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The effect of Ketotifen on reducing the inflammatory response in Newcastle disease in chickens

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Abstract In this study, the effect of ketotifen on Newcastle Disease (ND) lesions was evaluated. A total of 150 one-day-old Ross broiler chickens were randomly divided into 5 groups with three replicates. The chickens received Newcastle Disease Vaccine (NDV) and ketotifen with different regimens. Group 1, as a negative control, received no vaccine and virulent NDV. Group 2 received acute NDV and no vaccine. The chickens in group 3 received acute NDV and ketotifen at the same time. Group 4 received acute NDV and ketotifen (before and after virus administration). Group 5 received acute NDV and two doses of Lasota ND vaccine at 10 and 20 days of age. 24 h after challenge, 3 chickens from each group were randomly slaughtered and the proventriculus was collected in 10% formalin for histopathological examination. In addition, the proventriculus was collected 72 h after virus inoculation. Blood samples were collected 24 hours before and 1 week after NDV challenge to determine antibody titers against NDV. Mortality was monitored for 7 days after virus inoculation. Results showed that the comparison of HI antibody titer against NDV in different groups one week after challenge showed that there is a significant difference between groups 1 with others. The comparison of mast cell population in 24 h before challenge showed that the highest and lowest population of mast cells was seen in groups 1 and 5 (respectively), that there is a significant difference between these groups. In one week after challenge, there is no significant difference between all groups for mast cell population. NDV vaccination can increase the protection against NDV challenge. In addition, the administration of ketotifen can reduce the inflammatory process of NDV infection, causing less lesions and less mortality in chickens.

Introduction

Newcastle Disease (ND) is one of the most important poultry diseases, so it is an economically serious limiting factor in the poultry industry. On the other hand, the high resistance of this virus in the worst weather conditions, as well as the existence of different ways of transmitting it from one environment to another, emphasizes the importance and the problems that this disease can cause [1]. Because the clinical signs of the virus are highly variable, diagnosis can be difficult, ranging from 100% mortality in unvaccinated birds to reduced egg production in well-vaccinated laying hens [2]. Newcastle Disease Virus (NDV) is a member of the *Paramyxoviridae* family of avian paramyxovirus serotype 1 [3].

In ND, spleen cells produce interferon-alpha and interferon-beta 6 hours after infection, and one day after infection, interferon-gamma is produced by natural killer cells, activating macrophages and initiating a cellular immune response [4, 5]. T lymphocytes respond rapidly to the second exposure to viral antigen. This activity can be measured by measuring lymphocyte proliferation and cytokine secretion. T-helper cells produce interferon-gamma, which is seen 3 days after administration of the live vaccine. This activity leads to the onset of humoral immunity. Macrophages also induce the secretion of nitric oxide. Nitric oxide production is higher in chickens with high antibody titers than in chickens with low titers, indicating a correlation between cellular and humoral immunity [6]. However, in parallel with viral replication in the host, increased production of large amounts of interferon-gamma significantly reduces mortality, which plays a role in cellular immunity against Newcastle disease virus. After infection with NDV, B lymphocytes differentiate into plasma cells and produce specific antibodies. These antibodies are essential to prevent mortality in chickens after exposure to virulent NDV [7].

Researchers have long known that many tissues in the body contain substances that, when released by various stimuli, produce various physiological effects, such as redness of the skin, pain or itching, and bronchospasm. It was later discovered that many of these substances are also present in nerve tissue. Histamine and serotonin and hydroxytryptamine are amino acids that are biologically active in both neuronal and non-neuronal tissues and are often released locally [5, 8]. Evidence suggests that histamine also plays a role in the immune activity and chemotaxis of white blood cells [8].

Mast cells originate from the population of hematopoietic cells in the bone marrow [9]. These cells emerge from the bone marrow as progenitor cells and migrate to tissues where they are fully differentiated under the influence of specific cytokines. These cells require interleukin-3 to mature and differentiate. Progenitor mast cells express CD117 but have fewer granules than adult mast cells [10]. One of the main characteristics of adult mast cells is that they have dense secretory granules that are easily visualized with metachromatic dyes such as toluidine blue. Mast cells are abundant in tissues exposed to the environment, such as the skin, gastrointestinal tract, and respiratory tract, allowing them to respond rapidly to invading organisms. These cells play an important role in parasitic and bacterial infections, but their role in viral infections has not been elucidated [11, 7]. These cells can alter their response to pathogens and mount an appropriate inflammatory response after invading pathogens [12]. Two distinct subsets of mast cells have been identified, which include connective tissue mast cells and mucosal mast cells [13]. One of the differences between different types of mast cells is the type of protease produced by each type of mast cell. Connective tissue mast cells are found primarily in the skin and parenchymal tissues around arteries, while mucosal mast cells include mast cells in the lungs and gastrointestinal tract. In the lungs, these cells produce large amounts of kinase-containing proteases, including mucosal cell proteases 1, 2, 6, 7 and tryptase and carboxypeptidase, whereas mucosal mast cells produce only proteases 1,6,7. Both cell types play a role in allergic reactions and can be activated by the same mechanisms [9].

