Efficacy and pharmacokinetics of enrofloxacin and flunixin meglumine for treatment of cows with experimentally induced *Escherichia coli* mastitis

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The efficacy of flunixin alone and together with enrofloxacin in treatment of experimental *Escherichia coli* mastitis was compared using six cows. The cross-over study design was used. Pharmacokinetics of flunixin and enrofloxacin were also studied in these diseased cows. The response of each cow was similar after the first and second challenge and the individual reaction seemed to explain the severity of clinical signs. The most important predictive factor for outcome of *E. coli* mastitis was a heavy drop in milk yield. Treatment with enrofloxacin and flunixin enhanced elimination of bacteria, but the difference from those receiving flunixin alone was not significant. Two cows, which had received no antimicrobial treatment (Group 1), were killed on day 4 postchallenge. One cow was killed after the first and the other after the second challenge. Cows receiving combination therapy produced 0.9 L more milk per day during the study period than cows which had only received flunixin ($P < 0.05$). Based on our findings, antimicrobial treatment might be beneficial in the treatment of high-yielding cows in early lactation. The absorption of enrofloxacin was delayed after subcutaneous administration, the mean apparent elimination half-life being about 23 h, whereas after i.v. administration elimination $t_{1/2}$ was only 1.5 h. The majority of the antimicrobial activity in milk originated from the active metabolite, ciprofloxacin, which could be measured throughout the 120-h follow-up period after the last subcutaneous administration. No differences were present in the pharmacokinetic parameters of flunixin between treatment groups: mean elimination half-life was 5.7–6.2 h, volume of distribution 0.43–0.49 L/kg and clearance 0.13–0.14 L h/kg. No flunixin or merely traces were detected in milk: one of the three cows had a concentration of 0.019 mg/L 8 h after administration.

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INTRODUCTION

Mastitis caused by *Escherichia coli* can be a serious disease especially in early lactation cows (Katholm & Andersen, 1992; Shuster *et al.*, 1996; Pyörälä & Pyörälä, 1998). Treatment of coliform mastitis has comprised of antimicrobials and supportive treatments such as nonsteroidal anti-inflammatories and fluid therapy. Parenteral use of antimicrobials has been recommended because drug distribution when administered into the swollen udder quarter is poor (Ziv, 1980). The efficacy of antimicrobial treatment in treating coliform mastitis has been questioned because cure rates without antimicrobials have been high, or treatment with drugs shown to be ineffective *in vitro* has been effective (Griffin *et al.*, 1982; Pyörälä & Syväjärvi, 1987; Jones & Ward, 1990). No difference has been seen between cows not receiving antimicrobial treatment and those receiving either parenteral sulphonamide-trimethoprim or intramammary colistin sulphate (Pyörälä *et al.*, 1994). Frequent milking with oxytocin stimulation has also been recommended for treatment of coliform mastitis (Radostits *et al.*, 2000). This treatment has

been reported to give equal or better results than treatment with antimicrobials (Guterbock *et al.*, 1993; Stämpfli *et al.*, 1994).

The number of antimicrobial substances with suitable pharmacokinetic and pharmacodynamic properties for the treatment of coliform mastitis is limited. Fluoroquinolones, such as enrofloxacin, theoretically could be effective in the treatment of coliform mastitis. Enrofloxacin and its antimicrobially active metabolite, ciprofloxacin, have been shown to reach high concentrations in milk (Kaartinen *et al.*, 1995), and minimum inhibitory concentration (*MIC*) values for *E. coli* isolates from mastitis are low (Pyörälä & Myllys, 1995). Furthermore, the presence of mastitic milk does not greatly interfere with the activity of enrofloxacin because the *MIC* values of enrofloxacin against *E. coli* remained the same as found in broth (Fang & Pyörälä, 1996). Antimicrobials and nonsteroidal anti-inflammatory medicines are often given simultaneously to cows which suffer from serious coliform mastitis. Flunixin has been shown to diminish the detrimental effects of endotoxins (Anderson *et al.*, 1986).

The objectives of our study were: (1) to compare the effect of flunixin alone and together with enrofloxacin in the treatment of experimental *E. coli* mastitis and (2) to study pharmacokinetics of flunixin and enrofloxacin in diseased cows.

