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Effect of Ofloxacinon the Humeral Immune Response In Vaccinated Chickens

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Abstract: The present work was designed to study effects of a second generation quinolone ; ofloxacin on humeral immune response in infectious bronchitis virus vaccine (IBV) vaccinated chickens. Ofloxacin was administered orally in therapeutic dose (5mg/kg b.wt./day) for 5 successive days. Chickens were classified into 3 equal groups each of 50. The first group was left non vaccinated non treated and served as control . The second group was vaccinated with IBV vaccine by fine spray at one day old and 4 weeks old .The third group was concurrently vaccinated and given orally the therapeutic dose of ofloxacin (5mg/kg b.wt./day) for 5 successive days. Blood samples were collected from wing vein of 5 chickens from each group, at zero time just before vaccination or treatment and 1, 2, 3 and 4 weeks post-treatment for studying humeral immune response. The results showed that, serum total proteins and albumin levels were increased significantly ($P \leq 0.05$) in the vaccinated ofloxacin treated group of chickens after 1 and 2 weeks since beginning of administration as compared with the vaccinated non-treated and control groups. The administration of Infectious bronchitis virus vaccine alone or in combination with ofloxacin resulted in insignificant changes in levels of alpha (α) and beta (β) globulins as compared with control group. Chickens of the second group (vaccinated non-treated) showed significant elevation in gama (γ) globulins level ($P \leq 0.05$) after 2 and 3 weeks since beginning of administration while the third group (vaccinated ofloxacin treated) showed a significant decrease ($P \leq 0.05$) in gama (γ) globulins level after 1, 2, 3 and 4 weeks as compared with control group. It could be concluded that the therapeutic dose of ofloxacin may have a significant suppressive effect on the chicken's humeral immune response. So, care must be taken when using ofloxacin concurrently with vaccination in chickens.

Key words: Ofloxacin · Humeral Immune · Chickens

INTRODUCTION

Fluoroquinolonesare synthetic compounds and considered asone of the most important antibacterial groups since discovery of sulphonamides, this group characterized by their broad spectrum activities against both Grampositive and Gram-negativemicro-organisms including all bacteria resistant to beta-lactams and sulphonamides. This group ofantimicrobial characterized by its rapid intestinal absorption and rapid distributed through all body tissues. It have been used for all birds and animal diseases [1, 2]. The fluoroquinolones that are marketed for use in veterinarymedicine today are typically well absorbed orally, have a large volume of distribution, penetrate nearly every tissue and cell in the body and have extended elimination half-lives, allowing for every 24 or 48hour dosing. At appropriate drug concentration (MICratios), the fluoroquinolones are rapidly bactericidal, exhibit concentration-dependent killing and may exhibit a prolonged *in vivo* post-antibiotic effect (PAE) on certain bacteria [3].

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The fluoroquinolones are classified into different groups based on their chemical structure or their biological activities. Classification by chemical structure is dependent on the number of rings associated with the pyridine-β-carboxylic acid nucleus**.** Group II; second generation which is the majority of fluoroquinolones onthe market today, is composed of bicyclic derivatives. They have an extended spectrum of antibacterial activity.Most fluoroquinolones approved for use in people (including ciprofloxacin, norfloxacin and ofloxacin) and most of fluoroquinolones approved for usein veterinary medicine are belonging to the second-generation fluoroquinolones [4].

Ofloxacin is one of second generation fluoroquinolones. It is a racemic mixture, which consists of 50% levofloxacin (the biologically active component) and 50% of its "mirror image" or enantiomerdextrofloxacin. Ofloxacinisbroad-spectrum bactericidal antibioticactive against both Gram-positive, Gram-negative bacteriaand even mycoplasma. It has been used widely in veterinary field. The mechanism of action of ofloxacin is function by inhibiting DNA gyrase, a type II topoisomerase and topoisomerase thereby inhibiting DNA supercoiling and consequently bacterial cell division [5-7].

Many antibacterial drugs affect the immune response eg. tetracycline, oxytetracyclinechloramphenicol, kitasamycin,, tilmicosin, florfenicol, enrofloxacin and levofloxacin [8-12]. Thus, the aim of the present study was to investigate the effect of ofloxacinadministration on plasma proteins including albumin, Alpha (α), beta(β) and gama (γ) globulins in serum of chickens andconsequently on humeral immunity in infectious bronchitis virus vaccinated chickens.

