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Effect of Calcium Supplementation on Clinical and Hemato-biochemical Parameters Following Long Term Administration of Doxycycline in Mice

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Abstract: A research work was undertaken following oral doses of doxycycline and calcium with feed for 35 days from January to March, 2002, to study the adverse effect, hematological parameters, serum calcium level and post mortem changes in five groups (A, B, C, D and E) of Swiss albino mice (n = 25). Among these groups of mice group A was kept as control. Group B was treated only with doxycycline @ 500 mg kg^{-1} feed but groups C, D and E was given calcium @ 400 mg, 800 mg and 1200 mg kg^{-1} feed respectively in addition to doxycycline @ 500 mg kg^{-1} feed. All the treated groups of mice showed clinical signs like roughness of the body, depression, anorexia, weakness, staggering gait, recumbency at latter stage of the study period but these clinical signs were mild in calcium supplemented groups of mice. No visible adverse effect found in the offspring of any group of mice. Administration of doxycycline resulted significant decrease in total erythrocyte count (TEC), hemoglobin content (Hb%), percentage of neutrophil count and serum calcium level in all treated mice but calcium supplementation gradually improved these results in groups C, D and E respectively. Following post mortem examination, all the visceral organs were found to be highly congested and blood was blackish in colour in group B whereas mild or no congestion was found in calcium supplemented group, i.e., groups C, D and E. From the findings of the present study it may be concluded that long term administration of doxycycline results adverse effects as well as decrease serum calcium level. So, when doxycycline is to be administered for long period of time supplementation of calcium should be considered simultaneously.

Key words: Calcium supplementation, hemato-biochemical parameters, doxycyclin

Introduction

The tetracyclines are broad spectrum antibiotics and as a class, inhibit the growth of a wide variety of bacteria, protozoa and many intracellular organisms such as mycoplasma, chlamydia and rickettsia (Adams, 1995).Tetracyclines can easily chelate to polyvalent cations, which decreases their absorption several folds. Thus tetracyclines absorption in general can be decreased with the co-administration of food, dairy products, polyvalent cations (i.e. Ca^{++}, Mg^{++}, Fe^{++}, Al^{+++}), kaolin/pectin preparations and antacids (Neuvonen *et al.,* 1970; Hagermark and Hoglund, 1974; Gothoni *et al.,* 1972 and Aronson, 1980). The most commonly reported side effect of the tetracyclines, in both humans and animals, is gastrointestinal upset that results from irritation of the stomach and the upper small intestine. In mice, tetracyclines induced toxicosis resulted in increased transaminases, alkaline phosphatase, urea and total conjugate bilirubin, in addition to decreased cholesterol (Bocker *et al.,* 1982 and Hopf *et al.,* 1985). Tetracyclines administered by rapid intravenous injection may cause the animal to collapse, possibly due either to high initial blood concentrations or to chelation with calcium in the blood. Tooth mottling/discolouration occur when tetracyclines are administered during pregnancy when tooth development is occurring or when administered during the first post natal month. The discoloration is related to the chelation of tetracyclines to the calcium deposits in the developing teeth in the dentin (where it is mostly visible) and to lesser extent in the enamel (Hamp, 1967; Hennon, 1965; Finerman and Milch, 1963 and Moffit *et al.,* 1974). Few recent clinical reports are available on the use of doxycycline in animals. Doxycycline has been used to treat a variety of extracellular and intracellular infections in dogs, in particular ehrlichiosis and other infections in other species including respiratory tract disease and systemic colibacillosis in poultry, (Migaki and Babcock, 1977 and George *et al.,* 1977), psitacosis in avians and other anaplasmosis in spleenectomized calves. Doxycycline has increased activity against susceptible intracellular dwelling microbes due to their high penetration into cells due to increased lipophilicity. Waldroup *et al.* (1980) reported that reducing dietary calcium from 0.8 to 0.41% increased serum levels of chlorotetracycline and oxytetracycline. Adding sodium sulphate (1.25%) also increased serum levels; the best response occurred at the higher calcium level.

