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Modulatory Effects of Vitamin C on Enrofloxacin Induced Chromosome Damage and AgNOR Counts in Chick Bone Marrow Nuclei

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Abstract

Quinolones are broad spectrum antibiotics and are widely used in veterinary practice. But because of continuous improvement in their bactericidal and metabolic properties, it resulted in an increased amount of genotoxicity among animals. Vitamin C is a well-known antioxidant and a free radical scavenger and protects the cell from endogenous oxidative DNA damage. The aim of the study was to find the modulatory effect of Vitamin C against the enrofloxacin induced chromosomal aberrations and nuclear damage. The toxicity includes a significant decrease in mitotic index and increases in the number of chromosomal aberrations like break, deletion, centromeric attenuation, centric fusion and AgNOR counts in chicken bone marrow nuclei. The combined administration of enrofloxacin and Vitamin C resulted has markedly increased the mitotic index and reduced the chromosomal aberrations and AgNOR counts in the bone marrow cells.

Keywords: AgNOR, Chromosomal Aberrations, Enrofloxacin, Vitamin C

1. Introduction

Antibiotics are diverse groups of chemicals which includes tetracyclines, quinolones, macrolides etc. Quinolones are currently used antimicrobial and is not only applied to human medicine but is also widely functional in the treatment and prevention of veterinary diseases[1,2]. Several fluoroquinolones have been developed and synthesized to improve bactericidal and metabolic properties and because of these changes it has resulted in an increased amount of toxic and genotoxic effect of these chemicals[3]. Different studies were carried out concerning the genotoxic effect of quinolones[4–9].

Enrofloxacin (EFX) is a recently introduced wide spectrum antibiotic in veterinary practice[10]. Results have shown that EFX is genotoxic[4] to chicken chromosomes[6,11] as they are apt to take higher doses during summer months with water[6].

Vitamin C (VC) is very efficient antioxidant[12–15], scavenging reactive oxygen and protecting cells against free radical-mediated damage[16,17]. VC is commonly used as antitumor agent[18] and has antiproliferative[19], antigenotoxic[20–30], ameliorative[31] and antimutagenic[12,26,32] properties. The present communication reports the genotoxic effect of higher doses of EFX (curative dose X = 100 mg/kg bd. wt.) and the modulatory effect of Vitamin C on the chromosomal system and AgNOR counts in chicken bone marrow cells.

2. Materials and Methods

Eight weeks old male White Leghorn birds of weight 1±0.5 kg body were kept for acclimatization for three days in the laboratory under controlled temperature (25±2°C) and light (12 light: 12 dark). They were maintained in a well-ventilated animal house and were fed on standard feed and water given ad *libitum*. Animals were given

different doses (5X, 10X and 15X) of the curative dose of EFX as intra muscular injection for 5 successive days (Group II, IV and VI). The other groups were treated with Vitamin C (VC) along with EFX (Group III, V and VII). The experiments were performed on 07 groups (of 05 animals each) as follows:

Group I : Control

Group II : EFX 5X

Group III : EFX 5X + 200 mg/kg Vitamin C

Group IV : EFX 10X

Group V : EFX 10X + 200 mg/kg Vitamin C

Group VI : EFX 15X

Group VII : EFX 15X + 200 mg/kg Vitamin C

Animals were sacrificed 24 h after the last injection and the bone marrow preparations were made by usual hypotonic air drying technique[33-35]. Around 100 metaphases/animal were scored for different types of chromosomal aberrations such as chromosomal breaks, deletions, centromeric attenuations, centric fusion and fragmentation. For AgNOR, the smears were subjected to the one step silver nitrate method[36]. Black well-defined silver stained dots were observed per nuclei and scanned for AgNOR counts; 500 nuclei were counted in each case.

The mitotic activity of bone marrow cells was determined for each treated and control animal. It is expressed by the mitotic index (MI : number of dividing cells in 1000 cells).

Statistical analysis was done and Student's t-test was used to assess statistical significance.

3. Results

Table 1 presents the chromosomal aberrations produced due the effect of the drug EFX in the chicken bone marrow nuclei. The chromosomal aberrations values show a significant difference than that of the control value. The mitotic index value indicates the harmful effect of the drug, wherein the value in 15X EFX treated was more pronounced *(P<0.001)* than the other treated groups. Out of the different chromosomal aberration, breaks were found most of the times in the bone marrow nuclei. Breaks were found significantly *(P<0.01, P<0.001)* more than the control groups in a dose dependant manner. Similar pattern was observed in deletion in the chromosomal element. However, the values in deletion were observed less than that of breaks. It is interesting to note that centromeric attenuation were not observed in control groups, but all the 3 different treated groups showed values in a range of 1-6/500 nuclei. Centric fusion was in control and all the treated groups. This type of chromosomal aberrations was found to be highly significant *(P<0.001)* as compared to that of the control group. The chromosomal aberrations showed a dose dependant reaction wherein the values increased significantly from 5X to 15X, except for centromeric attenuation.