MATERIALS AND METHODS

Animals

Six Finnish Ayrshire cows, which had previously calved more than once, and were in their early lactation period during the present study (median 29 days), served as subjects. At the beginning of the study all the cows were determined to be clinically healthy by clinical examination and their milk somatic cell count was < 150 000 cells/mL. The animals were kept in a stanchion barn and fed with a diet of good-quality silage, hay and grain. Water was offered *ad libitum*. The cows were milked twice a day and their mean milk yield was 17 L/day (range 12–28 L). The Ethics Committee of the Faculty of Veterinary Medicine, Helsinki, Finland, approved the study protocol.

Experimental design

One udder quarter of each cow was infected via the teat canal with 1500 CFU of *Escherichia coli* strain FT238. The strain had been isolated from clinical mastitis and was nonhaemolytic, intermediately serum resistant and sensitive to enrofloxacin *in vitro* (*MIC* < 0.25 μg/mL). Before inoculation, the tip of the teat was cleaned carefully with 70% ethanol.

The cross-over study design was used. The six cows were randomly allocated to two treatment groups. After the appearance of clinical signs of mastitis (12 h postinoculation), all six cows received a single dose of flunixin meglumine (Finadyne®, Schering-Plough, Farum, Denmark, 2.2 mg/kg i.v.) and three of them were also given enrofloxacin (Baytril®, Bayer, Leverkusen, Germany), 5 mg/kg i.v. initially and then 5 mg/kg s.c. once a day for two further days. Each cow served as its own control: after 3 weeks, the contralateral udder quarter was inoculated as described above. Antimicrobial treatment was now administered to the three cows which had not received enrofloxacin after the first challenge. Again, all cows received a single dose of flunixin meglumine. Systemic and local clinical signs and the daily milk yield were recorded throughout the experiment as previously described by Hirvonen *et al.* (1999). During the study the affected udder quarter was milked by hand two additional times between morning and evening milkings using oxytocin 5 IU i.m. (Synox® 5 IU/mL, Orion Pharma, Turku, Finland) before milkings. The cows received fluid therapy (Ringer's lactate) if they had serious depression and total inappetence on the second day after the challenge. The cows, which had grave clinical signs (were recumbent and showed signs of decompensatory shock) were removed from the experiment on day 3 postchallenge, and intensive treatment with enrofloxacin (if in group not receiving antimicrobials, Group 1), flunixin meglumine (at 12 h intervals) and continuous intravenous fluids was introduced.

Sample collection

Blood samples were drawn from the jugular vein of each cow for analysis of concentrations of enrofloxacin, ciprofloxacin and flunixin. Blood samples were collected prior to challenge, at 2 and 30 min post-treatment, and then at 1, 2, 4, 8, 12, 24, 32, 48, 50, 52, 56, 60, 72, 80, 99 and 120 h. Milk samples were collected at morning and evening milkings and from each quarter of all cows prior to challenge. Post-treatment collection also occurred at 2, 8, 15 and 30 min, and then simultaneously with blood samples during the first 50 h. Thereafter, milk samples were collected at 51, 52, 54, 56, 72, 80, 96, 104, 120, 144, 168 and 240 h. Milk, EDTA-plasma and serum samples were stored at –20 °C prior to analysis.

Determination of enrofloxacin and ciprofloxacin from serum and milk

Serum

Concentrations of enrofloxacin and its metabolite ciprofloxacin were determined from serum by high-performance ion-pairing liquid chromatography (HPLC) with fluorometric detection, according to the method previously described by Tyczkowska *et al.* (1994) with minor modifications. All reagents were of HPLC quality. Immediately before analysis, serum samples (500 μL) were diluted with an equal volume of acetonitrile – 0.1 M NaOH (1:1). Samples were then vortexed and centrifuged in a Millipore 10 000 NMWL filter unit (Millipore, Bedford, MA, USA) at 10 000 g for 1 h. A 50–150 μL aliquot of the colourless ultrafiltrate was pipetted into an autosampler vial for analysis. Injection volume was 10 μL.

A Hewlett-Packard HPLC system with HP 1046 A fluorescence detector and autosampler (Hewlett Packard, Avondale, Pennsylvania, USA) was used. The mobile phase consisted of 0.02 M heptanesulphonate in 0.002 M phosphoric acid–acetonitrile–methanol (62:27:8); pH of the mobile phase was 2.5. The flow rate was 0.8 mL/min and run time 8 min. The PLRP-S polymer column (5 μm, 150 mm × 4.6 mm, Polymer

Laboratories, Amherst, MA, USA) was used. Stock solutions of 1 mg/mL enrofloxacin and ciprofloxacin standards (both from Bayer AG, Leverkusen, Germany) were prepared in 0.03 M NaOH and working concentrations were further diluted in 0.1 M NaOH – acetonitrile solution (1:1). The column effluent was monitored with fluorometric detection (excitation 277 nm, emission 445 nm). Limits of detection (LOD) and quantification (LOQ) were 10 and 20 ng/mL for both enrofloxacin and ciprofloxacin. The recovery rate for enrofloxacin was 79–109% and coefficient of variation (CV) was 18.3 ($n = 6$). The respective figures for ciprofloxacin were 70–105% and 13.5 ($n = 6$).

