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Enrofloxacin drug induced reactive oxygen species

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Abstract

Fluroquinolones drugs are known to have adverse effects including chondriotoxicity, however, the mechanism of this effect was not fully understood. To investigate the possibility of the involvement of oxidative stress in this mechanism, enrofloxacin was administered orally to 80 broiler chicks for 15 and 30 days at 100, 200 and 400 mg/kg body weight and the activities of some antioxidant enzymes and malondialdehide (MDA) blood levels were studied. An increase in MDA levels and decrease in the activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) suggest that, the adverse effects of enrofloxacin might be due to oxidative stress. Our results support the hypothesis that free radical mediated reactions might be involved in the adverse reactions of fluoroquinolones.

Key words: Antioxidant Enzymes; Broilers, Enrofloxacin, Malondialdehide, Reactive Oxygen Species

Introduction

Enrofloxacin drug belongs to the group of synthetic 6-fluoroquinolones or 4-quinolones, with potent antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria (Goodman and Gilman, 1985; Bauditz, 1987; Scheer, 1987). Fluoro-quinolones are considered relatively well tolerated. However, incidence of adverese effects has been reported. It is stated that fluoroquinolones produce reactive oxygen species (ROS). Side effects of these drugs, such as photo toxicity (Hooper and Wolfson, 1993) and cartilage damage (Gough et al., 1992) may relate to production of these ROS.

Superoxide dismutase (SOD) and catalase (CAT) are enzymes that participate in the systemic antioxidant defence, neutralization and elimination of toxic ROS (McCord, 1983). The end product of lipid peroxidation is MDA which is used as a bio-marker for radicals-induced damage (Day, 1996). Chicken possess both enzymatic and non enzymatic antioxidant mechanism of defence that prevent ROS formation or limit their toxic effects (Fridovich, 1986).

The aim of this study was to investigate the possible involvement of an oxidative stress induced adverse effects of fluoroquinolones. For this purpose, the effects of orally enrofloxacin administration on the activities of some antioxidant enzymes and MDA levels in chickens were determined.

Materials and Methods

The study was given ethical approval by the Internal Committee of Postgraduate Studies of the Faculty of Veterinary Medicine, University of Ankara. In this study, 80 one day old (Ross-308) broiler chicks were used. Chicks were fed on a standard diet and tape water *ad libitum* during experminental period. On day, chicks were randomly divided into 4 groups (20 each), group 1 was control, chicks in group 2, 3, and 4 were administered enrofloxacin orally once daily for 15 and 30 days at 100, 200 and 400 mg/kg body weight respectively. Ten chicks from each group were sampled and slaughtered after 15 and/or 30 days of the drug administration.

Blood samples were collected from the wing vein of each chick in the morning using evacuated tubes containing heparin as an anticoagulant. The blood was centrifuged at 3000 rpm for 15 minutes and the plasma was then separated. The total amount of lipid peroxidation products in the plasma was assayed using the thiobarbturic acid (TBA) method, measuring MDA reactive product spectrophotometerically at 532 nm, according to the method described by Yoshioka et al. (1979). The red blood cells were washed three times with phosphate buffer (PSB) and were haemolysed by adding freezed distal water. After further centrifugation of the cell lysate at 3000 rpm at 4 ºC for 10 minutes, SOD and CAT activities were measured in UV visible spectrometer (Schimaduz UV-1601) in the supernatant

according to the method described by Fitzgerald et al. (1992) and Luck (1965), respectively.

Data were analyzed by one–way ANOVA, differences between respective means were determined using Duncan test. P value less than 0.05 was considered to be statistatically significant.

Results

The variation of MDA, SOD and CAT levels in the blood of broiler chicks after administration of different doses of enrofloxacin for 15 and 30 days are illustrated in tables 1, 2 and 3 respectively. MDA level was significantly increased (P<0.05) in the group 4 administered for 15 days compared to control. There was significant increased (P<0.05) in MDA levels in the groups administered for 30 days compared with the control. The levels of MDA were some what less in the groups administered for 30 days compared with those administered for 15 days.

