESULTES

Danofloxacin and marbofloxacin concentrations in serum and milk shown that the two drugs were passed rapidly from blood into milk in injected cows (Table 1 and Fig. 1-2), reach maximal concentration in milk after two hours after injection, danofloxacin levels in serum and milk were 0.35 ± 0.144 and 0.70 ± 0.292 µg/ml and marbofloxacin levels were 1.07 ± 0.033 and 1.31 ± 0.029 µg/ml respectively. On the other hand, at 24 hrs. danofloxacin cannot detect in serum but still in milk until 30 hrs. while marbofloxacin was detected after 24 hrs. in serum and milk with levels 0.03 ± 0.017 and 0.025 ± 0.003 µg/ml respectively.

Table (2) showed that, T_{max} of danofloxacin and marbofloxacin in serum were 1± 0.05 and 1± 0.01hour respectively while in milk was variable (8 ± 0.11 hrs. for danofloxacin and 2 ± 0.06 hrs. for marbofloxacin).

Table 1: Serum and milk concentrations after single i.m injection of danofloxacin (1.25 mg/kg b.w.) or marbofloxacin (2 mg/kg b.w.) in lactating cows (µg/ml).

Time of sampling	Danofloxacin		Marbofloxacin	
	Serum (n =5) mean ± S.E.	Milk (n =5) mean ± S.E	Serum (n =5) mean ± S.E	Milk (n =5) mean ± S.E
Pre-injection	0.00	0.00	0.00	0.00
1/2h	0.33± 0.027	0.09±0.029+	0.77± 0.029*	0.055± 0.003
1h	0.35 ± 0.144	0.31 ± 0.027	1.42 ± 0.029*	0.72 ± 0.033+
2h	0.35 ± 0.041	0.70 ± 0.292	1.07 ± 0.033*	1.31 ± 0.029+
4h	0.34± 0.034	0.80 ± 0.332	0.75 ± 0.025*	0.86 ± 0.027
8h	0.19 ± 0.029	0.79 ± 0.029+	0.37 ± 0.035*	0.42 ± 0.049
12h	0.055 ± 0.003	0.12 ± 0.027	0.12 ± 0.041*	0.18 ± 0.035
24h	ND	0.052± 0.002+	0.03 ± 0.017*	0.025 ± 0.003
30h	ND	0.052± 0.002+	ND	ND
36h	ND	ND	ND	ND

ND=Not Detected. * And + = significant at ($p<0.05$).

Table 2: Pharmacokinetic parameters in serum and milk after single i.m. injection of danofloxacin (1.25mg/kg b.w.) or marbofloxacin (2mg/kg b.w.) in lactating cows.

pharmacokinetic parameters	Danofloxacin		Marbofloxacin	
	Serum (n =5) mean ± S.E	Milk (n =5) mean ± S.E	Serum (n =5) mean ± S.E	Milk (n =5) mean ± S.E
C_{max} µg/ml	0.369 ± 0.02	0.80 ± 0.06	1.41± 0.03*	1.31± 0.04+
t_{max} h	1± 0.05	8 ± 0.11+	1± 0.01	2 ± 0.06
$t_{1/2el}$ h	3 ± 0.11	4.2 ± 0.23	3 ± 0.12	4 ± 0.12
AUC µg/ml.h	3.20 ± 0.14	11.06 ± 0.45+	7.42 ± 0.22*	5.95 ± 0.02
$C_{max\text{-}milk}/C_{max\text{-}serum}$	2.17		0.93	
$t_{1/2el\text{-}milk}/t_{1/2el\text{-}serum}$	1.4		1.33	
AUC_{milk}/AUC_{serum}	3.46		0.80	

* And + = significant at ($p<0.05$).