Ketotifen is a second generation, non-competitive, selective histamine and anti-histamine (H1 receptor) antagonist and mast cell membrane stabilizer that prevents the release of mediators by cells involved in the hypersensitivity reaction [5]. It inhibits the release of some allergic mediators such as histamine, leukotrienes, and platelet activating factor [6]. The oral absorption of this drug is at least 60%. After consumption. [8]. It should be noted that both neutral agonists and H1 antagonists reduce or inhibit the effects of histamine through reversible competitive binding to the H1 receptor [8]. Therefore, this study investigated the role of ketotifen in reducing the effects of mucosal mast cells in ND.

Materials and Methods

This study was conducted in the experimental farm of the Veterinary Hospital of Islamic Azad University, Shahrekord Branch, Shahrekord, Iran. A total of 150 one-day-old Ross broiler chickens

were randomly divided into 5 groups with three replicates (10 chickens in each replicate). During the experimental period, all experimental groups were equal in terms of water, feed, temperature and management and differed only in receiving ND vaccine and taking ketotifen. The groups were as follows:

Group 1: This group did not receive any NDV, ND vaccine or drugs during the experimental period.

Group 2: The chickens received only acute NDV (with genotype VII and subtype VIId) at the age of 30 days at a dose of 50 × 105 EID50 (0.5 ml).

Group 3: The chickens received acute NDV (with genotype VII, accession number MW430766) at the age of 30 days at a dose of 50 × 105 egg infectious dose 50% (EID50) (14) (0.5 ml) by gavage, but before receiving the virus they received a dose of ketotifen (Emad Darman Pars Co., Iran) at a dose of 1 mg/kg BW by gavage one hour before receiving the virus.

Group 4: In addition to receiving acute NDV (with genotype VII, accession number MW430766) at 30 days of age at a dose of 50 × 105 EID50 (0.5 ml), the chickens received ketotifen at a dose of 1 mg/kg BW [15] by gavage one hour before and 12 hours after receiving the virus.

Group 5: Chickens received acute NDV (with genotype VII and subtype VIId) at 30 days of age at a dose of 50 × 105 EID50 (0.5 ml) and two doses of B1 and Lasota vaccine (Vetrina, Croatia) against ND at 10 and 20 days of age.

All chickens were healthy prior to the start of the field study. At 24 h post challenge, 3 chickens from each group were randomly slaughtered and the proventriculus was collected in 10% formalin for histopathological examination. In addition, the proventriculus was collected from each group 72 h after virus inoculation. Blood samples were collected 24 h before and 1 week after NDV challenge for determination of antibody titers against NDV by the conventional HI method (4 HA unit). Mortality was monitored for 7 days after virus inoculation (Table 1).

Toluidine blue staining of proventriculus samples was performed to observe mast cells.

The histologic sections were evaluated by light microscopy at ×40, ×100 and ×400 magnifications.

Images were captured using Dino Late camera and Dino Capture software version 2. Marking of images and graphics was done with Corel Draw software version 20.

The data were analyzed using SPSS (version 26). The differences between the groups were investigated with one-way ANOVA and Tukey statistical programs ($p<0.05$).

In group 1, the number of mast cells was higher than other groups and showed a statistically significant difference with other groups ($p<0.05$).

In groups 3 and 4, which received ketotifen, the number of tissue mast cells counted was statistically lower than in the other groups ($p<0.05$).

In group 5, which did not receive ketotifen and received only the NDV vaccine, the number of mast cells was statistically higher than in groups 3 and 4, but the severity of mortality was lower and showed a statistically significant difference from the other groups ($p<0.05$). The severity of clinical signs and mortality one week after challenge has been shown in Table 3.