Milk

Enrofloxacin and ciprofloxacin residues in milk samples were analysed using the HPLC method as described by Hormazabal and Yndestad (1994). The HPLC analyses were performed on a Hewlett Packard 1090 liquid chromatograph consisting of an autosampler and a Hewlett Packard 1046 fluorescence detector. The detector was operated at an excitation wavelength of 277 nm and an emission wavelength of 445 nm with cut-off filter at 370 nm. The analytical column was PLRP-S with adsorbent polymer (150 × 4.6 mm, 5 μm). Quantification was based on peak heights compared with a standard curve. The standards were diluted in antibiotic-free milk, which had been treated similarly to the samples. The LOD for enrofloxacin and ciprofloxacin was 5 and 8 ng/mL, and LOQ 10 and 16 ng/mL, respectively. For enrofloxacin, the recovery rate and CV were 87–94% and 3.4 ($n = 6$). The corresponding figures for ciprofloxacin were 77–88% and 4.8 ($n = 6$).

Determination of flunixin

Plasma

The method, which has been developed and validated for residue surveillance of tissues, was used. Flunixin meglumine dissociates in solution and was determined as flunixin. One millilitre of 1 M sulphuric acid was added to 2 mL of plasma and mixed with 6 mL of water. Then samples were centrifuged at 10 000 g for 20 min and the supernatant was purified using the solid phase extraction column (SPE, C18, 3 cm^3/500 mg, Varian, Bond Elut, Harbor City, CA, USA) with methanol as eluant. The eluate was evaporated to dryness by the nitrogen flow. The residue was dissolved in 0.25 mL of the mobile phase solution.

Concentrations of flunixin were measured in plasma by using HPLC. Hewlett Packard's 1090 system with UV-light diode array, autosampler unit and HP Chemstation data collection software was used. The mobile phase consisted of methanol–water–tetrahydrofurane (52:43:5). The flow rate was 1 mL/min. The sample was injected automatically onto the column. The analytical column was 200 mm × 4 mm Hypersil ODS (Hewlett-Packard). The LOQ was 20 ng/mL. Recovery was 65% at 40 ng/mL.

Milk

We used the method published by Rupp *et al.* (1995), with the following modifications. Milk samples (5 mL) were acidified with 0.3 mL of 1 M HCl and the sample was then mixed with 5.5 g silica gel (mesh 70–230). The milk–silica mixture was used to prepare a chromatographic column, which was washed with dichlormethane–hexane solution (70:30). The analyte was eluted from the column with 50 mL of ethylacetate. The eluate was handled with acidic water and the sample was extracted from ethylacetate to 0.1 M NaOH. The pH of the aqueous layer was adjusted to 5. Finally, the milk sample was purified with solid-phase extraction and prepared for HPLC analysis similarly to the serum samples described previously. The LOD and LOQ were 6 and 13 ng/mL, respectively.

Data analysis

Clinical data

Treatment effects and temporal patterns were analysed by a repeated measures analysis of variance with two within factors. Significances of *F*-ratios were evaluated using Greenhouse–Geisser adjusted *P*-values. Because of the small sample size and some missing data, challenge time could not be included in the models, but its effects were analysed separately.

Pharmacokinetic data

The serum and milk concentration–time data were analysed using software based on statistical moment theory (Yamaoka *et al.*, 1978). Statistical analysis was performed using Student's *t*-test.

RESULTS

Clinical findings

The most important factor explaining the severity of mastitis was the cow itself because the response of each cow followed the similar pattern after both challenges. No statistically significant differences were present between the two challenges in any parameters measured. All cows developed clinical mastitis within 12 h postinoculation. Three cows had mild signs and three other cows had moderate to severe signs. Mean scores for clinical signs and local signs are shown in Fig. 1. Moreover, no significant differences in these clinical parameters were found between the two treatment groups. A detailed presentation of acute-phase proteins in relation to outcome has been published earlier (Hirvonen *et al.*, 1999).

