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RESEARCH ARTICLE

Hematological and Biochemical Alterations due to Over Dosage of Enrofloxacin in Cats

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ABSTRACT

Enrofloxacin, an antimicrobial agent used to treat bacterial diseases is well tolerated by cats at recommended dosage (5 mg/kg). To investigate the tolerance of high-dose of the Hipralona Enro-I® (5% enrofloxacin solution) in cats, 28 urban cats (11 males and 17 females) between the weight of 1.3 and 2.4 kg were randomly assigned to a control group (n=4) and three treatment groups (n=8). Each treatment groups were injected low-dose enrofloxacin (5 mg/kg, IM), high-dose enrofloxacin (15 mg/kg, IM), and very high-dose enrofloxacin (25 mg/kg, IM) once daily for seven consecutive days. Blood samples were collected from jugular vein before injection of enrofloxacin in the hind limb muscles of cats as self-control, and at 3rd, 7th, 14th, and 21st days after first drug injection. Samples were tested for various hematological and serum biochemical parameters. The systemic tolerance during experiment was investigated via monitoring of behavior and general physical examination. Hematological and serum biochemical parameters were not significantly different between groups. In addition, there was no statistically difference between various sampling days for any treatment group in each parameter. Furthermore, there was no change in the behavior and the general health condition of cats in the control and the three experimental groups. As serum biochemical indications of hepatotoxication and nephrotoxication were not observed. It was concluded that muscular injection of enrofloxacin in doses up to 25 mg/kg ($5ED_{50}$) for seven consecutive days was tolerated by cats.

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INTRODUCTION

Fluroquinolones are a relatively new class of potent synthetic antimicrobial agents, with a long lasting bactericidal effect and a broad spectrum of antibacterial activity, including strains resistant to many other antibacterial agents (Guay, 1992; Choi *et al.*, 2011). Several fluoroquinolones are used widely in human medicine. Enrofloxacin is the only member of the fluoroquinolone family in veterinary medicine. Enrofloxacin was synthesized in Bayer's laboratories in the mid 1980's and intended particularly for veterinary use. Good results of using enrofloxacin were reported in treating uncomplicated bacterial infections (Stanley and Pacchiana, 2008; Forster-van Hijfte *et al.*, 2011) and in opportunistic mycobacterial infections in cats (Studdert and Hughes, 1992). Enro- floxacin has been used against gram-negative aerobes (*E. coli*, *Proteus* spp., *Pasteurella multocida*, *Bordetella bronchiseptica*, some *Pseudomonas* spp.); *Staphylococcus intermedius* that causes bacterial pneumonia in cat; *Haemobartonella felis* as an agent of feline infectious anemia (Dowers *et al.*, 2002).

Administered at the originally recommended dosage of 2.5 mg/kg, PO, every 12 hours, the drug shown to be effective and well tolerated in cats. In 1997, the popular trend to administer bactericidal antibiotics on a once-daily basis to achieve higher peak plasma concentrations influenced the manufacturer of enrofloxacin to alter its dosing guidelines. Once-daily administration was associated with improved efficacy and less development of bacterial resistance (Turnidge, 1999). Although enro-floxacin typically has linear pharmacokinetics and serum

concentrations that can be correlated with bacterial effects, a variable dosing range of 5 to 20 mg/kg as a single or split dose was added to the enrofloxacin label.

Because of the ease of injection a once-daily dose and the increasing resistance of isolates to other antimicrobials, veterinarians commonly began administering large daily doses of enrofloxacin. Unfortunately, the new dosing guidelines were frequently exceeded, with doses being given often more than twice the maximum recommended dosage of 20 mg/kg/day (Gelatt *et al.*, 2001). Therefore, the aim of the present study was to investigate the tolerance of high-dose of enrofloxacin injection in cats.

MATERIALS AND METHODS

The experiments were performed on 28 urban cats, (11 males and 17 females) aged 2-5 years weighing 1.3-2.4 kg. These cats were hunted by special safe traps from different parts of Shiraz. Cats were acclimated to laboratory housing for at least five weeks prior to the experiment. The cats were housed in individual cages with free access to drinking water and food. In the first week of the adaptation time, physical examination, CBC test, deworming, and ectoparasite treatment were performed to rule out other concurrent diseases and previous infections. Experimental procedures were according to National Institutes of Health and European Union guidelines for animal care.