Results

Antibody titer against NDV

The comparison of HI antibody titer against NDV showed the highest and lowest HI titer was seen in group 5 and 1, (respectively) in 24 hours before and one week after challenge that there is a significant difference between group 5 and 1 ($P<0.05$). Also, there is a significant difference between group 2 with others, while there is no significant difference between chickens in groups 1, 3 and 4 for HI antibody titer against NDV in 24 hours before challenge.

The comparison of HI antibody titer against NDV in different groups one week after challenge showed that there is a significant difference between group 1 with others ($P<0.05$) (Table 1).

Table 1. The antibody titers (Mean±SEM) against NDV in different groups by HI method

Time of sampling	Group 1	Group 2	Group 3	Group 4	Group 5
24 hr. before challenge (29 days old)	2.83±1.16^{b*}	1.00±0.39^{c}	1.66±0.51^{b}	2.60±1.14^{b}	5.60±1.04^{a}
One week after challenge (36 days old)	5.04±0.54^{c}	9.00±0.80^{a}	8.20±0.75^{ab}	6.80±0.55^{b}	9.66±0.51^{a}

*Different superscript indicates show significant difference between the groups (P<0.05).

Table 2. The count of Mast cells (Mean±SEM) in different groups

Time of sampling	Group 1	Group 2	Group 3	Group 4	Group 5
24 hr. before challenge (29 days old)	10.75±1.70^{a*}	8.50±2.64^{ab}	8.75±1.70^{ab}	6.25±1.50^{ab}	**5.50±1.10^{b}**
One week after challenge (36 days old)	4.25±0.95^{a}	4.25±1.25^{a}	3.00±0.81^{a}	3.00±0.81^{a}	**3.00±0.81^{a}**

*Different superscript indicates show significant difference between the groups (P<0.05).

Table 3. Severity of clinical signs and mortality in various experimental groups

	Group 1	Group 2	Group 3	Group 4	Group 5
Clinical signs	2	3	2	2	**1**
Mortality	Without mortality	15	10	5	**3**

1: Depression and mild neurological sign
2: Depression and moderate neurological sign
3: Depression and severe neurological sign

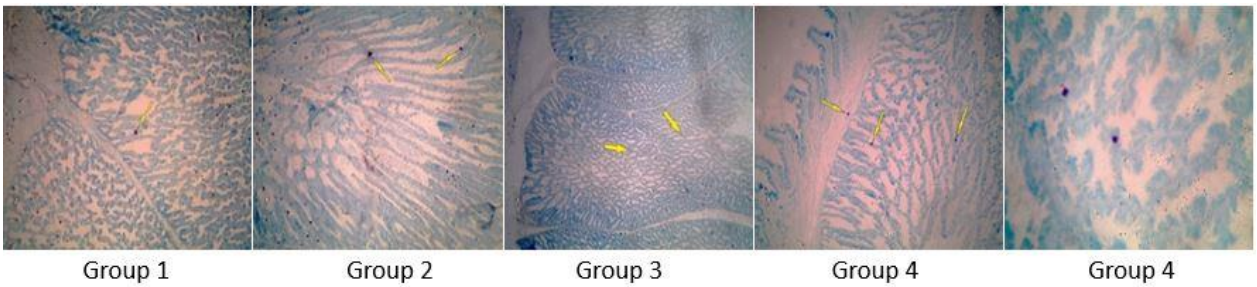

Fig 1. The proventriculus sections in different groups 24 hr. before challenge. (Toluidine blue staining, 10X).

The Mast Cell Population

The mast cells in the proventriculus samples were shown in Figure 1 and 2 in 24 h before and 72 h after challenge. The comparison of mast cell population in 24 h before challenge showed that the highest and lowest population of mast cells was seen in group 1 and 5, respectively, that there is a significant difference between these groups ($P<0.05$). One week after challenge, there is no significant difference between all groups for mast cell population (Table 2).

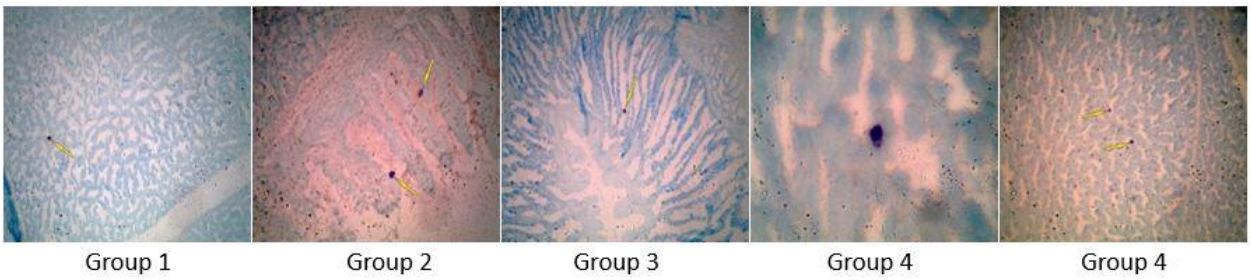

Fig 2. The proventriculus sections in different groups 72 hr. after challenge. (Toluidine blue staining, 10X).