MATERIALS AND METHODS

Drugs:

- Ofloxacin: (Ofloxacin 10% solution®, Chemical Industry Company, Korea) was used at a dose of (5mg/kg b.wt.), administered orally once daily for 5successivedays.
- Infectious bronchitis virus vaccine (Bioral H 120®, Reaux Merix, France). It is Modified live vaccine against avian infectious bronchitis H 120 strain - Freeze-dried pellet chickens,used forvaccination of 2nd and 3rd groups of chickensby fine spray on 1st day and 4th week of age.

Experimental Design: One hundred and fifty, one day old Hubbardchickens were usedin this study.The chickens were Kept under hygienic conditions, fed on standard ration and water *ad-libitum.* Chickens were divided into three equal groups each of fifty chickens.

- The 1st group was servedas control non vaccinated and non treated.
- The 2nd group was vaccinated with IBV vaccine at 1st day of age and 4th week and not treated.
- The 3rd group was vaccinated as the 2nd group (1st day of age and 4th week) and treated with ofloxacin (5mg/kg. b. wt.) orally for five successive days.

Blood Sampling: Blood samples were collected from wing vein of 5 chickens from each group just before vaccination (Zerotime) as well as one, two, three and four weeks after vaccination and treatment. Samples were taken without anticoagulant and left to clot at room temperature and then centrifuged at 3000 r.p.m for 15 min to obtain clear serum. The sera were stored in deep freezer at -20°C till used for analysis. For studying the effect of the drug on the humeral immunity of vaccinated chickens. Total plasma proteins were measured using modified biuret method as described by Weichselbunm [13]. Serum albumin concentration was determined according to method of Doumas *et al*. [14]. Alpha (α), beta (β) and gama (γ) globulins were also determined using disc electrophoresis according to method of Ornstein [15].

Statistical Analysis: Statistical analysis was performed using the SAS computer program [16]. The data were analyzed using analysis of variance (One way ANOVA) with Duncan's multiple range test to compare treatment means. All data were expressed as the mean ± standard error (SE).

RESULTS

The results of the present study showed the effect of Infectious bronchitis virus vaccine alone and in co-administration with ofloxacinon serum total proteins, albumin, Alpha (α), beta (β) and gama (γ) globulins in serum of chickens.

It was found that the total proteins and albumin levels were increased significantly ($P \leq 0.05$) in the vaccinated ofloxacin treated group of chickens after 1 and 2 weeks since beginning of administration as compared with the vaccinated non-treated and control groups (Table 1 & 2). The administration of Infectious bronchitis virus vaccine alone or in combination with ofloxacin resulted in insignificant changes in levels of alpha (α) and beta (β) globulins as compared with control group (Table 3 & 4). Chickens of the second group (vaccinated

Table 1: Effect of ofloxacin (5mg/kgb.w.) administered orally for 5 successive days on Serum total proteins (gm/dl) in chickens (n=5)

	Time post treatment in weeks				
Groups	0	1W	2W	3W	4W
Control	5.93±0.061	5.01±0.22	5.91±0.23	5.82±0.13	5.67±0.21
Nontreatedvaccinated	5.41±0.01	5.62±0.3	5.21±0.12	5.61±0.13	5.99±0.10
Treatedvaccinated	5.63±0.61	6.41±0.32*	6.81±0.24*	5.91±0.11	5.67±0.51

*= P≤0.05

Table 2: Effect of ofloxacin (5mg/kg b.w.) administered orally for 5 successive days on serum Albumin (gm/dl) in chickens (n=5)

	Time post treatment in weeks				
Groups	0	1W	2W	3W	4W
Control	3.89±0.06	3.61±0.21	3.89±0.61	3.56±0.22	3.71±0.16
Nontreated-vaccinated	3.64±0.12	3.72±0.71	3.74±0.75	3.42±0.32	3.44±0.21
Treated-vaccinated	3.76±0.24	4.24±0.83*	4.11±0.21*	3.63±0.09	3.50±0.64

*= P≤0.05

Table 3: Effect of ofloxacin (5mg/kg b.w.) administered orally for 5 successive days on serum Alph-globulins (gm/dl) in chickens (n=5)

	Time post treatment in weeks				
Groups	0	1W	2W	3W	4W
Control	0.93±0.061	0.95±0.022	0.99±0.01	0.90±0.02	0.87±0.03
Nontreated-vaccinated	0.84±0.021	0.94±0.010	0.95±0.16	0.97±0.04	0.86±0.02
Treated-vaccinated	0.97±0.63	0.89±0.031	0.96±0.17	0.88±0.07	0.97±0.04

*= P≤0.05

Table 4: Effect of ofloxacin (5mg/kg b.w.) administered orally for 5 successive days on serum Beta-globulins (gm/dl) in chickens (n=5)