The cats were randomly allocated into three treatment (n=8) groups: low-dose enrofloxacin (5 mg/kg, =ED50) (Hipralona Enro-I®, Hipra Co., Spain), high-dose enrofloxacin (15 mg/kg, =3ED50), and very high-dose enrofloxacin (25 mg/kg, =5ED50), and a control group (n=4, 0.5 ml distilled water as placebo) intramuscularly in the hind limb muscles, once daily for seven consecutive days. Blood samples were collected from jugular vein before injection of drug or placebo on day 0 as self-control for each group. Further blood samples with or without anticoagulant were collected on days 3, 7, 14, and 21 after first day of injection of drug or placebo. Samples were analyzed for hematological parameters (RBC counts, WBC counts, hemoglobin concentration, hematocrit, MCV, and MCHC) by using standard routine hematological techniques (Weiss and Wardrop, 2010). Serum concentration of glucose was assayed by the O-toluidine method, total protein by the Biuret method, blood urea nitrogen (BUN) with the di-acetyl monoxime method, and creatinine with the modified Jaffe method. Serum enzyme activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was evaluated by the colorimetric method. All blood samples were assayed by a technician who was blinded to the treatment groups.

Amount of food and water consumption, body temperature, pulse, respiration, mucosa, general behavior (locomotion, coordination, special adequacy & purposefullness of locomotion, obstacle overcoming), defecation, micturition, skin and hair status were recorded by another

Table 1: Hematological parameters (Mean±SE) at sampling of pre-administration day and various days during and after injection of enrofloxacin for three groups of cats

Parameters	Sampling Days				
	0†	3	7	14	21
PCV (%)					
LD‡	37.13±1.6	36.75±1.7	37.00±1.6	36.63±1.7	37.25±1.8
HD	39.88±2.0	40.13±1.8	39.63±1.9	39.37±1.9	39.25±1.8
VHD	38.38±1.9	38.63±1.8	38.13±1.8	37.88±1.8	37.75±1.8
Control	38.00±1.4	38.50±1.6	37.25±1.3	37.25±1.3	37.00±1.7
P-value	0.73	0.59	0.72	0.71	0.83
Hb (g/dl)					
LD	8.98±0.4	8.99±0.4	8.99±0.4	8.99±0.4	8.99±0.4
HD	10.05±0.7	10.05±0.7	10.05±0.7	10.05±0.7	10.06±0.7
VHD	9.75±0.2	9.75±0.2	9.75±0.2	9.75±0.2	9.75±0.2
Control	10.60±0.4	10.60±0.5	10.60±0.4	10.60±0.5	10.60±0.5
P-value	0.23	0.24	0.24	0.24	0.23
RBCs (10^6/µl)					
LD	10.26±0.2	10.25±0.2	10.25±0.2	10.26±0.2	10.26±0.2
HD	9.69±0.4	9.70±0.4	9.66±0.4	9.69±0.4	9.69±0.4
VHD	9.56±0.3	9.56±0.3	9.53±0.3	9.51±0.3	9.53±0.3
Control	9.58±0.3	9.65±0.3	9.60±0.3	9.65±0.3	9.63±0.3
P-value	0.34	0.39	0.34	0.33	0.38
MCV (fl)					
LD	35.88±1.6	35.63±1.7	35.88±1.7	35.38±1.7	36.13±1.7
HD	40.88±2.0	41.13±2.0	41.00±2.0	40.50±2.0	40.38±1.8
VHD	39.75±2.1	40.00±2.0	39.75±1.7	39.63±1.7	39.25±1.9
Control	39.75±2.3	39.75±2.6	38.50±2.1	38.25±2.3	38.25±2.7
P-value	0.29	0.21	0.24	0.22	0.43
MCHC (g/dl)					
LD	24.00±1.0	24.25±0.9	23.75±1.0	24.13±0.9	24.00±0.9
HD	24.75±1.0	24.63±1.0	25.00±1.1	24.88±1.0	25.00±1.0
VHD	25.00±0.9	25.00±0.9	25.38±0.9	25.50±0.9	25.63±0.9
Control	27.25±0.5	26.75±0.3	28.00±0.4	28.00±0.6	28.25±0.3
P-value	0.23	0.42	0.10	0.11	0.07
WBC (10^3/µl)					
LD	1.21±0.2	1.17±0.2	1.16±0.2	1.13±0.1	1.09±0.1
HD	1.98±0.3	1.66±0.2	1.51±0.2	1.30±0.2	1.16±0.2
VHD	1.23±0.3	1.11±0.2	0.82±0.1	0.78±0.1	0.83±0.1
Control	1.21±0.3	1.21±0.2	1.15±0.2	1.19±0.2	1.19±0.2
P-value	0.08	0.24	0.07	0.10	0.35