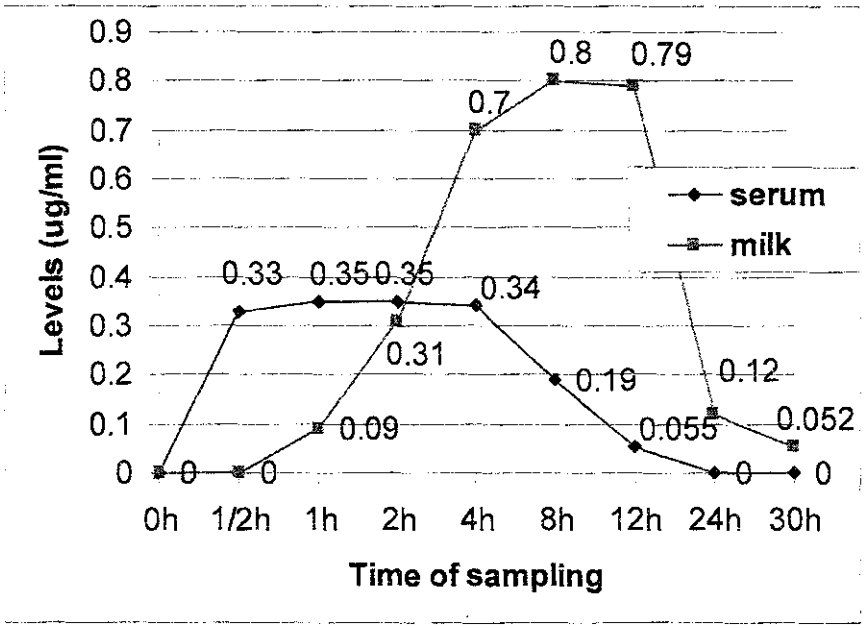

Fig. 1: Serum and milk concentrations after single i.m injection of danofloxacin (1.25 mg/kg b.w.) in lactating cows (µg/ml).

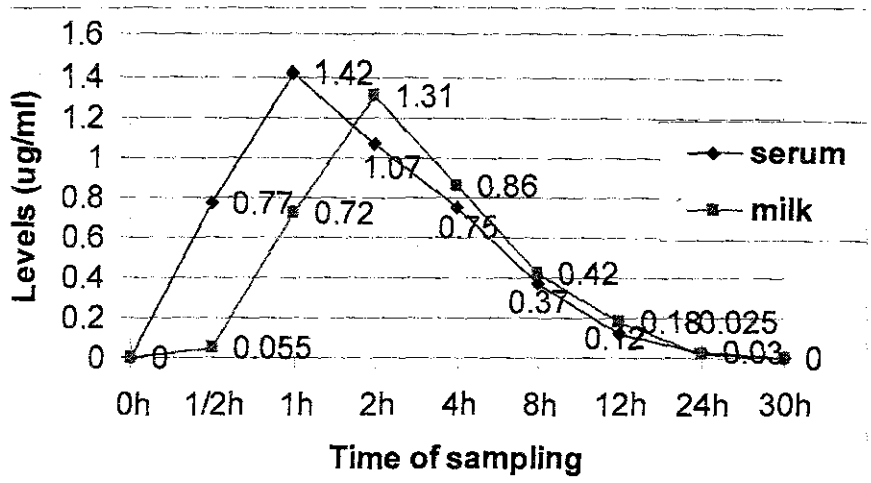

Fig. 2: Serum and milk concentrations after single i.m injection of marbofloxacin (2 mg/kg b.w.) in lactating cows (μg/ml).

DISCUSSION

It is generally accepted that xenobiotics cross the blood-milk barrier in the udder by non ionic passive-diffusion and the extent of diffusion is greatly influenced by the physicochemical properties of the drug (Rasmussen, 1966 and Atkinson and begg, 1990) and due to presence of a carboxylic acid and basic amine-functional groups in danofloxacin and marbofloxacin, they are amphotric and considered zwitterionic and so between pH. 6 and 8, these drugs are sufficiently lipid-soluble to be able to penetrate tissues resulting extensive penetration from blood into the cow's milk (Brown, 1996) which therefore predictable on the basis of the "ion trap" mechanism (Rasmussen, 1966 and Atkinson and begg, 1990).

In the present study the pharmacokinetic interpretation of danofloxacin and marbofloxacin concentrations data; in serum and milk, reveals the rapid and extensive penetration of the two drugs from blood into milk. This is similar to results obtained by Soback *et al.*, (1994); Brown, (1996); Shem-Tov *et al.*, (1997) and Schneider *et al.*, (2004) where they recorded the very large distribution of these two drugs in the body of lactating cows.