Two cows, which were not treated with enrofloxacin (Group 1), had to be killed on day 4 postchallenge despite intensive treatment. One of these cows was killed after the first challenge and the other after the second challenge. The latter cow had moderate clinical signs after the first challenge and recovered from these normally when treated with flunixin and enrofloxacin. Clinical signs of the cows that were later killed had initially improved but deteriorated again on day 3 postchallenge. No statistical difference was present in the number of deaths between the two treatment groups when calculated by Fisher's exact test ($P = 0.27$). In all recovering cows systemic

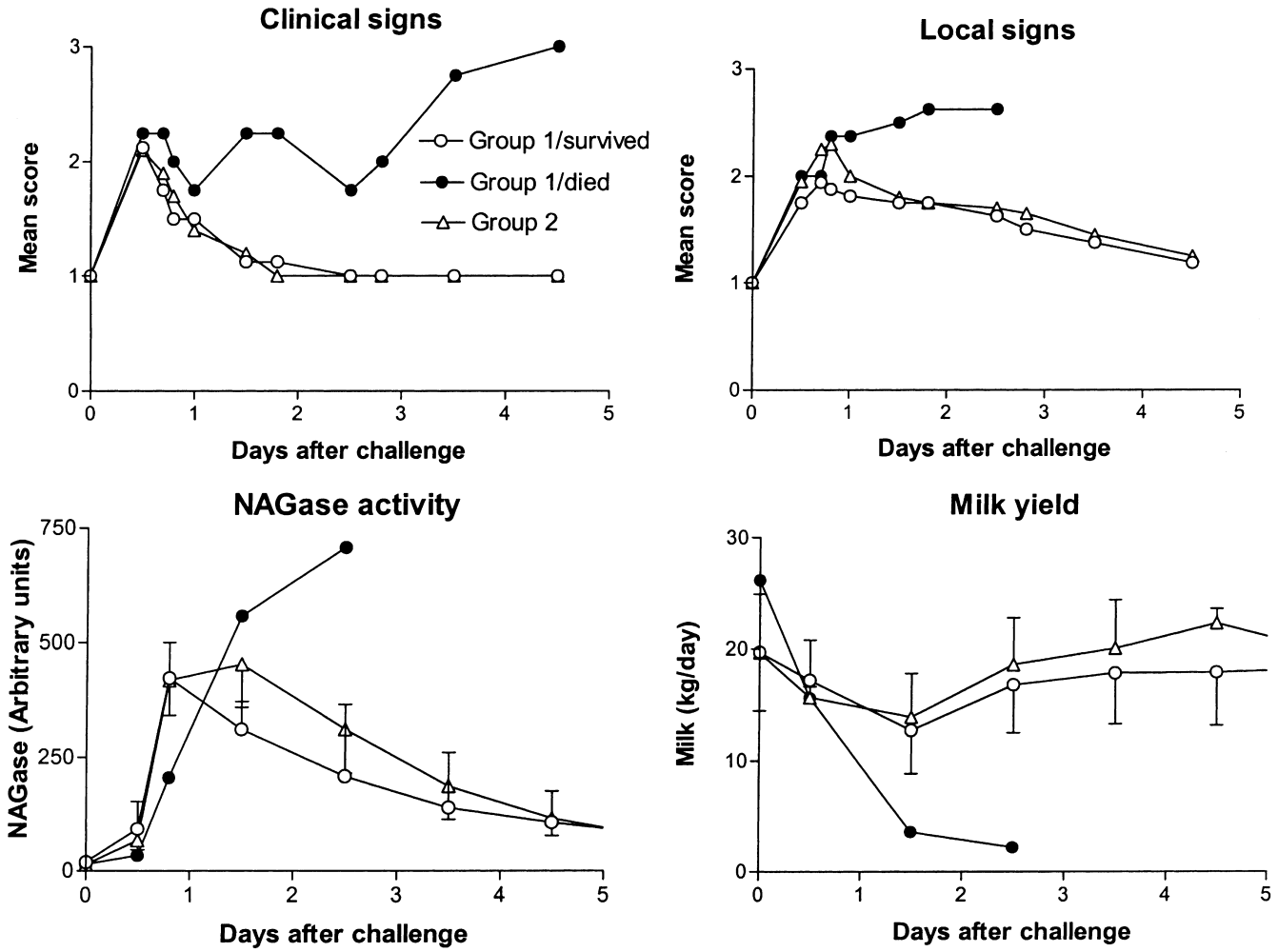

Fig. 1. Clinical and local signs, milk NAGase and milk yield per day. Cows were challenged at time-point zero. Treatment was initiated 12 h later. Group 1 = flunixin administered once at a dose of 2.2 mg/kg i.v. 12 h after the challenge when clinical signs had developed; Group 2 = flunixin as to cows in Group 1 with concomitant i.v. enrofloxacin 5 mg/kg, and again at 24 and 48 h using s.c. route. Group 1/survived = data from cows which recovered from the challenge; Group 1/died = data from two cows which did not recover (score for clinical signs is given for these cows until they were killed).

signs disappeared after 2 days; local signs disappeared more slowly.

