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Embryotoxic effects of Ciprofloxacin in Chicken: A potential risk for antibiotic resistance

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Abstract

Background: Ciprofloxacin is considered as a relatively safe drug having broad spectrum antimicrobial activity in humans and animals including poultry, besides its reported cartilage damage in children and debated use in pregnancy.

Methods: Current study is designed to highlight the embryotoxic effects of ciprofloxacin in chicken. In the study fertilized eggs were categorized in 5 groups incubated at standard parameters. On 4th day of incubation, group1 and 2 were injected with Ciprofloxacin (30 and 60 mg/egg respectively). Group 3 was pinched with needle only and group 4 was injected with sterilized saline solution. Group 5 was kept as a control, without any injection or pinching.

Results: On the 18th day of incubation, the growth of embryos was monitored. Hemorrhages on neck and head areas were noticed in treated group treated with 60 mg/egg. Low body weight along with the defective beak and nail formation was noticed. No other clear external deformity was observed in any treated and normal groups. It can be inferred that embryotoxic effects of ciprofloxacin cannot be ignored. High or repeated doses can reduce the turnover number of successful hatching chicks and the consumption of antibiotics treated chicken can lead to the development of antimicrobial resistance in humans.

Conclusion: It can be concluded that recommended dose limit is very important to avoid the harmful effect of antibiotics and chemicals. Embryotoxic effect of ciprofloxacin cannot be ignored. Use of antibiotic/s in poultry and live stocks should be carefully monitored and judged on medical basis; also its use for growth promotion should be discontinued.

Introduction

Ciprofloxacin or $C_{17}H_{18}FN_3O_31$ (cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid C1CC1N2C=C(C(=O)C3=CC(=C(C=C32)N4CCNCC4)F)C(=O)O}) is first fluoroquinolones to be marketed. Ciprofloxacin has been proved to be very effective against various infections in human and animals (poultry) caused by gram negative bacteria and some mycobacteria by targeting bacterial topoisomerase II and IV [1]. Appropriate use of antibiotics and synthetic chemicals in chicken feed can be beneficial and results in prevention or reduction of infectious disease and increasing the efficiency and growth rate. Different chemicals, currently being used by poultry industry, are tetracycline, penicillin, chlortetracycline, oxytetracycline, nitro-furan, sulfa compounds and arsenical compounds.

Clinically adverse effects have been observed by the co-administration of ciprofloxacin with some drugs like, leflunomide, propranolol, theophylline, verapamil, propafenone, tizanidine and naproxen etc. High doses of the drug can lead to the developmental issues of the chicken fetus and can directly affect the turnover number of successful hatching chicks thus annual production rate of chicken. The consumption of antibiotic treated chicken, can lead to the development of antimicrobial resistance in humans. Risk assessment on non-human use of antibiotics and development of antimicrobial resistance has been addressed internationally by [2,3] and [4]. To avoid the risk of developing drug resistance against ciprofloxacin, unnecessary drug exposure to humans should be avoided. Utilizing in poultry and meat can lead to the development of drug resistance [5]. Inappropriate use of the drug results in toxicity in the patients with gout and hyperuricemia undergoing probenecid therapy.

During probenecid therapy, the concentration of ciprofloxacin in systemic circulation observed to be increased due to reduced renal clearance. Animal farms and slaughterhouses are the sites of primary exposure. Current study is designed to highlight the embryotoxic effect of ciprofloxacin in chicken embryo.

Methods

A total of 30 fertilized eggs (from healthy Lyallpur Silver Black (LSB) chicken, grown under standard conditions) were taken, weighed (51 ± 0.3) and incubated at 37 ºC. On 4th day of incubation, eggs were grouped in five categories having 8 eggs each and further processed as follows.

Group 1: injected with 30 mg/Kg of antibiotic (SIGMA: 17850-5G-F)

Group 2: injected with 60 mg/Kg of antibiotic

Group 3: injected/pinched with needle only

Group 4: injected with sterilized saline solution

Group 5: without any injection.

For injection, the established protocol [5] was followed. On the 18th day of incubation, the growth of embryos was monitored. Body weight of embryo, formation of beak, eyes, palate, skull, feathers, limbs, claws, nails and size of head were analyzed and compared in both treated and untreated group. All procedures used in the study were approved by the ethical review board of Zoology department AUST, Pakistan.

Results

On 18th day of incubation, normal developmental features were noticed in group 1, 3, 4 and 5. Hemorrhages on body (neck and head areas), low body weight, defective beak and nail formation were prominent in group 2 (60mg/ Kg egg) (Figure 1). The observed difference in weight in normal and treated groups was not statistically significant. No other clear external deformity was found in any treated and normal groups.

