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Full Length Research Paper

Effect of Ciprofloxacin and Levofloxacin on haematological parameters of dogs

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ABSTRACT

In this study, the effect of two fluoroquinolones employed in small and companion animal medicine were evaluated. The haemogram of eight healthy dogs administered with oral ciprofloxacin (25mg/kg) or levofloxacin (25mg/kg) 12hourly for 14days was assessed using the packed cell volume (PCV), red blood cell counts, haemoglobin concentration, total white blood cells and the differential counts and platelet count. Blood samples were collected before commencement of treatment on day 0 and subsequently on days 2, 5, 7, 10 and 14. Dogs administered with ciprofloxacin exhibited a gradual decline in the PCV between days 0 (34.0±4.0%) and 10 (28.7±1.5%), followed by a return to pre-treatment value by day 14 (34.0±1.0%). Red cell indices showed a similar pattern of decline with the lowest level recorded on day 10 (4.5±0.3X10^6/µL) from 5.3±0.8X10^6/µL on day 0. White cell indices on the other hand were within the same range throughout the study. Levofloxacin reduced the mean PCV between days 0 (39.3±5.9%) and 2 (28.7±3.2%) with a gradual return to pre-treatment values by day 14 (33.7±5.3%). The decline in the PCV of dogs administered with levofloxacin may be due to the reduction in all the blood parameters except the mean lymphocyte and eosinophil counts (33.3±6.4% and 2.3±1.5%) which increased from 17.8±4.3% and 1.8±0.6%. The platelet count also reduced by day 2 for ciprofloxacin (1.70±0.17X10^5/µL) and levofloxacin (1.65±0.26X10^5/µL). The two fluoroquinolones showed some potential for causing anaemia and a mild blood clotting defect which may resolve within a few days. From this study, prescription and administration the drugs should be with caution especially in dogs with any underlying blood dyscrasia.

Keywords: Ciprofloxacin, Levofloxacin, haematology, dog

INTRODUCTION

Fluoroquinolone antibiotics are a group of antibacterial agents derived from the basic structure of nalidixic acid, which was first synthesized in early 1960s (Tafani *et al.*, 1982; Kawahara, 1998). Further substitution of the quinolone molecule has led to the development of generations of fluoroquinolones (Andersson and MacGowan, 2003). This group of drugs is important for the treatment of serious gram-negative infections in small animals (Liu and Mulholland, 2005; MacDougall *et al.*, 2005). Ciprofloxacin is a second generation fluoroquinolone and the first fluoroquinolone to be commercialized. Levofloxacin on the other hand is a third generation fluoroquinolone, the pure S-enantiomer of the racemic drug substance ofloxacin (Morrissey *et al.*, 1996).

Serious adverse events occur more commonly with fluoroquinolones than with any other antibiotic drug classes. Most adverse reactions are mild to moderate;

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however, on occasion, serious adverse effects occur (De Sarro and De Sarro, 2001; Owens and Ambrose, 2005). Both ciprofloxacin and levofloxacin have been reported to cause spontaneous tendon ruptures, rhabdomyolysis and worsen myasthenia gravis (Petitjeans *et al*., 2003; Hsiao *et al*., 2005; Gunduz *et al*., 2006). These antibiotics are contraindicated in geriatric, pediatric, pregnant or lactating patients due to severe adverse effects associated with them unless for very severe bacterial infections (Shin *et al*., 2003; Owens and Ambrose, 2005; Iannini, 2007). Bone marrow suppression, interstitial nephritis and haemolytic anaemia may also occur during ciprofloxacin therapy in humans (Dutta and Badhe, 1999; Lim and Alam, 2003), while levofloxacin has been reported to cause auto-immune anaemia (Oh *et al*., 2003)

There is, however sparse information on detailed investigation of haematological changes that may accompany administration of these fluoroquinolone antibiotics in dogs. This study was therefore designed to assess the possible haematological changes which may be observed following prolonged administration of the fluoroquinolone antibiotics ciprofloxacin or levofloxacin to dogs.