The first important finding in the present study was the shift between plasma and milk kinetic profiles that appeared after 2 hrs. specially with danofloxacin which at 4 and 8 hrs. its milk concentration

was 2.4 and 4.2 times higher than the plasma concentration, but with marbofloxacin this shift occurs by relatively little degree (was 1.15 and 1.14 at the same time) respectively. In details, C_{max} for danofloxacin in serum and milk were 0.369 ± 0.02 and 0.80 ± 0.06 μg/ml after 1 and 8 hrs. and for marbofloxacin were 1.41± 0.03 and 1.31± 0.04 μg/ml after 1 and 2 hrs. respectively, and whereas danofloxacin at 24 hrs. not detected in serum, its mean concentrations (0.052± 0.002 μg/ml) in milk still until 30 hrs., in contrast marbofloxacin concentrations were measured in two fluids until 24 hrs. with nearly similar values (0.03 ± 0.017 in serum and 0.025 ± 0.003 μg/ml in milk). Nearly similar results were previously reported by Shem-Tov *et al.*, (1998) with danofloxacin in lactating cows, where they found that danofloxacin concentration in milk at 2 hrs. was equal to those in serum and at 4 and 6 hrs. were 2.2 and 5.5 times greater than the corresponding concentrations in serum. But they not detect the drug in serum and milk after 12 and 24 hrs. respectively. For marbofloxacin, Schneider *et al.*, (2004) mentioned that C_{max} in serum was 1.667 μg/ml after 45min and in milk was 1.024 μg/ml after 2.5 hrs., and C_{23h} in serum was 0.017 and in milk was 0.013 μg/ml. In keeping with this lines (VMRI, 2006) recored C_{max} for marbofloxacin in serum 1.5 μg/ml after less than 1 hr. and in milk 1.02 μg/ml after 2.5 hrs.

Comparative studies strongly suggest that the lactations may increase the rate of elimination of some fluoroquinolones from the serum, where the mean elimination half-life ($t_{1/2el}$) in serum may be shorter in lactating than non-lactating animals of the same species. The intravenous $t_{1/2\beta}$ of enrofloxacin in non lactating cattle was 5.5 ± 0.9 hrs. (Sheer, 1987) whereas the corresponding value in lactating cows was 1.7 hr.(Walser *et al.*, 1993) and 1.68 ± 0.18 hr. in another study (Kaartinen *et al.*, 1996). For marbofloxacin, it was 5.72 ± 1.17 hrs. in non lactating adult cattle (Thomas *et al.*, 1994) and was 2.07 ± 0.66 hrs. in lactating cows (Shem-Tov *et al.*, 1997). In the present study, the $t_{1/2el}$ in serum was 3 ± 0.11 and 3 ± 0.12 hrs. for danofloxacin and marbofloxacin respectively, which relatively shorter than 3.5 - 4.5 hrs. which reported by (Grimshaw *et al.*, 1990 and Giles *et al.*, 1991) following administration of danofloxacin (i.v., i.m. and s.c.) to non lactating cattle. A shorter than (4-7 hrs.) that reported for marbofloxacin in non lactating cows (VMRI, 2006).

In the present study, the ratios $C_{max\text{-}milk}/C_{max\text{-}serum}$ was 2.17 and 0.93, $t_{1/2el\text{-}milk}/t_{1/2el\text{-}serum}$ was 1.4 and 1.33 and AUC_{milk}/AUC_{serum} was 3.46 and 0.80 for danofloxacin and marbofloxacin respectively which

supported with earlier findings on danofloxacin by Shem-Tov *et al.* (1998) and on marbofloxacin by Schneider *et al.* (2004) their ratios were 2.54 and 0.61, 1.26 and 1.23, and 3.52 and 0.83 for danofloxacin and marbofloxacin respectively. These results indicating greater persistence of drug concentrations in the milk relative to those in serum, with superiority for danofloxacin in reaching to milk and persisting for a longer time, but for fairness if danofloxacin has this good specialty we found that marbofloxacin has another good properties of short withdrawal period in milk that give it superiority when used in treating diseases other than mastitis.