Bacterial counts peaked in milk of recovered cows between 12 and 32 h postchallenge. Counts were higher in milk of the cows that were later killed (Fig. 2). A similar trend was seen in milk NAGase activity (Fig. 1). Neither of these parameters showed statistically significant differences. Milk yield of cows with fatal mastitis decreased steadily. The lowest yield in recovered cows was recorded on average 36 h postchallenge. Cows receiving combination therapy produced 0.9 L more milk per day during the study period than cows receiving only flunixin ($P < 0.05$).

Pharmacokinetics

Concentration–time curves of enrofloxacin and its active metabolite ciprofloxacin in plasma and in milk are shown in Fig. 2. Pharmacokinetic data are presented in Table 1. Absorption of enrofloxacin was strongly delayed after s.c. administration: the mean apparent elimination half-life was about 23 h, while after i.v. administration elimination $t_{½}$ was only 1.5 h. The same phenomenon was seen in ciprofloxacin. The majority of antimicrobial activity in milk seems to originate from the active metabolite ciprofloxacin, which could be measured throughout the 120-h follow-up period after the last s.c. administration (Fig. 2). It is difficult to calculate the C_{max}/MIC ratios because both the parent compound and its metabolite, ciprofloxacin, are antimicrobially active. The sum of these compounds was used to estimate the C_{max} of total antimicrobial activity. The ratio of this C_{max}/MIC in milk was smaller after s.c. (14) than i.v. administration (24).

Flunixin concentration–time curves are depicted in Fig. 3. Two of the cows in Group 2 had to be excluded from the data set. One of these received flunixin meglumine perivascularly and the other had been killed during first challenge. Pharmacokinetic parameters are shown in Table 2. Flunixin concentration in milk was also measured from milk samples taken during