EFX was also found harmful as depicted by the AgNOR (Table 2) counts in the treated groups. The count was found to be significantly more *(P<0.05)* as compared with the control group. The AgNOR counts increased in dose dependant manner from 5X to 10X but it falls to that of the 5X level at 15X of the drug, this shows the suppression of transcriptional activity of rDNA genes. The rDNA genes become active when the drug was injected and this to be expected since in the normal cells hypertranscription is not necessary.

Figure 1 depicts the modulatory effect of VC in EFX induced different types of chromosomal aberrations in chick bone marrow nuclei. Combined application of EFX and VC resulted in the significant reduction of all the chromosomal aberrations taken in the study produced due the effect of EFX. Mitotic index was found to be significantly increased *(P<0.05)* when the birds were treated with VC for 5X and 10X treated birds, however, the values in 15X treated birds were highly significant at *P<0.001*. Reduction in deletions was found mostly in the macro chromosomes and most of it was observed in 15X treated group (Table 1). Breaks were found in all the treated groups (Table 1), it is reduced significantly *(P<0.05, P<0.001)* in VC treated groups. Significant reduction in centromeric attenuation and centric fusion in 5X and 10X treated birds was observed with VC respectively. However, the effect of VC for reduction in centromeric attenuation was found to be non- significant in case of 10X and 15X treated groups. Highly significant *(P<0.001)* reduction was observed in break in 10X treated group whereas in mitotic index, deletion and centric fusion in 15X treated groups.

There was a significant decrease in AgNOR counts in treated groups with VC as compared to EFX alone. The decreases in the counts of AgNOR values at 5X and 10X

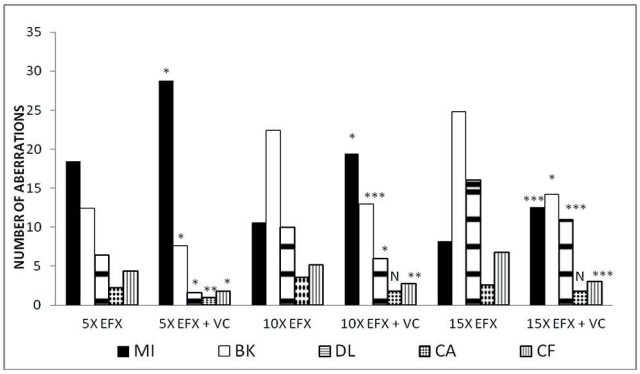


Figure 1. Effect of Vitamin C on EFX induced mitotic index, CAs in chicken (n = 5) bone marrow nuclei.
* *Statistically significant at P < 0.05,*
** *Statistically significant at P < 0.01,*
*** *Statistically significant at P < 0.001, N Statistically not significant*,
MI- Mitotic Index, BK- Break, DL - Deletion, CA- Centromeric Attenuation, CF - Centric Fusion

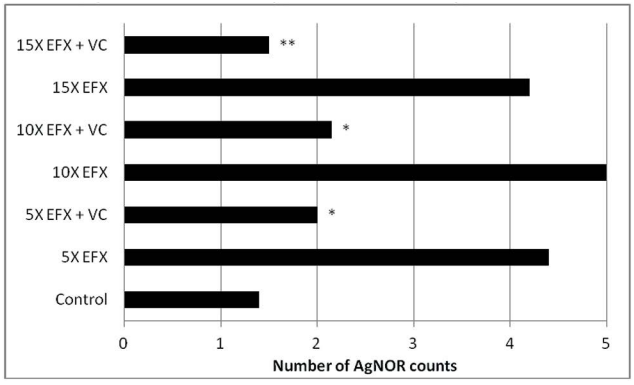


Figure 2. Effect of Vitamin C on EFX induced AgNOR counts in chicken (n-5) bone marrow nuclei.
* *Statistically significant at P<0.05*
** *Statistically significant at P<0.01*

is nearly the same and is significant at *(P<0.05),* which depicts the effect of VC in ameliorating the harmful effect of the drug. Therefore, VC is helpful in reducing the stress produced by the toxic effect of the drug in the chick bone marrow nuclei. The effect of VC is more pronounced *(P<0.01)* when it is given the group receiving the 15X the drug.