The previously reported minimal inhibitory concentrations (MIC) of danofloxacin for 90% of the field isolates of *E.coli* 0.06 μg/ml (Toutain, 2003), for *S. aureus* was 0.18 μg/ml (Cruz *et al.*, 1998) and for *Mycoplasma sp.* was 0.008-0.5 μg/ml (Cooper *et al.*, 1993), and that record for marbofloxacin with *E.coli* was 0.016 μg/ml, *S. aureus* was 0.229 μg/ml (Schneider *et al.*, 2004) and for *Mycoplasma sp.* was 0.48 μg/ml (Drugeon *et al.*, 1997). Depending on these results the present study showed that, danfloxacin concentrations were maintained in the cow's milk equal to or higher than the MIC_{90} for *E.coli* and *Mycoplasma sp.* for 24hrs. and higher than the MIC_{90} for *S. aureus* for 12 hrs.. These results were agreemented to that mentioned by Shem-Tov *et al.* (1998). Marbofloxacin concentrations were maintained equal to or higher than MIC_{90} of *E.coli* for 24 hrs. and for *S. aureus* and *Mycoplasma* spp.. for nearly 8hrs., and although of this result its effect alone as parenteral injection for mastitis treatment is still controversial due to steep marbofloxacin milk profile which is nearly parallel to the plasma profile and most trials for its use in mastitis treatment usually were accompanied with local treatment. As Thomas *et al.* (1998) which mentioned that marbofloxacin parenterally was effective on mastitis due to coibacilli when combined with intramammary treament which only has an effect on *Gram-positive* mastitis (cloxacillin), also Grandemange and Davot, (2002) found; in a field trial, that parenteral injection of marbofloxacin in combination with a local cloxacillin treatment showed efficacy in treatment of acute clinical coliform mastitis in dairy cows, and in another trial for the same authors found that marbofloxacin parenterally showed some expected efficacy against *S. aureus*-induced infections, especially when associated with a local ß-lactamine treatment. On the contrast we found Schneider *et al.* (2004) told that, a possible efficacy for marbofloxacin against *E. coli*-induced mastitis is

expected after its repeated intramuscular administrations at a daily dose rate of 2 mg/kg.

The main pharmacokinetic/pharmacodynamic (PK/PD) parameters correlating with efficacy, minimising the risk of development of resistance and the best surrgate of drug activity are the C_{max} /MIC and AUC/MIC ratios (Toutain *et al.*, 2002) specially if its endpoints are equal to or thigher than 10 and 125 hrs. respectively (Hyatt *et al.*, 1995; Schentag, 1999; Pickerill *et al.*, 2000 and Schentag *et al.*, 2001). In this study for danofloxacin $C_{max\text{-}milk}$ /MIC and AUC_{milk}/MIC for *E. coli* was 13.33 and 184.33 hrs., for *S. aureus* was 4.4 and 61.44 hrs. and for *Mycoplasma* spp.. was 1.6 and 22.12 hrs. whereas for marbofloxacin this ratios were 81.88 and 371.88 hrs. for *E. coli,* 5.72 and 25.98 hrs. for *S. aureus* and 2.73 and 12.4 hrs. for *Mycoplasma* spp.. respectively. Although, these endpoints are only reached for *E. coli* strains and relatively far away for *S. aureus* and *Mycoplasma* spp..for the two drugs, some activity against *S. aureus* and *Mycoplasma* spp.. may be expected because the activity of fluoroquinolones against *Gram-positive* strains is rather time dependent (Schneider *et al.*, 2004).

Depeding on results of this study specially a high peak milk concentration that maintained as MIC over the assuming dosage interval (either 12 or 24 hrs.) for the two drugs and with recalling that fluoroquinolones are concentration dependent antimicrobial agents (Hooper and Wolfson, 1993 and Bousquet-Melou *et al.*, 2002) the potential use of danofloxacin and marbofloxacin in the treatment of bovine mastitis can be suggested. With taking in account that danofloxacin is justified at dose of 1.25 mg/kg q 24 hrs. for treatment of bovine mastitis associated with *Gram-negative* pathogens and 1.25 mg/kg q 12 hrs. for treatment of that caused by *S. aureus*. or *Mycoplasma* spp.., but the uses of marbofloxacin alone as mastitis treatment is still controversial and need futher field trials .

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