MATERIALS AND METHODS

Experimental animals

Eight healthy local dogs between the ages of 6 - 12 months, weighing 8-10 kg were acquired from Oyo town in Oyo State. The dogs were housed at the boarding kennels of the Veterinary Teaching Hospital of the University of Ibadan. The dogs were stabilized and acclimatized for a period of 14 days. They were dewormed using a combination anthelminthic drug, Prazisam® (pyrantel pamoate, prazinquantel and febendazole) at a dose rate of 10mg per kg body weight during this period. They were fed with compounded dog food and water was provided *ad libitum.* The dogs were randomly and equally divided into two groups, each to be administered with ciprofloxacin or levofloxacin. The experiment was conducted as a cross-over study. The animals were treated humanely and the study design was in accordance with the ethical codes of care and use of animals.

Drug Administration and Sample collection

To each group, 20mg/kg ciprofloxacin and levofloxacin tablets were respectively administered orally twice daily for a period of 14 days. Blood samples were collected via the jugular vein before administration of the first dose and subsequently on the days 2, 5, 7 and 14 of the administration of the drugs. About 3ml of blood was collected into Lithium heparinized bottles for haematological analysis by Cole's method (Cole, 1986). Haematological parameters determined include packed cell volume, red blood cell count, haemoglobin count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell, lymphocyte, neutrophils, eosinophil and platelet counts.

Statistical analysis

Data were presented as the mean ±standard error of mean. The statistical differences of paired data within a group were evaluated by one-way repeated measures analysis of variance (ANOVA). The statistical significance was considered at $p<0.05$.

RESULTS

Packed cell volume (PCV)

The PCV of dogs administered with ciprofloxacin reduced from 34.00±4.02% on day 0 until the day 10 (28.67±1.45%) after which a return to the baseline level was observed on day 14 (34.00±1.00%). The PCV of dogs administered with levofloxacin was observed to decline from day 2 (28.67±3.18%) but did not return fully to baseline level by day 14 (33.67±5.33%).

Red blood cell count (RBC)

The same pattern of decline in PCV was also observed in mean RBC of the dogs in both groups. RBC value was least on day 5 (4.55±1.13 $X10^6/\mu L$) for dogs administered with ciprofloxacin, with a drastic return to values beyond baseline values from day 7 (5.33±0.69 $X10^6/\mu L$), but dogs administered with levofloxacin showed reduced RBC values for the entire duration of the experiment.

Haemoglobin concentration (Hb)

Mean Hb of the dogs administered with ciprofloxacin decreased from 11.00±1.30g/dl on day 0 to 10.35±1.21g/dl by day 2. A further decline was observed in the course of this experiment with a return to baseline levels on day 14 (11.10±0.21g/dl). Dogs administered with levofloxacin, however showed a continuous decline in the mean Hb from 11.05±1.34g/dl on day 0 to 10.77±1.98g/dl on day 14.

White blood cell count (WBC)

The white blood cell counts initially decreased between day 0 (10.30±2.80 $X10^3/\mu L$) and day 5 (8.93±1.02 $X10^3/\mu L$), but increased beyond the base line levels between day 7 (10.66±2.22 $X10^3/\mu L$) and day 10

(10.58±0.82 $X10^3/\mu L$) for dogs administered with ciprofloxacin. The levofloxacin group on the other hand showed continuous fluctuations in the mean WBC with the highest value observed on day 5 (12.60±2.86 $X10^3/\mu L$).

Differential cell counts

Lymphocytes and Neutrophils: For dogs administered with ciprofloxacin, mean lymphocyte count on day 0 (28.75±3.30%) was initially significantly ($p<0.05$) decreased to 23.25±1.03%. A gradual return to normal was observed between day 5 (26.00±1.78%) and day 7 (25.00±2.27%). By day 10 (30.33±1.45%) the lymphocyte levels had exceeded that observed on day 0. Neutrophil levels were increased throughout the course of the experiment from 66.00±3.32% on day 0 to 72.67±3.18% on day 14.

Dogs administered with levofloxacin recorded non-significantly increased lymphocytes counts in the course of the experiment from day 0 (22.75±1.65%) till day 14 (26.00±2.00%). The neutrophil levels also increased from 71.75±1.32% on day 0 to 74.00±1.53% on day 10. A decline was however, observed on day 14 (66.33±2.60%).