4. Discussion

The fluoroquinolones comprise large groups of synthetic antimicrobial agents[38]. EFX, the fluoroquinolone, is widely used in antimicrobial therapy. It inhibits the bacterial gyrase and eukaryotic topoisomerase-II in high concentrations, which resulted in genotoxic effects in chicken[6]. In the present investigation the effect of EFX is clearly shown by production of various chromosomal anomalies along with reduction in mitotic index and increase in number of AgNOR counts. The CAs was recorded in a dose dependant fashion wherein breaks and deletions were encountered most as compared to other CAs taken in the study. These finding run in agreement with results of Mukherjee *et al.*,[39] who observed a positive dose-dependent chromosomal aberration in mouse bone marrow cells. Ciprofloxacin significantly induced DNA damage in a dose-dependent manner[12,40–43].

Concurrent administration of VC with EFX has resulted in marked decrease in the values of various CAs and AgNOR counts with increase in MI. Administration of Vitamin C modulated the cytotoxicity induced by genotoxic agents and markedly decreased the number of chromosomal aberrations[13,20,21,23–25,27,44,45]. In an experiment by Roy *et al.*,[28] it was showed that Vitamin C pre-treatment apparently reduced the frequency of

Table 1. Mean number of mitotic index and chromosomal aberrations (CAs) in chicken (n = 5) bone marrow nuclei after treatment with EFX

	MI	BK	DL	CA	CF
Control group	36±1	5.4±0.74	1.2±0.58	Nil	0.8±0.37
Treated group					
5X	18.4±0.60***	12.4±0.81**	6.4±0.51*	2.2±0.37	4.4±0.24***
10X	10.6±0.51***	22.4±0.51***	0±0.54***	3.6±0.86	5.2±0.19***
15X	8.2±0.37***	24.8±1.28***	16±0.31***	2.6±0.24	6.8±0.73***

* *Statistically significant at P<0.05*
** *Statistically significant at P<0.01*
*** *Statistically significant at P<0.001*
BK- Break, DL- Deletion, CA- Centromeric Attenuation, CF- Centric Fusion

Table 2. AgNOR in chicken (n = 5) bone marrow nuclei after treatment with EFX

	Control	5X EFX	10X EFX	15X EFX
AgNOR counts	1.4±0.25	4.4±0.18*	5±0.28*	4.2±0.20*

* *Statistically significant at P<0.05.*

CA, MN and SA induced by choloroquine. The repeated doses of VC reduced the percentage of chromosome aberrations induced by RMP in a significant and dose-dependent manner[12]. Vitamin C at the concentration of 100 or 200 mu g/ml of VC, administered in pre-, post- or simultaneous treatment, there was a significant reduction in both chromosome aberrations and number of abnormal metaphases induced by doxorubicin[32]. In the present study it was found that Vitamin C at a dose of 200 mg/kg reduces the various chromosomal aberrations induced by EFX. In a study by Abasova *et al.*,[46], the levels of cells with total level of cytogenetic disorders decreased by 38% after vitamin course, which confirmed their antimutagenic effect. However, in the present study the total cytogenetic disorder was decreased by 56.6%. Ascorbic acid is hydrophilic and functions as a support to the antioxidant defence mechanism[13,26,27] and free radical scavenger which also prevents free radical induced DNA damage[44]. Ascorbic acid was found to be effective in reducing micronucleus frequency induced by cyclophosphamide and bleomycin[37]. This is in agreement with the present findings which showed that VC reduces the CAs significantly to that of the treated birds thus presenting the protective role of VC in the birds.

The further protective action of VC was marked by increasing the value of MI. According to Misra and Choudhury[47] each pre-treatment dose of VC protected bone marrow cells from the cisplatin-induced cytogenotoxicity. The similar findings were observed in the present where Vitamin C induces higher mitotic index as compared to the treated birds.

Techniques like Nucleolar Organizer Region (NOR) staining have helped in diagnosis of cancer (Hung *et al.*, 2000). There was a significant increase (AgNOR counts) in HT-29 human carcinoma cell line when treated with ciprofloxacin[48]. The counts of AgNOR increases in dose dependant manner which was supported by Josza *et al.*[49], where they found that remobilization of muscle cell increases intracellular protein synthesis thereby increases the AgNOR counts. In the present study it was found that VC acts by reducing the counts of AgNOR.

VC appeared to be a promising agent against genotoxic potential of EFX. Administration of VC along with EFX proved to be beneficial as it has resulted in reduction of CAs near to that of the normal values and modulated the antioxidant defence system of the body. These data are in agreement that Vitamin C increased the total antioxidant status in plasma and it could be useful as free radical scavenger[50,51]. It was reported that extracellular Vitamin C levels scavenge the ROS derived by oxidation-reduction cycle and acted as a chemoprotective agent[52].

Thus, the results depicted that antioxidative Vitamin C may be useful in controlling the toxic effects of EFX. However, other studies are required with aim to promote the use mixtures of different antioxidative vitamins among the birds, as these organisms are apt to take up higher doses with water during summer months.

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