† After blood sampling enrofloxacin was intramuscularly injected once daily for seven consecutive days and the same, control group was injected by placebo (0.5 ml distilled water). ‡ LD low dose group (5 mg/kg), HD high dose group (15mg/kg), VHD very high dose group (25mg/kg).

Table 2: Biochemical parameters (Mean±SE) at sampling of pre-administration day and various days during and after injection of enrofloxacin for three groups of cats

Parameters	Sampling Days				
	0†	3	7	14	21
ALT (U/L)					
LD‡	48.94±2.3	52.19±4.3	45.88±2.7	45.82±3.3	48.07±3.6
HD	43.19±2.4	46.50±3.7	43.57±2.8	46.00±4.1	43.32±2.6
VHD	44.57±3.7	45.12±3.6	44.82±2.9	43.44±2.7	42.88±3.4
Control	45.25±2.9	42.25±3.8	44.5±6.4	43.00±3.6	40.50±3.9
P-value	0.51	0.41	0.96	0.90	0.49
AST (U/L)					
LD	36.75±1.8	39.25±1.7	43.50±1.5	43.38±1.4	41.75±1.6
HD	35.88±2.2	39.38±2.7	43.50±3.6	40.00±2.4	38.00±2.3
VHD	37.13±1.7	45.13±2.7	46.63±1.9	38.13±2.2	35.63±2.2
Control	35.75±3.1	36.25±2.5	35.75±1.6	37.00±0.8	38.00±1.1
P-value	0.96	0.14	0.10	0.18	0.20
Glucose (mg/dl)					
LD	96.79±4.5	96.96±4.5	97.01±4.5	96.97±4.5	97.05±4.6
HD	88.16±6.7	88.39±6.7	88.50±6.7	88.47±6.6	88.61±6.7
VHD	100.16±2.3	100.18±2.4	100.81±2.4	101.15±2.6	100.58±2.6
Control	97.50±2.8	98.17±2.9	99.45±2.9	98.92±2.4	100.10±2.3
P-value	0.31	0.32	0.28	0.27	0.29
Total protein (g/dl)					
LD	7.73±0.3	7.17±0.2	7.08±0.2	7.00±0.2	6.93±0.2
HD	7.70±0.2	7.45±0.3	7.37±0.3	7.29±0.3	7.24±0.3
VHD	6.97±0.3	6.96±0.3	6.95±0.3	6.94±0.3	6.93±0.3
Control	6.96±0.5	6.92±0.5	6.95±0.5	6.95±0.5	6.96±0.5
P-value	0.13	0.60	0.72	0.80	0.82
BUN (mg/dl)					
LD	32.19±3.8	32.51±3.9	30.90±3.9	32.32±3.8	32.44±3.9
HD	36.15±9.4	36.83±8.9	36.92±9.2	36.87±8.7	36.95±9.1
VHD	35.65±3.1	36.09±3.0	36.12±2.9	36.42±3.8	35.70±3.4
Control	41.45±9.5	41.96±9.3	41.77±9.9	41.60±9.5	42.05±9.6
P-value	0.37	0.33	0.26	0.36	0.33
Creatinine (mg/dl)					
LD	1.42±0.5	1.45±0.5	1.47±0.5	1.47±0.4	1.40±0.5
HD	1.37±0.4	1.37±0.5	1.40±0.3	1.42±0.5	1.43±0.4
VHD	1.97±0.3	2.12±0.3	2.0±0.2	1.92±0.5	2.0±0.3
Control	1.57±0.2	1.68±0.3	1.57±0.4	1.60±0.5	1.57±0.3
P-value	0.19	0.10	0.16	0.44	0.22

†After blood sampling enrofloxacin was intramuscularly injected once daily for seven consecutive days and the same, control group was injected by placebo (0.5 ml distilled water). ‡LD low dose group (5 mg/kg), HD high dose group (15mg/kg), VHD very high dose group (25mg/kg).

author who was blinded to the treatment groups In addition, cats were daily assessed for immediate medical attention. The entire cats were adapted out to private homes at the end of the study.