Doxycycline is ideal for treating susceptible infection when renal failure or renal insufficiency is a complicating factor in antimicrobial therapy (Shaw and Rubin, 1986). Zeeuwen *et al.* (1993) studied on the veal calves after administration of doxycycline cyclate (by error 5 mg twice daily, approximately 50 mg kg^{-1} body weight) because of respiratory disorder after post-mortem examination of 4 calves; they found pulmonary edema, myocardial degeneration and myocarditis. Cho *et al.* (1995) reported that doxycycline inhibited the random migration of polymorphonuclear leukocytes (pmn) in a dose dependent fashion at concentrations higher than 50 μg ml^{-1}. Doxycycline 1-100 μg ml^{-1} suppressed the phagocytosis of *Staphylococcus aureus* of pmn by 25-30%. Electron

microscopic studies revealed that doxycycline induced nuclear swelling, vacuole formation and the swelling and loss of pseudupodia in pmn at a concentration of 100 $\mu g\ ml^{-1}$ and cell lyses at a concentration of 1000 $\mu g\ ml^{-1}$. It is concluded that therapeutic dose of doxycycline might affect some functions of pmn (random migration and phagocytosis). Powers *et al.* (2000) administered doxycycline cyclate to 5 groups of 6 cockatiels by i/m injection (100 mg kg^{-1} every 10 days for 5 injections) or by mixing with drinking water (0.28 or 0.83 mg ml^{-1}), Seeds (500mg kg^{-1}) or mash (1000 mg kg^{-1}). Plasma doxycycline concentrations were measured periodically during the 45 days trial. Birds given doxycycline by i/m injection had variable, localized tissue reactions and mean trial plasma concentration $<1\ \mu g\ ml^{-1}$, birds that received doxycycline in drinking water and in seeds maintained group mean doxycycline concentrations $>1\ \mu g\ ml^{-1}$. Birds that received doxycycline in mash had high plasma doxycycline concentrations and showed severe clinical illness. Considering the facts, the present study was conducted to determine the adverse effects of long term administration of Doxacilvet(R) (Square Pharma.) in mice on clinical and haematological parameters, serum calcium level and post-mortem changes.

Materials and Methods

This experiment was conducted on 25 Swiss albino mice in the Department of Pharmacology in collaboration with the Department of Physiology, Bangladesh Agricultural University, Mymensingh during the period of 42 days from January to March, 2002. After the procurement of mice from ICDDR,B, Mohakhali, Dhaka all the animals were kept under close observation in order to acclimatize to the new environment for a period of one week prior to commencement of the experiment. All the mice were maintained on good housing conditions and were provided with normal feed and water *ad libitum.* After acclimatization, all the mice were divided into five equal groups, (A, B, C, D and E), each containing two male and three female mice. Group A was kept as control without giving any treatment and receiving only standard feeding. Rest four groups of mice (B, C, D, E,) were treated with doxycycline at the dose rate of 500 mg kg^{-1} feed. The three groups of mice (C, D, E) were also treated with calcium at the dose rate of 400 mg kg^{-1} feed, 800 mg kg^{-1} feed and 1200 mg kg^{-1} feed respectively for consecutive 35 days. After administration of doxycycline and calcium all mice were kept under close observation for entire 35 days of treatment and more 7 days post treatment period.

The effects of long term administration of doxycycline in mice were assessed on general health of mice and their offspring, hematological parameters, serum calcium level and post mortem changes. Blood of each mouse was collected from subclavian vein 42nd day of post treatment period, and the total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin content (Hb%) and differential leukocyte count (DLC) were carried out as per method described by Coffin (1955) and Schlamn (1965). Serum calcium level was estimated by following CPC method. Each mouse of the control and treated groups were sacrificed using ether overdose. The visceral organs like liver, kidney, lungs, heart, stomach and intestine were collected to observe the gross pathological changes.

Statistical Analysis: The data were analyzed statistically using student's 't' test as per method described by Bailey (1981).

Results and Discussion

Doxycycline is a good antibiotic and widely used both in veterinary and human medicine. Each and every drug has some side effect or adverse effect. It is true for doxycycline also. It chelates with blood calcium, as a result hypocalcemic symptoms results.