	Time post treatment in weeks				
Groups	0	1W	2W	3W	4W
Control	0.76±0.07	0.77±0.07	0.86±0.05	0.69±0.02	0.71±0.01
Nontreated-vaccinated	0.86±0.03	0.70±0.06	0.66±0.01	0.78±0.01	0.81±0.08
Treated-vaccinated	0.71±0.08	0.75±0.03	0.83±0.06	0.88±0.04	0.79±0.01

*= P≤0.05

Table 5: Effect of ofloxacin (5mg/kg b.w.) administered orally for 5 successive days on Serum Gamma-globulins (gm/dl) in chickens (n=5)

	Time post treatment in weeks				
Groups	0	1W	2W	3W	4W
Control	0.56±0.06	0.56±0.07	0.51±0.06	0.57±0.02	0.50±0.04
Nontreated-vaccinated	0.54±0.05	0.52±0.08	0.73±0.08*	0.77±0.01*	0.59±0.02
Treated-vaccinated	0.57±0.07	0.33±0.01*	0.38±0.02*	0.38±0.05*	0.37±0.03*

*= P≤0.05

non-treated) showed significant elevation in gama (γ) globulinslevel (P≤0.05) after 2 and 3 weeks since beginning of administrationwhile the third group (vaccinated ofloxacin treated) showed a significant decrease (P≤0.05) in gama (γ)globulins levelafter 1,2, 3 and 4 weeks as compared with control group (Table 5).

DISCUSSION

The present work was designedto investigate the effect of ofloxacinco-administrationwith Infectious bronchitis virus vaccine on plasma proteins including gama (γ) globulins and consequently the effect on humeral immunity. The duration of the present study lasted for 4 weeks to cover the vaccination schedule including broilers primary vaccination from 1 day age and possible booster vaccination 3 weeks later according to manufacturing company directions.

The result ofsignificantly increased serum levels of the total plasma proteins and albumin in the 3rd group(vaccinated ofloxacin treated group) of chickens after 1 and 2 weeks since beginning of administration as compared with the vaccinated nontreated and control groups,can be explained on basis thatofloxacin as fluoroquinolonescan cause diarrhea [17] resulting in haemoconcentration and dehydration. Any rise in albumin

level is only a relative hyperalbuminemia because of haemoconcentration as a result of water loss and dehydration [18].

The gama (γ) globulins level in serum of control group chickenswereranged from 0.50±0.04 to0.57±0.07, these are in agreementwith findings of gamaglobulins levels 0.49±0.08in healthy control non treated chickens reported byAbd El-Baky [19]. Immunoglobulins are found in the γ-globulin fraction. The immunoglobulins produced by B-lymphocytes in the spleen, lymph nodes and bone marrow following theirstimulation by the presence of pathogen or antigens in the circulation or tissues.Immunoglobulinsact as antibodies and are produced in response to antigens. They are highly specific to only one antigenic determinant. The lymphocytic cell line plays the central role in the immune system [18]. Our results of significant elevation of gama (γ) globulins level in chickens of the second group (vaccinated non-treated) after 2 and 3 weeks since beginning of administrationare in agreement with findings of increased γ-globulinseum levels in chickens as a result of vaccinationor experimental infection of *E. coli* and *salmonella* in chickens as compared to control group [11, 19, 20].

Conversely, there wassignificant decrease ofgama globulinsin third group (vaccinated and ofloxacin treated) after 1,2, 3 and 4 weeks as compared with controlgroup. These findings are in agreement with previous reports that antibiotics cause decreased gama globulins level and antibody response,feeding young conventional chickens on diet containing 50 mg/kg procaine penicillin G affect the electrophoretic pattern of serum of young conventional chickens at 4 months the antibiotic-treated birds showed lower gamma globulin values than the untreated group, alsoadministration of tetracycline hydrochloride to rabbits intramuscularly at a single dose of 25 mg/kg or repeatedly for 7 and 20 days resultedin decrease of gamma-globulin fraction level in the lymph and blood serum that, may be attributed to inhibition of the immunesystem function due to antibiotic therapy [21, 8]. The florfenicol, tilmicosin and enrofloxacinhampered the Newcastle disease (ND) antibody production, measured by both HI and ELISA in the first 3 weeks after the last dose.Alsolevofloxacin significantly decreased the antibodytiter in rats immunized with bovine serum albumin (BSA) docked gelatin microparticles (GM) which suggests that these antibiotics exert adverse effect on antibodies (gama globulins) production [11, 12, 18]. The decreased of gama globulins may be explained on basis of bone marrow failureinduced by use of other flouroquinolone ciprofloxacin [22].