Monocytes and Eosinophils: Monocyte and eosinophil levels initially increased from 3.50±0.50% and 1.75±0.48% on day 0 to 3.75±0.25% and 2.75±1.38% on day 2. Subsequently a decline was observed for the other observation days of the experiment except the sudden increase in eosinophil levels on day 14 (4.33±0.88%). Dogs administered with levofloxacin showed a decline in the monocyte counts from on day 0 (3.75±0.48%) and the least observed on day 10 (1.67±1.20%). Eosinophil count however increased throughout the course of the experiment, the highest count observed on day 14 (4.67±1.45%).

Platelet count: Mean platelet count were also initially decreased between day 0 (2.01±0.55 $X10^5/\mu L$) and day 5 (1.83±0.22 $X10^5/\mu L$), but returned to baseline levels by day 7 (2.09±0.22 $X10^5/\mu L$) for ciprofloxacin group, while the decline observed in the levofloxacin group on day 0 (2.27±0.64 $X10^5/\mu L$) continued till day 10 (1.93±0.38 $X10^5/\mu L$) with a return to baseline by day 14 (2.42±0.30 $X10^5/\mu L$).

Table 1:
Full blood count of dogs administered with Ciprofloxacin (25mg/kg) 12hourly for 14 consecutive days

	Day 0	Day 2	Day 5	Day 7	Day 10	Day 14
PCV %	34.00±4.02	32.75±4.03	30.00±6.01	33.50±4.35	28.67±1.45	34.00±1.00
$RBCX10^6/\mu L$	5.29±0.77	5.12±0.75	4.55±1.13	5.33±0.69	5.31±0.27	5.45±0.25
Hb g/dl	11.00±1.30	10.35±1.21	9.63±2.04	10.68±1.35	9.03±0.49	11.10±0.21
$WBCX10^3/\mu L$	10.30±2.80	8.83±1.18	8.93±1.02	10.66±2.22	10.58±0.82	8.97±0.88
Lymphocyte%	28.75±3.30[a]	23.25±1.03 [ab]	26.00±1.78	25.00±2.27	30.33±1.45[b]	30.30±2.33
Neutrophil%	66.00±3.32	71.75±3.73	69.50±2.66	71.25±1.97	67.67±6.89	72.67±3.18
Monocytes %	3.50±0.50	3.75±0.25	2.50±0.22	2.50±0.87	2.67±1.20	2.67±0.67
Eosinophil%	1.75±0.48[a]	2.75±1.38[b]	2.00±1.23[c]	1.75±0.85[d]	1.67±0.67[e]	4.33±0.88[abcde]
Platelet $X10^5/\mu L$	2.01±0.55	1.70±0.17	1.83±0.22	2.09±0.22	2.02±0.58	1.93±0.13

Same superscripts on a row are statistically significant

Table 2:
Full blood count of dogs administered with levofloxacin (25mg/kg) 12hourly for 14 consecutive days

	Day 0	Day 2	Day 5	Day 7	Day 10	Day 14
PCV %	34.25±3.77	28.67±3.18	30.00±2.94	32.75±2.78	30.33±2.91	33.67±5.33
$RBCX10^6/\mu L$	5.56±0.66	5.46±1.03	5.94±0.51	5.48±0.56	4.80±0.37	5.51±0.90
Hb g/dl	11.05±1.34	10.53±1.85	9.60±0.89	11.25±0.92	9.63±0.83	10.77±1.98
$WBCX10^3/\mu L$	11.90±3.12	11.00±2.08	12.60±2.86	10.99±0.70	11.35±1.66	12.02±3.10
Lymphocyte%	22.75±1.65	24.00±3.06	23.00±4.08	23.25±1.84	22.67±3.18	26.00±2.00
Neutrophil%	71.75±1.32	71.00±2.08	72.25±4.37	72.50±2.08	74.00±1.53	66.33±2.60
Monocytes %	3.75±0.48	3.67±1.20	2.75±0.25	2.00±0.41	1.67±1.20	3.67±0.67
Eosinophil%	1.75±0.63	2.33±1.21	2.00±0.71	2.50±0.50	2.67±1.20	4.67±1.45
Platelet $X10^5/\mu L$	2.27±0.64	1.65±0.26	2.13±0.40	1.95±0.06	1.93±0.38	2.42±0.30

Same superscripts on a row are statistically significant ($p<0.05$)