Clinical Parameters: The oral administration of doxycycline (@ 500 mg kg^{-1} feed) alone in group B and also the administration of doxycycline (@ 500 mg kg^{-1} feed) plus calcium @ 400, 800 and 1200 mg kg^{-1} feed in group C, D and E respectively daily for 35 days showed varying degree of clinical illness. The administration of doxycycline alone (@ 500 mg kg^{-1} feed) in group B, produced clinical signs i.e. roughness of the body, depression, anorexia, weakness, staggering gait and recumbency. Similar clinical signs are also observed in the three treated groups (groups C, D and E) given doxycycline (@ 500 mg kg^{-1} feed) plus calcium (@ 400, 800 and 1200 mg kg^{-1} feed respectively), but those signs were very mild in nature. Similar to the present findings Power *et al.* (2000) also observed clinical illness in cockatiels following doxycycline administration. But in contrast to present findings the clinical signs were severe in nature. In group B, receiving doxycycline (@ 500 mg kg^{-1} feed) alone, two female mice became pregnant and produced 15 (8+7) offspring which were normal but weak and emaciated during treatment and post treatment period. Similarly in group C, following administration of doxycycline (@ 500 mg kg^{-1} feed) plus calcium (@ 400 mg kg^{-1} feed), one female mouse became pregnant and delivered 8 offspring and they were apparently normal during treatment and post treatment period. In group D, administration of doxycycline (@ 500 mg kg^{-1} feed) along with calcium (@ 800 mg kg^{-1} feed), one female mouse became pregnant and delivered 10 offspring during later period of treatment. All the offspring were normal during delivery time and post treatment period. In group E, doxycycline (@ 500 mg kg^{-1} feed) plus calcium (@ 1200 mg kg^{-1} feed), two female mice

became pregnant and delivered 18 (10+8) offspring during later period of treatment. At birth time all the offspring were normal and found healthy throughout the study period. The present findings on doxycycline could not be compared because of lack of literature directly on doxycycline. However, Hossain (2001) observed similar findings i.e. normal offspring following oxytetracycline injection in mice in recommended doses (15 mg kg^{-1} body weight). However, Hossain (2001) also observed death of few offspring following double the recommended doses (30 mg kg^{-1} body weight). In the present study, the dose of doxycycline was same in each treated group (C, D and E) but in group C, D, and E , calcium was supplied along with same dose of doxycycline (@ 500 mg kg^{-1} feed) in different doses i.e. @ 400, 800 and 1200 mg kg^{-1} feed respectively. With calcium supplementation the offspring were found normal and healthy in all three treated groups indicating that calcium supplementation had a positive effect on offspring of the doxycycline treated mice. Administratin of doxycycline results chelation of blood calcium and as a result clinical signs associated with hypocalcemia were observed in all the treated groups (B, C, D and E). But the calcium supplement at gradually increasing doses (@ 400, 800 and 1200 mg kg^{-1} feed respectively) along with doxycycline (@ 500 mg kg^{-1} feed) reduced the severity of clinical illness respectively. This might be due to chelation of doxycycline with the supplemented calcium keeping blood calcium level normal.

Hematological Parameters: Oral administration of doxycycline (@ 500 mg kg^{-1} feed) significantly reduced the TEC values in the treated mice but the percentage of reduction was gradually decreased when the dose of calcium gradually increased (Table 1). Doxycycline, like oxytetracycline may disturb in erythropoiesis resulting decrease in erythrocyte number. When calcium is supplemented at various doses, it may bind the blood doxycycline accordingly and so, higher the dose of calcium increase the number of erythrocyte. Kreutzmann (1977) reported that oxytetracycline caused disturbance in erythropoiesis in the cell resulting decrease in erythrocyte number and disturbance of erythrocyte fat metabolism. His findings strengthen the present finding. The reduction of TLC in doxycycline treated mice was not significant but in the calcium supplemented mice the percentage of reduction of TLC was gradually decreased when the dose of calcium gradually increased, because of higher dose of calcium binds more doxycycline. So, lower the doxycycline level higher the TLC value. Oral administration of doxycycline significantly reduced the haemoglobin values in the treated mice but the percentage of reduction gradually decreased when the dose of calcium gradually increased. Doxycycline like oxytetracycline may disturb in erythropoiesis resulting decrease in erythrocyte number and thus reducing Hb gm% content. When calcium is supplemented at various doses it may bind the blood doxycycline accordingly and so higher the dose of calcium increase the number erythrocyte. Haemoglobin content is correlated with the erythrocyte number. Neutrophil percentage was significantly ($P<0.05$) decreased following oral administration of doxycycline but in the calcium supplemented mice the decrease in neutrophil percentage was less. The reason behind it may be due to binding of doxycycline by the calcium supplementation and thus minimizing the effect of doxycycline. Lymphocyte percentage was significantly ($P<0.01$) increased following oral administration of doxycycline but in the calcium supplemented mice the increased in lymphocyte percentage was less. The reason behind it may be due to chelation of doxycycline with the supplemented calcium and thus minimizing the effect of doxycycline.