CONCLUSION

On basis of the present study, it could be concluded that ofloxacinmust not be used during vaccination in chickens because of itspotential negative influence on the humeral immunity that expose the treated birds to differentdiseases and reduction in the economic status.. Our advice was to avoid usingofloxacin during vaccination in chickens farms.

REFERENCES

1. Horie, M., K. Saito, N. Nose and H. Nakazawa, 1994. Simultaneous determination of benofloxacin, danofloxacin and ofloxacin in tissues by high performance liquid chromatography. J. chromatographt B., 653: 69-76.
2. Grohe, K., 1998. The chemistry of the quinolones: methods of synthesizing the quinolone ring system. In: Kuhlman J, *et al*. (eds). Quinolone Antibacterials. New York: Springer-Verlag.
3. Giguère, J.F., Prescott and M. Patricia Dowling, 2013. Antimicrobial Therapy in Veterinary Medicine, Fifth Edition. Wiley & Sons, Inc. Publisher.
4. Bryskier, A., 2005. Fluoroquinolones. In: Bryskier A (ed). Antimicrobial Agents: Antibacterials and Antifungals. Washington, DC: ASM Press, pp: 668.
5. Drlica, K., X. Zhaoand Zhao, 1997. DNA gyrase, topoisomerase IV and the 4-quinolones. Microbiol Mol. Biol. Rev., 61: 377-92.
6. Hui, G. and R. Das, 2000. Prevalence of *E. coli* in duck in two district of west bengal with their serotyping and antibigram. Ind. J. Anim. Health, 39: 61-64.
7. Kim, K.S., 2000. In-Vitro and in-Vivo activity of ofloxacin against E.Coli in chickens. Kor. J. Vet. Pub. Heal., 24: 71-78.
8. Balashev, V.N. and R.G. Polosova, 1981. Experimental effect of tetracycline on the protein makeup of the lymph and blood serum. Antibiotiki, 26: 374-8.
9. Grondel, J.L., G.C. Angenent and E. Egberts, 1985. The influence of antibiotics on the immune system. Investigations on the cellular functions of chicken leukocytes *in vitro*. Vet. Immunol Immunopathol., 10: 307-16.

10. Laval, A., M. Viso, A. Berhanu and S. Kerveillant-Lenoire, 1988. Immunomodulator effects of two antibiotics, chloramphenicol and kitasamycin, in the chicken Ann. Rech. Vet., 19: 259-66.
11. Khalifeh, M.S., M.M. Amawi, E.A. Abu-Basha and I.B. Yonis, 2009. Assessment of humoral and cellular-mediated immune response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. Poult. Sci., 88: 2118-24.
12. Madan, J., D. Kaushik, S. Sardana, A. Ali, S.M. Sudheesh and S.R. Pandey, 2010. Effect of levofloxacin and pefloxacin on humoral immune response elicited by bovine serum albumin docked in gelatin microparticles and nanoparticles. Pharmazie., 65: 284-9.
13. Weichselbunm, T.E., 1946. An accurate and rapid method for the determination of protein in small amount of blood serum and plasma. Amer. J. Clin. Path., 16: 40-43.
14. Doumas, B.T., W.A. Watson and H.C. Biggs, 1971. Albumin standards and Management of serum albumin with bromocresol green. Chin. Chim. Acta., 31: 87-96.
15. Ornstein, L., 1964. Disc electrophoresis background and theory. J. Ann. NY Acad. Sci., 28(121): 321.
16. SAS, C, 2002. Statistical Analysis. User's Guide Statistics. SAS Institute Inc., Cary, North California, U.S.A.
17. De Sarro, A., G. De Sarro and De Sarro, 2001. Adverse reactions to fluoroquinolones.an over view on mechanistic aspects. Curr. Med. Chem., 8: 371-84.
18. Kaneko, J.J., J.W. Harvey and M.L. Bruss, 2008. Clinical-biochemistry-of-domestic-animals-sixth-edition, pp: 117-155.
19. Abd El-Baky, A.A., 2007. Clinicopathological studies on probiotics in broilers, Ph.D thesis, Cairo university faculty of veterinary medicine.
20. Mogoda, O.S., 2011. Comparative clinicopathological studies on the effect of different probiotics in broilers, thesis for master degree, Beniseuf university faculty of veterinary medicine.
21. Wostmann, B.S. and H.A. Gordon, 1958. Electrophoretic Studies on Serum Proteins of Young Germfree, Conventional and Antibiotic Treated Conventional Chickens. Exp. Biol. Med. (Maywood), 97: 832-835.
22. Dutta, T.K. and A.B. Badhe, 1999. Ciprofloxacin-induced bone marrow depression. Postgrad Med. J., 75: 571-573.