DISCUSSION

The haemogram of dogs administered with ciprofloxacin or levofloxacin in this study showed clinically significant ($p<0.01$) depression of the haematological parameters assessed. Absorption of drugs into the blood stream or direct intravenous administration poses a potential hazard to blood cells which primarily come into contact with these drugs as they are transported to their sites of action. Many drugs administered parenteral for reasons other than their haematological effect have been reported to cause adverse effect(s**)** in the blood, which in some cases may be severe (Seltsam and Salama, 2000; Garratty and Arndt, 2007). Antibiotics including cephalosporins, penicilins and some fluoroquinolones have been severally reported to cause haemolytic anaemia (Owens and Ambrose, 2005; Powers and Silberstein, 2008; Schrier and Price, 2008; Tuccori *et al.,* 2008; Mayer *et al.,* 2010). Fluoroquinolones are currently one of the most commonly prescribed classes of antibiotics in both humans and animals. This class of antibiotics is the first line drug for several infections including genitourinary infections and pulmonary infections **(**Liu and Mulholland, 2005; MacDougall *et al.,* 2005**)**. Ciprofloxacin and levofloxacin are broad spectrum antibiotics which reach therapeutic levels in many secretions of the body, including articular fluids after being distributed or re-distributed by the blood to these sites. Thus, these drugs are very relevant to present day clinical practice.

In this study, ciprofloxacin caused reduction in the packed cell volume (PCV) with depression in the mean red and white blood cell indices, and the platelet count. The decline in PCV continued through the course of treatment, although a slight recovery was observed only on day 7 of the treatment. This decline may be attributed to decreases in both red and white blood cell counts which persisted until day 5 of treatment. Recovery from these anomalies was observed by the end of the first week of treatment, with further depressions in the blood cell levels by day 14 observation. Levofloxacin on the other hand caused a persistent depression of the PCV, and red and white blood cells. Although attempts at recovery was observed between days 5 and 7 of treatment, a progressive anaemia with reduced platelet counts was observed by day 14 of treatment. This is a similar finding to that reported by Oh *et al*. (2003) in humans.

Three major mechanisms have been postulated for drug-mediated anaemia (Packman, 2001). The first is the hapten-drug adsorption mechanism which involves covalent binding of drug to RBC membrane and attachment of anti-drug antibody to the membrane-bound drug, which opsonizes the cells for destruction by splenic macrophages. Secondly, a trimolecular complex may be formed which composes of the drug, RBC membrane antigen and an antibody which recognises the complex formed by the drug and RBC membrane. These two mechanisms require the presence of the drug, while the third mechanism does not require the drug. Some drugs directly stimulate formation of true autoantibodies which are same as those seen in autoimmune haemolytic anaemia, with increased formation of lymphocytes (Packman, 2001; Pierce and Nester, 2011). This third mechanism of induction of anaemia may correspond to the lymphocytosis observed in the dogs treated with levofloxacin and day 14 observation of dogs treated with ciprofloxacin.

This study showed that the anaemia induced may resolve within 14 days for ciprofloxacin therapy, but levofloxacin induced anaemia may require a longer period to resolve. Therefore administration of either of these antibiotics to patients with existing anaemia should be with caution. Mild blood clotting defects may also occur particularly with levofloxacin which reduced the platelet count of the dogs throughout the course of treatment. The mild thrombocytopenia observed in this study may be due to either decreased production of platelets as observed in bone-marrow suppression or increased destruction mediated by the drugs (Roberts and Murray, 2003; Watson *et al.*, 2006).

In conclusion, this study confirmed that prolonged administration of ciprofloxacin and levofloxacin may cause anaemia as well as thrombocytopaenia considering that the dogs used in this study were healthy with normal blood parameters. These drugs should be used with great caution in patients with anaemia as a further deterioration of an underlying anaemic condition may result. In an earlier report, haemolytic anaemia with acute renal failure was reported in human patients that were placed on the fluoroquinolone drug temafloxacin (Maguire *et al.,* 1994). The pathogenesis of anaemia in this study is however unknown. A full hemogram may be necessary and would be beneficial before and in the course of therapy with these fluoroquinolones. This will guide the judgement of the clinician while deciding whether to discontinue therapy or monitor the patient closely in the course of therapy and ensure safety.

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