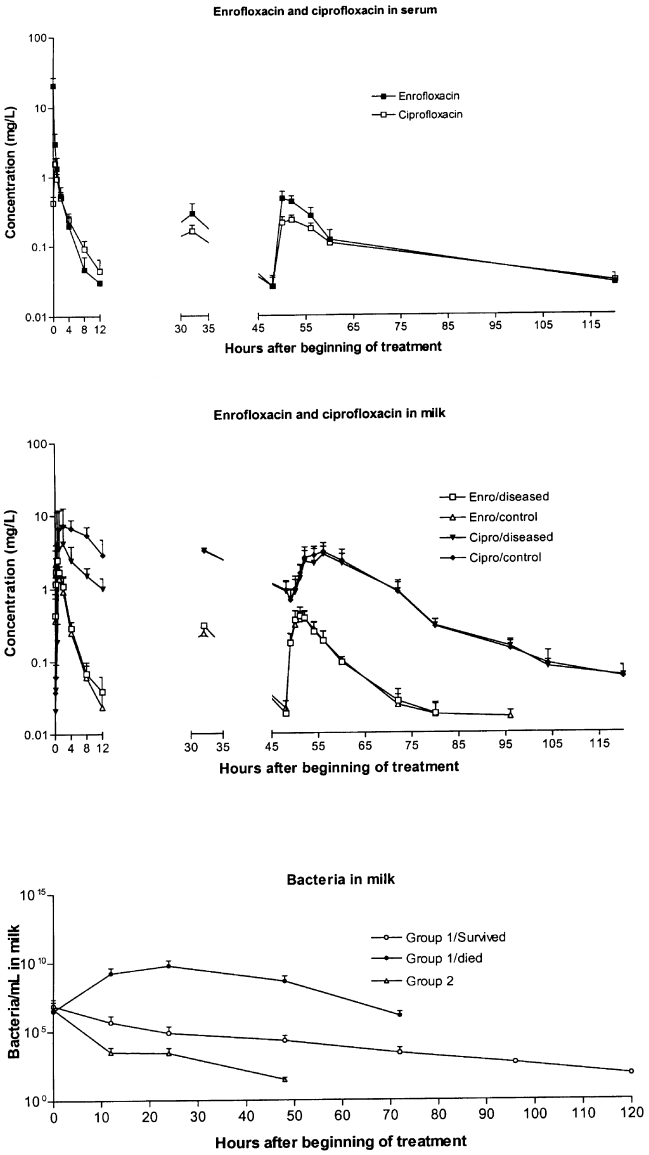

Fig. 2. Upper panel: concentration–time curves of enrofloxacin and its metabolite ciprofloxacin in serum. Each cow received three doses. The first dose (5 mg/kg) was administered at 0 h by the i.v. route. The second and third doses (5 mg/kg) were given s.c. 24 and 48 h later. Results are presented as means of six cows. Standard deviations are indicated by bars. Middle panel: Concentration of enrofloxacin and its metabolite ciprofloxacin in milk from infected and healthy control quarters. No samples were taken after the first s.c. administration (between 24 and 48 h). Lower panel: Number of bacteria in milk of infected quarter.

post-treatment morning and evening milkings but further analyses were not carried out because no flunixin or only traces were detected; one cow had a concentration of 0.019 mg flunixin per litre 8 h after administration.

DISCUSSION

No conclusive answer on the efficacy of antimicrobial treatment in coliform mastitis was provided. This may have been due to the small number of cows, which reduced the power of the statistical tests so that only strong effects could reach statistical significance. The only significant difference was seen in postchallenge milk yield, which remained at a higher level in the cows treated with enrofloxacin. Also Hoeben *et al.* (2000) found significant difference in milk production between the cows treated with enrofloxacin and nontreated cows when cows received enrofloxacin 5 mg/kg i.v. 10 h and s.c. 30 h postchallenge. No deaths occurred in our study among the cows treated with enrofloxacin, but the number of deaths did not differ significantly between treatment groups. The milk yield of the two cows with fatal mastitis dropped to less than 5 L/day 36 h postchallenge (Fig. 1). Interestingly, the prechallenge milk production of these cows was higher than that of the other cows. According to Jones and Jones (1986), high milk yield may be a predisposing factor to *E. coli* mastitis; in the four herds studied, the yield of cows subsequently developing coliform mastitis was higher than that of control cows. Vandeputte-van Messon *et al.* (1993) have also shown that cows classified as severe responders to experimentally induced *E. coli* mastitis produced more milk than cows classified as moderate responders. Also a low individual somatic cell count has been associated with an increased risk for clinical mastitis (Suriyasathaporn *et al.*, 2000). In the present study the cows with fatal mastitis did not have lower prechallenge somatic cell counts than the cows that survived.

The recovered cows were able to limit the bacterial growth in milk so that bacterial numbers started to decrease after 12 h postchallenge. Hoeben *et al.* (2000) found a significant difference in bacterial numbers at 18 and 48 h postchallenge between enrofloxacin and nontreated cows in experimental *E. coli* mastitis. Our results are also in accordance with the findings of Hill and Shears (1979) and Katholm and Andersen (1998), who found a positive correlation between number of bacteria in milk and severity of mastitic signs. According to the former study (Hill & Shears, 1979), bacterial numbers exceeding one million per millilitre were sufficiently high to impair killing of bacteria. In our study, mean bacterial numbers in both groups were between one million and 10 million at 12 h postchallenge. Thereafter, bacterial numbers increased only in the milk of those cows which later died (Fig. 1). Rapid individual immune response could explain the positive outcome of the disease.

The cows treated with enrofloxacin eliminated bacteria from the mammary gland faster than cows receiving no antimicrobial therapy, although the difference was not statistically significant (Fig. 2). A similar finding has been reported by Monfardini *et al.* (1999), i.e. bacterial numbers decreased more rapidly after i.v. administration of enrofloxacin than in nontreated controls. In addition, the pharmacokinetics of enrofloxacin differed markedly after i.v and s.c. administration, as also shown previously by Kaartinen *et al.* (1995). However, we measured total enrofloxacin, while in the earlier study free enrofloxacin was measured. After s.c. administration, the apparent half-lives of enrofloxacin and its metabolite, ciprofloxacin, were prolonged due to delayed absorption from the injection site (Table 1). No difference was present in concentrations of enrofloxacin and ciprofloxacin in milk of infected and