Discussion

The main cause of lesions and death due to acute NDV is the cleavage of protein F0 into two proteins, F1 and F2, under the influence of trypsin and trypsin-like enzymes present in various organs of the body and in the compounds themselves. One of the sites of tryptase accumulation is in mast cell granules. With the entry of NDV into the body, mast cells secrete and proliferate, and eventually the secretion of these compounds from the granules in mast cells causes the division of the F0 protein. We can use drugs or substances to reduce the proliferation of mast cells or stabilize the membrane of mast cells to prevent the secretion of tryptase. This administration will reduce the incidence of mortality and complications of NDV infection [1]. Therefore, in this study, which is on the role of ketotifen (which is an antihistamine) and by stabilizing the mast cell membrane, it can reduce the effects of the virus. The following results were obtained in the first stage of sampling, which was performed 24 hours after virus challenge [2, 13].

There were no statistical differences in the number of mast cells between chickens receiving ketotifen at 72 hours after treatment. This study indicated that the effect of ketotifen disappeared in the treated groups. In the study, 7 days after viral challenge, antibody titers showed no statistically significant difference between groups, indicating an increase in titers in all affected groups.

Sun Qu et al. (2009) in China challenged 60 chickens with NDV. Acute mucosal damage was observed in infected birds, but in the ketotifen pretreatment group, the mast cell population and their tryptase and histamine levels were significantly reduced, indicating relief from disease and its side effects [13]. In this study, we used a challenge dose with local NDV, which demonstrated that these responses are similar and not related to a geographic origin.

Similarly, Wang Di et al (2008) examined the Infectious Bursal Disease (IBD) virus in a number of chickens and found that inflammatory lesions increased significantly in chickens infected with IBD virus. It was also observed that the number of mast cell cells increases during infection with this virus at the site of infection. Therefore, it seems that the increase of mast cells cause to increase IBD virus lesions [16].

Wang et al. (2009) investigated the role of ketotifen in reducing the effects of mast cells in IBD virus, in which the release of mediators produced in mast cells accumulated in infected tissue and lesion margins in the ketotifen group

was significantly reduced, leading to increased survival during infection with IBD virus [17].
Graham et al. studied the role of mast cells in the H5N1 avian influenza virus and showed that the mast cell population increased in the airways of mice infected with H5N1 [18].

The review of other studies showed that H1N1, H3N2 and influenza B viruses are also capable of activating mast cell lines, leading to a significant increase in total mast cell numbers and during the first 5 days of viral challenge. Thus, mast cells were shown to be involved in the inflammatory response to AIV infection in mice [19].

Hu et al (2012) conducted a study on 12-month-old female mice and investigated the effect of influenza virus plus ketotifen. Three mice from each group were selected and their lungs, trachea, and nasal tissues were collected for histopathologic examination. The lesions observed included swelling of the lungs with extensive hemorrhage with infiltration of inflammatory cells and interstitial edema around the blood vessels, resulting in a significant increase in the number of mast cells in the nasal mucosa, trachea and lungs. On days 1, 3 and 5, a marked release of granules from these cells was observed, followed by the levels of tryptase and alpha tumor necrosis factor in the nasal mucosa, trachea and lungs of infected mice compared to the control group [19]. In contrast, mast cell inflammatory cells were inhibited in the drug-treated group, and by preventing the release of the granules of these cells, the damage following the release of the granules and its tissue destruction, the drug significantly reduced disease lesions, so that mice in the group without ketotifen showed severe bronchitis and inflammation of the bronchi with bronchopneumonia, leading to death [5]. In addition, infiltration of various inflammatory cells, together with interstitial edema, increased the thickness of the alveolar wall. It has also been found that programmed cell death is a major cause of influenza virus damage in the lung, so in infected mice, programmed cell death causes very severe damage. However, in the ketotifen group, programming reduces virus damage by reducing the number of dying cells and can prevent many lesions and consequent mortality [19].