Parametric one-way ANOVA with Tukey test was used to investigate differences of hematological and biochemical parameters between groups (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). P Value <0.05 was considered significant.

RESULTS

There was no change in the behavior and the general health condition of cats in the control and the three experimental groups. Statistical analysis indicated that there was no difference in PCV, Hb, RBC indices, and WBC count between the three treatment groups and the control group (P>0.05, Table 1). Furthermore, there was no difference in serum biochemical parameters (glucose, total protein, ALT, AST, BUN, and creatinine) between the three treatment groups and the control group (P>0.05, Table 2). In addition, there was no statistically difference between various sampling days for any treatment group in each parameter (P>0.05).

DISCUSSION

Results of the present study showed that there was no hematological and serum biochemical changes in cats treated with different doses of enrofloxacin. However, WBC count decreased between the expected normal variation in all groups during the study period, it was not considered as a treatment-related effect. Decreased WBC counts and PCV ratio were reported in humans receiving fluoroquinolones (Blum *et al*., 1994; Maguire *et al*., 1994). However, these adverse effects were not observed in dogs receiving enrofloxacin (Traş *et al*., 2001).

Injection of enrofloxacin did not change the serum glucose concentrations of cats during the study period. Consistent with our findings, using enrofloxacin did not reveal any significant effects on serum glucose in laboratory animals (Altreuther, 1987) and in dogs (Traş *et al*., 2001). Meanwhile, enrofloxacin at dosage of 5 and 10 mg/kg, PO, q 12 h was administered to dogs for 1 day may result in underestimate urine glucose concentration (Rees and Boothe, 2004). Reported side effects of enrofloxacin in animals are lameness, acidosis, polydipsia and crystalluria (Orsini and Perkons, 1992; Boothe, 1994). However, the lack of changes in serum BUN & creatinene concentrations during the present study showed that enrofloxacin injection in cats had no nephrotoxic effect.

Our results confirmed the lack of clinical manifestations of intolerance to enrofloxacin, reported by other authors (Kordick *et al*., 1997; Todorov, 2004). There were no symptoms of lameness, edema, painfulness and increased body temperature in all treated cats during

the period of study. Furthermore, the lack of changes in post treatment total protein values, AST and ALT activities vs the pretreatment values did not support the hepatotoxic effect of enrofloxacin (Greene *et al.*, 2002). Enrofloxacin is reported to reduce specific liver enzymes (Vancutsem and Babish, 1996). However, a clinical study using enrofloxacin showed an increase of ALT and AST (Neer, 1988). In addition, Traş *et al.* (2001) showed that enrofloxacin injection at the recommended dose of 5 mg/kg IM, once daily to healthy dogs for 14 days, causes a transient increases in AST and MCV levels. Similar effects are reported in humans receiving ciprofloxacin and norfloxacin (Davoren and Mainstone, 1993; Jones and Smith, 1997). Furthermore, enrofloxacin (20 mg/kg, PO, q 24 h) caused a significant decrease in fibrinogen concentration in dogs (Webb *et al.*, 2006). Meanwhile, serum total protein concentrations of the cats in the present study did not change after the injection of enrofloxacin with doses of 5, 15 and 25 mg/kg body weight, once daily for seven consecutive days.

Unlike the data for ophthalmic disorders consequently to degenerative retinal changes in cats treated with enrofloxacin (Ford *et al.*, 2007; Şaroglu and Erdikmen, 2008), the tested doses and regimen of application of Hipralona Enro-I® did not result in clinically detectable changes in the vision of experimental animals (mydryasis, lack of coordinated and purposeful locomotion, obstacle overcoming).

Conclusions

Results of the present study indicated that the injection of enrofloxacin in doses up to 25 mg/kg (5ED50) for seven consecutive days was tolerated by cats & did not alter hematological & biochemical parameters.

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