Table 1. Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin in five cows suffering from clinical *E. coli* mastitis. One cow was not in the data set because it was killed during the first challenge (Group 1, flunixin only). Enrofloxacin (Baytril vet, Bayer, Germany) was administered in a single i.v. dose of 5 mg/kg. Cows received the same dose s.c. 24 and 48 h later

	Mean ± standard deviation (range)	
Parameter	Enrofloxacin	Ciprofloxacin
Intravenous administration		
Elimination $t_{1/2}$ (h)	1.5 ± 0.2 (1.3–1.7)	2.8 ± 0.2 (2.6–3.0)
MRT (h)	0.8 ± 0.2 (0.6–1.1)	3.3 ± 0.4 (2.8–3.8)
AUC (mg h/L)	9.2 ± 2.8 (6.6–13.8)	3.7 ± 0.7 (2.7–4.6)
V_{dss} (L/kg)	0.47 ± 0.2 (0.32–0.74)	
Cl (L h/kg)	0.58 ± 0.16 (0.36–0.76)	
C_{max} (mg/L)		1.6 ± 0.3 (1.3–2.0)
T_{max} (h)		0.5
$C_{8\ h}$ (mg/L)	0.05 ± 0.02 (0.02–0.08)	0.09 ± 0.03 (0.07–014)
Second subcutaneous administration		
Apparent elimination $t_{1/2}$ (h)	23.1 ± 3.5 (18.2–27.1)	24.9 ± 4.0 (21.3–31.2)
MRT (h)	23.2 ± 2.4 (19.1–25.2)	28.8 ± 4.2 (25.1–35.6)
AUC (mg h/L)	8.7 ± 2.0 (5.2–10.6)	6.9 ± 1.0 (5.8–7.9)
MAT (h)	22.4 ± 2.5 (18.0–24.4)	
C_{max} (mg/L)	0.48 ± 0.12 (0.32–0.65)	0.23 ± 0.4 (0.20–0.28)
T_{max} (h)	2.4 ± 0.9 (2.0–4.0)	3.2 ± 1.1 (2.0–4.0)
$C_{8\ h}$ (mg/L)	0.12 ± 0.04 (0.06–0.18)	0.11 ± 0.01 (0.09–0.13)

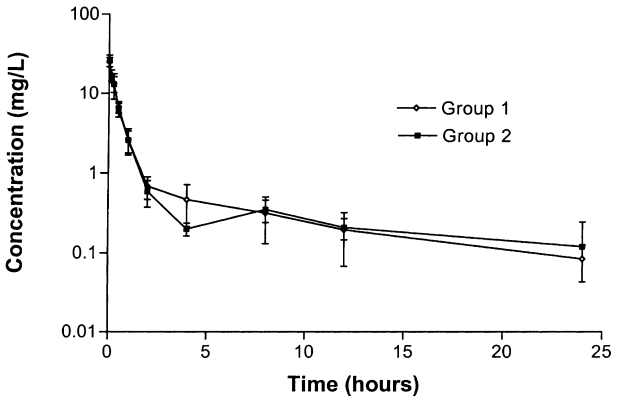

Fig. 3. Concentration–time curves of flunixin in cows which had received only flunixin meglumine (Group 1) and in cows which had received both flunixin meglumine and enrofloxacin (Group 2). Flunixin was administered in a single dose of 2.2 mg/kg i.v. Presented are the means of six and four cows, respectively. In Group 2, two cows were not in the data set because one was killed during the first challenge (Group 1) and the other was excluded due to perivascular injection. Standard deviations are indicated by bars.

noninfected control quarters (Fig. 2). The *MIC* of the challenge strain to enrofloxacin was less than 0.25 μg/mL. Antimicrobial activity in milk exceeded this for an extended period of time after s.c. administration. However, because fluoroquinolones are concentration-dependent drugs, a large C_{max}/MIC ratio is considered more important than the time that concentration at the infection site exceeds the *MIC* of the pathogen (Craig, 1993; Walker, 2000). This ratio in milk was larger after i.v. administration than s.c. administration.