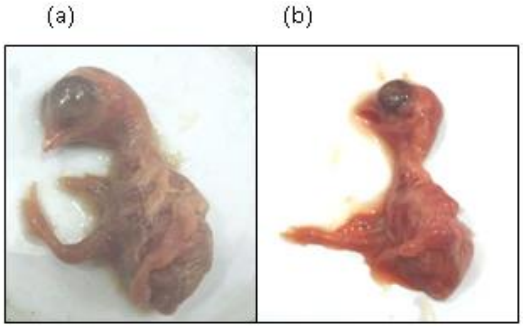

Figure 1: (a) Normal development in untreated chick at 18th day of incubation, (b) defective beak and nail formation in embryo treated with 60mg/kg egg weight.

Features observed	**Group I** 30 mg/Kg ciprofloxacin	**Group II** 60 mg/Kg Of ciprofloxacin	**Group III** 0 mg/Kg (Pinched with needle)	**Group IV** 0 mg/Kg of antibiotic 0.5ml Saline	**Group V** Control/ 0 mg/Kg (without injection)
Weight (Average)	37gm	29gm	36gm	35gm	37gm
Beak formation	No significant change	Defective beak	No significant	No significant	No significant
Claws and Nails	No significant change	Defective nail	No significant change	No significant change	No significant change
Hemorrhage on body	No	Yes	No	No	No

Table 1: Dosing composition and effect on growth parameters

Discussion

Resistance in human infections is linked to farm use increasing fluoroquinolone. Campylobacter infections is generally attributed to the use of these antibiotics in farm animals, and in particular poultry [5]. Residues of oxolinic acid and ciprofloxacin have been observed in tissues and eggs of laying hen for many days after absorption in the gut [6,7]. Campylobacter present in

human to the campylobacter isolated from poultry was compared by using Pulsed-field gel electrophoresis, Multi-locus sequencing typing and fla-typing and found to be related to poultry [8]. According to a report, almost 80% of the campylobacter infections in the UK are acquired by contaminated poultry. Other observations links contaminated poultry to human infections up to 38% and 77% [8,9]. The accepted dose is, 2 to 50 grams per ton for prevention or treatment of common diseases. The dose can be exceeded to 50-200 grams/ton or more in case of some specific diseases. Eggs and poultry meat are important foods sources for fulfilling the dietary needs of human population. In poultry, ciprofloxacin is recommended for respiratory, urinary and gastrointestinal tract infections [10].

In chicken, the most vulnerable age is 3 and 4 to 6 weeks for respiratory and gastrointestinal diseases respectively [11,12]. In young chicks, the recommend dose of ciprofloxacin is 17mg to 250mg in 24 hours. Initially, this dosage is recommended for 5 days but can be extended up to 2 weeks, depending upon severity of the disease [13]. In absolute conditions, the withdrawal period of ciprofloxacin is of 12-15 days [13]. The withdrawal period can be increased up to 23 days in some cases, for example, the protein binding of the drug will be enhanced in animals having impaired hepatic and/or renal functions. In poultry, the drug should only be used in severe infection and must be discontinued after at the end of second week of age. The reason behind is the farmed chicken used for meat purposes is of average age between 6 to 8 weeks [14]. Ciprofloxacin disturb the expression of genes involved in regulation and differentiation of osteoblast function including Runx2, Osterix, b-catenin [15], and Rnf146 [16]. Ciprofloxacin also disrupts the functions of BMP1 and DMP1 [17]. A damage to Runx2 and Osterix genes with ciprofloxacin, direct the differentiation osteoblast cell into adipose cell due to which the characteristics of chondrocytes and osteogenesis would be lost [18], while due to induction of ciprofloxacin the unexpressed Rnf146 gene will decrease the activity of b-catenin, Fgf8 and TAZ protein that will work towards the osteoblast proliferation and inhibition of bone mineralization [16]. Ciprofloxacin interferes with BMP1 gene expression; it will stop mineralization of bone. This disruption will also inhibit differentiation of osteoblast results in low calcium accumulation in bone. The inappropriate administration of doses results in development of resistance to some microbes. The resistant strains can easily be transmitted to human and other animals.

Hence the potential danger of presence of ciprofloxacin in meat or eggs can't be ignored. A comprehensive toxicological study is required to check the presence of residues of antibiotic in the eggs and to analyze the cardiovascular defects, spina bifida, polydactyly, and hypospadias in farm animals.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

Bibi Nazia Murtaza: Manuscript writing and Data analysis
Sidra tul Muntaha: Monitoring of research
Muhammad Siraj: Drafting and Manuscript scanning
Mujaddad ur Rehman: Data evaluation

Aneela Rehman: Manuscript writing and Correspondence

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