Table 1:Effects of oral administration of Doxycycline and calcium on hematological parameters in Swiss albino mice

Groups of mice	Treatment	Changes in the hematological parameters				
		TEC (million/cumm)	TLC (thousand/cumm)	Hb (gm %)	Neutrophil (%)	Lymphocyte (%)
A	Control	10.18±0.14	7.96±0.17	12.3±0.15	33.0±0.32	60.6±0.38
B	Doxycycline @ 500mg kg^{-1} feed	9.53±0.14* -(6.38%)	7.49±0.14 -(5.9%)	10.0±0.22** - (18.69%)	31.6±0.33* -(4.24%)	62.6±0.40** +(3.19%)
C	Doxycycline@ 500 mg kg^{-1} feed +Calcium@ 400 mg kg^{-1} feed	9.72±0.16 -(4.51%)	7.58±0.17 -(4.77%)	10.4±0.17** -(15.44%)	35.6±0.43** +(7.30%)	59.8±0.50 -(1.32%)
D	Doxycycline@ 500 mg kg^{-1} feed +Calcium@ 800 mg kg^{-1} feed	9.98±0.15 -(1.96%)	7.79±0.17 -(2.13%)	10.7±0.19** -(13.00%)	36.0±0.50** +(8.33%)	58.8±0.51* -(2.97%)
E	Doxycycline@ 500mg kg^{-1} feed +Calcium@ 1200 mg kg^{-1} feed	10.05±0.19 -(1.27%)	7.95±0.16 -(0.12%)	10.9±0.16** -(11.38%)	36.4±0.50** +(9.34%)	57.8±0.52** -(4.62%)

Values given above represent the mean ± standard error (SE) of 5 animals
* Indicates significant value ($P<0.05$), ** Indicates highly significant values ($P<0.01$)
% Indicates percentage of reduction (-) or addition (+)

Table 2: Effects of oral administration of Doxycycline and calcium on serum calcium level (mg dl^{-1}) in Swiss albino mice

Groups of mice	Treatment	Serum calcium level (mg dl^{-1})
A	Control	7.76 ± 0.14
B	Doxycycline@ 500mg kg^{-1} feed	6.68 ± 0.15** -(13.92%)
C	Doxycycline@ 500mg kg^{-1} feed + Calcium@ 400 mg kg^{-1} feed	9.84 ± 0.14** +(21.14%)
D	Doxycycline@ 500mg kg^{-1} feed + Calcium@ 800 mg kg^{-1} feed	11.14 ± 0.10** +(30.34%)
E	Doxycycline@ 500mg kg^{-1} feed + Calcium@ 1200 mg kg^{-1} feed	12.34 ± 0.13** +(37.11%)

Values given above represent the mean ± standard error (SE) of 5 animals
** Indicates highly significant values ($P<0.01$)
% Indicates percentage of reduction (-) or addition (+)

Biochemical Parameters: Serum calcium level significantly decreased ($P<0.01$) in group B following oral administration of doxycycline (@ 500 mg kg^{-1} feed) alone but when calcium was supplemented at various doses in other groups (C, D and E) the decrease in serum calcium level gradually improved because the supplemented calcium became available in the body (Table 2). As a result administered doxycycline chelates with supplemented calcium. So higher the calcium supplementation, the serum calcium level is improved resulting increase in serum calcium level. So, it can be concluded that when doxycycline is administered calcium supplementation will be helpful to prevent hypocalcemia and hypocalcemic symptoms especially in lactating and growing animals and birds. Other workers like Kumer (1994) and Tras *et al.* (1999) found similar type of findings. Hossain (2001) also observed similar finding after working on another antibiotic (oxytetracycline) of the same group in Swiss albino mice.

Postmortem Findings: In group B, following administration of doxycycline (@ 500 mg kg^{-1} feed) alone, all the visceral organs were highly congested (especially liver and heart) and black and the blood was also blackish in colour. But in group C, D and E following administration of doxycycline (@ 500 mg kg^{-1} feed) plus calcium (@ 400, 800 and 1200 mg kg^{-1} feed respectively), all the visceral organs were slightly congested in comparison to group B. Zeeuwen *et al.* (1993) studied on the veal calves following administration of doxycycline cyclate, after post-mortem examination of 4 calves, they found pulmonary edema, myocardial degeneration and myocarditis.

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