Peracute coliform mastitis is a serious disease with a death rate of up to 80% in spite of intensive treatment with fluids, anti-inflammatory drugs and antimicrobials (Radostits *et al.*, 2000). The efficacy of different antimicrobial treatments has been studied in experimental models and field trials. Jones and Ward (1990) found no significant difference in whether the cows suffering from clinical coliform mastitis were treated with parenteral gentamicin or with erythromycin, to which coliforms are intrinsically resistant. All these cows were also treated with cephapirin-containing intramammaries. Although intramammary treatment is usually not considered to be very

Table 2. Pharmacokinetics of flunixin in cows suffering from clinical *E. coli* mastitis. Cows in Group 1 received only flunixin meglumine. Cows in Group 2 received both flunixin meglumine and enrofloxacin. Flunixin was administered to cows in both groups once in a single intravenous dose of 2.2 mg/kg. Presented are the means of six and four cows, respectively. In Group 2, two cows were not in the data set because one was killed during the first challenge (Group 1) and the other was excluded due to perivascular injection. None of the parameters showed statistically significant differences

	Mean ± standard deviation (range)	
Parameter	Group 1 ($n = 6$)	Group 2 ($n = 4$)
Elimination $t_{1/2}$ (h)	5.7 ± 2.6 (3.2–9.4)	6.2 ± 1.8 (4.6–8.8)
MRT (h)	3.5 ± 1.8 (1.7–6.2)	4.0 ± 1.3 (3.0–5.9)
AUC (mg h/L)	17.1 ± 5.1 (10.6–22.9)	17.9 ± 4.3 (13.8–23.0)
V_{dss} (L/kg)	0.43 ± 0.10 (0.31–0.59)	0.49 ± 0.09 (0.37–0.56)
Cl (L h/kg)	0.14 ± 0.04 (0.10–0.21)	0.13 ± 0.03 (0.10–0.16)

effective in coliform mastitis, the use of a first-generation cephalosporin could have had some effect in that study. To our knowledge, the only published study where efficacy of antimicrobials to treat coliform mastitis was shown is one trial carried out on a serious experimentally induced *E. coli* model (Shpigel *et al*., 1997). In field conditions, the susceptibility of the causative organism is not known when the decision to treat the suspected coliform mastitis with antimicrobials is made. Shpigel *et al*. (1998) had shown that the odds ratio of recovery in cases caused by coliforms, which were susceptible to trimethoprim-sulphonamide and treated with the same drug, was 2.75 compared with cases caused by resistant organisms. In contrast, Pyörälä and Syväjärvi (1987) found no correlation between *in vitro* susceptibility of the isolated coliform strain to the drug used in the treatment, and recovery of the cow.

Anti-inflammatory medication is often used in the treatment of mastitis. Swedish veterinarians, for example, gave anti-inflammatory drugs in 71% of peracute mastitis cases (Ekman *et al*., 1994). We chose flunixin because of its proven ability to diminish detrimental effects of endotoxins (Anderson *et al*., 1986). Dascanio *et al*. (1995) compared the effect of phenylbutazone or flunixin meglumine with a placebo in cows suffering from acute mastitis and treated with intramammary gentamicin, but they found no significant difference in the loss of milk among the three treatment groups. Neither was any difference found when the effect of fluid therapy, flunixin meglumine and a combination of fluid therapy and flunixin meglumine were compared in the treatment of toxic mastitis (Green *et al*., 1997). However, in the latter study, which included only 22 confirmed coliform cases, the cows were seriously ill or even moribund, which may explain the results.

The anti-inflammatory effect of nonsteroidal anti-inflammatory drugs outlives the drug's presence in the bloodstream because of the high degree of protein binding at the site of inflammation (Nolan, 2000). The mean elimination half-life of flunixin in our study was 5.7–6.2 h. This is close to that (5.2 h) reported in heifers by Odensvik (1995) and slightly lower than the 8.1 h reported by Hardee *et al*. (1985). Anderson *et al*. (1990) observed an elimination half-life of 3.1 h with a dosage half that of ours. We have not been able to find any published reports on flunixin concentrations in milk other than a summary report of a maximum residue limit (MRL) for flunixin (http://www.emea.eu.int). In the study of Anderson *et al*. (1990), no flunixin was detected in any of the milk samples, but the detection limit of their assay was quite high. Our findings that flunixin concentrations in milk were low are in agreement with the MRL summary report. Because flunixin is a weak acid, it has difficulty crossing from blood to milk, which has a lower pH.

Evidence for the general benefit of antimicrobial treatment in coliform mastitis is still lacking. Cows in early lactation are most susceptible and variation between cows' capacity to respond to infection is marked, as seen in our study. According to our findings, a heavy drop in milk yield of the cow was only statistically significant variable explaining the outcome of *E. coli* mastitis. While treatment with enrofloxacin did enhance the elimination rate of bacteria, the difference was not significant. In conclusion, antimicrobial treatment may be beneficial in the treatment of high-yielding cows in early lactation, particularly if serious prognostic signs are recorded.

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