Conclusion

In conclusion, according to the results of the present study and review of records, it shows that the use of antihistamine compounds, if used correctly and appropriately, can be useful in reducing the complications of NDV disease. In the present study, we used one dose of ketotifen, but we suggest to administer the following doses for evaluation of the results.

Acknowledgements

Not applicable

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

All ethical considerations including utilizing animals were considered cautiously.

References

1. Abdoshah M, Pourbakhsh SA, Peighambari SM, Shojadoost B, Momayez R, Mojahedi Z. Pathogenicity indices of Newcastle disease viruses isolated from Iranian poultry flocks in Iran. J Vet Res. 2012; 67(2):159-64.
2. Bayram H, Devalia JL, Khair OA, Abdelaziz MM, Sapsford RJ, Czarlewski W, Campbell AM, Bousquet J, Davies RJ. Effect of loratadine on nitrogen dioxide–induced changes in electrical resistance and release of inflammatory mediators from cultured human bronchial epithelial cells. J Allergy Clin Immunol. 1999; 104(1):93-9.
3. Panda A, Huang Z, Elankumaran S, Rockemann DD, Samal SK. Role of fusion protein cleavage site in the virulence of Newcastle disease virus. Microb pathol. 2004 1; 36(1):1-0.

4. Dzogbema KF, Talaki E, Batawui KB, Dao BB. Review on Newcastle disease in poultry. Int. J. of Biol. and Chem. Sci. 2021; 15(2):773-89.
5. Abraham SN, John AL. Mast cell-orchestrated immunity to pathogens. Nat Rev Immunol. 2010; 10(6):440-52.
6. Amoia CF, Nnadi PA, Ezema C, Couacy-Hymann E. Epidemiology of Newcastle disease in Africa with emphasis on Côte d'Ivoire: A review. Vet World. 2021; 14(7):1727.
7. Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. Annu Rev Immunol. 2005; 23:749-86.
8. Muhammadiy NS. Pengaruh Pemberian Ekstrak Kayu Secang (Caesalpinia Sappan L.) Terhadap Kadar Kolesterol Total Dan Trigliserida Darah Tikus Diabetes Yang Diinduksi Aloksan (Doctoral dissertation, Fakultas Farmasi Universitas Jember).
9. Håkanson R, Rönnberg AL, Sjölund K. Fluorometric determination of histamine with OPT: optimum reaction conditions and tests of identity. Anal Biochem. 1972 1; 47(2):356-70.
10. Jang J, Hong SH, Kim IH. Validation of a real-time RT-PCR method to quantify Newcastle disease virus (NDV) titer and comparison with other quantifiable methods. J Microbiol Biotechnol. 2011; 21(1):100-8.
11. Marshall JS, Jawdat DM. Mast cells in innate immunity. J Allergy Clin Immunol. 2004; 114(1):21-7.
13. Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. Nat Rev Immunol. 2014; 14(7):478-94.
14. Costa-Hurtado M, Afonso CL, Miller PJ, Shepherd E, DeJesus E, Smith D, & Pantin-Jackwood MJ. Effect of infection with a mesogenic strain of Newcastle disease virus on infection with highly pathogenic avian influenza virus in chickens. Avian Dis., 2016; 60(1), 269-278.
15. Sun Q, Wang D, She R, Li W, Liu S, Han D, & Ding Y. Increased mast cell density during the infection with velogenic Newcastle disease virus in chickens. Avian pathol. 2008; 37(6), 579-585.
16. Wang D, Xiong J, She R, Liu L, Zhang Y, Luo D, Li W, Hu Y, Wang Y, Zhang Q, Sun Q. Mast cell mediated inflammatory response in chickens after infection with very virulent infectious bursal disease virus. Vet Immunol Immunopathol. 2008 ; 124(1-2):19-28.
17. Wang D, Zhou X, She R, Xiong J, Sun Q, Peng K, Liu L, Liu Y. Impaired intestinal mucosal immunity in specific-pathogen-free chickens after infection with very virulent infectious bursal disease virus. Poultry Sci. 2009; 88(8):1623-8.
18. Gurish MF, Austen KF. Developmental origin and functional specialization of mast cell subsets. Immunity. 2012; 37(1):25-33.
19. Hu Y, Jin Y, Han D, Zhang G, Cao S, Xie J, Xue J, Li Y, Meng D, Fan X, Sun LQ. Mast cell-induced lung injury in mice infected with H5N1 influenza virus. J Virol. 2012; 86(6):3347-56.

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