Table 1: MDA levels (nmol/ml) in plasma of broiler chicks administered enrofoxacin orally dailly once at 100, 200 and 400 mg/kg body weight for 15 and /or 30 days

Groups	15 Days	30Days
Control	10.53±0.39^{c}	9.39±2.29^{b}
Group2	11.69±1.62^{b}	10.85±3.15^{a}
Group3	12.50±2.61^{b}	10.84±2.54^{a}
Group4	14.59±2.30^{a}	11.50±3.25^{a}

Values (mean ±SD) with different superscripts in the column differ significantly (P<0.05).

Table 2: SOD activity (U/gHb) in red blood cell of broiler chicks administered enrofoxacin orally dailly once at 100, 200 and 400 mg/kg body weight for 15 and /or 30 days

Groups	15 Days	30Days
Control	14.11±4.07^{a}	5.97±0.53^{a}
Group2	7.55±4.15^{bc}	4.36±1.40^{bc}
Group3	6.81±5.48^{bc}	4.23±1.23^{bc}
Group4	3.47±1.61^{c}	3.19±0.23^{c}

Values (mean ±SD) with different superscripts in the column differ significantly (P<0.05)

Table 3: CAT activity (K/mgHb)) in red blood cell of broiler chicks administered enrofoxacin orally dially once at 100, 200 and 400 mg/kg body weight for 15 and /or 30 days

Groups	15 Days	30Days
Control	10.64±7.64	17.43±12.18
Group2	9.74±7.33	15.68±6.91
Group3	8.05±5.78	14.85±4.03
Group4	6.56±4.12	8.72±7.14

In table 2 SOD activity decreased (P<0.05) with an increase in the drug dosage in the groups administered for 15 days and in the group administered for 30 days (U/gHb). In addition, SOD activity was lower in the groups administered for 30 days compared with those given for 15 days. More over the decreased in activity was significant (P<0.05) and was associated with the increase of the drug doses.

Table 3 shows decreased in CAT activity, associated with increase of drug dosage in all groups administered for 15 days and 30 days. In addition, CAT activity was higher in the groups administered for 30 days compared with those for 15 days. Although these differences were not significant when compared with control group.

Discussion

Lipid peroxidation is one of the best parameters indicative for the level of ROS-induced systemic biological damage (Georgieva et al., 2005). In this study the elevated MDA plasma concentration is probably due to the oxidative stress that occurred after enrofloxacin administration, which may be attributed to increased ROS production and reduced antioxidant defence system, resulting in lipid peroxidation (Sarban et al., 2005). Similarly, Alicia et al. (2002) reported an increase in MDA levels in the plasma associated with ciprofloxacin administration as indicated by the onset of lipid hydroperoxides (LOOH) (oxidative mediator of peroxidation).

Metabolism of enrofloxacin residues, generate free radicals and contribute to an increase in oxidative stress, promoting inhibition of cellular enzymes by the reduction in glutathione peroxidase (GSHPx) and CAT activities (Carreras et al., 2005; Yazar and Tras, 2001). In addition, Danafloxacin and enrofloxacin drugs may directly affect SOD and GSHPx activity or may produce the ROS and indirectly affect SOD and GSHPX activities (McCord, 1983, Yazar and Tras, 2001).

In this study all chicks administered with enrofloxacin showed a decrease in SOD activity with significant reduction in group 4 in the chicks administered for 15 and 30 days compared with control. The changes in the level of SOD activity in the treated groups in this study is probably due to enhanced ROS productionas as a result of the high dose of enrofloxacin. Similarly, ciprofloxacin induces ROS production in a dose and time–dependent pattern (Gurbay et al., 2001). Further more, Danfloxacin injected to mice caused an increase in SOD activity after 24 and 48hrs of injection (Yazar and Tras, 2001).

Catalase activity showed no significant change between all the groups studied. Non significant change was noted in CAT activity when fibroblast cells were incubated with ciprofloxacin (Gurbay et al., 2002).

Moreover, a decrease in CAT activity in chickens treated with enrofloxacin was reported (Fatima, 2005). In addition, enrofloxacin residues produced an adverse effect on the capability of the antioxidant enzyme CAT to reduce oxidative susceptibility in raw and cooked product (Carreras et al., 2005). Our results are in support of the hypothesis that free radical mediated reaction might be involved in the adverse reactions of enrofloxacin.

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