Journal of Veterinary Science

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Infectious Disease

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Dexamethasone reduces infectious bursal disease mortality in chickens

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ABSTRACT

Very virulent infectious bursal disease virus (vvIBDV) causes high mortality in chickens but measures to reduce the mortality have not been explored. Chickens (8–9 weeks) were treated with 3 agents before and during vvIBDV inoculation. Dexamethasone treatment reduced the mortality of infected chickens (40.7% vs. 3.7%; $p < 0.001$), but treatment with aspirin or vitamin E plus selenium did not affect the mortality. The bursa of Fabricius appeared to have shrunk in both dead and surviving chickens ($p < 0.01$). The results indicate that dexamethasone can reduce mortality in vvIBDV-infected chickens and may provide therapeutic clues for saving individual birds infected by the virus.

Keywords: Very virulent infectious bursal disease virus; nitric oxide; bursa of Fabricius; bursa-to-body-weight ratio; aspirin; vitamin E; selenium

INTRODUCTION

Infectious bursal disease (IBD) is a highly epidemic disease that poses an important threat to chickens [1]. The pathogenic agent, infectious bursal disease virus (IBDV) is a double-stranded RNA virus and divided into serotypes 1 and 2. The serotype 1 viruses are classified further as classical virulent (cv) and very virulent (vv) on the basis of their virulence and antigenicity [2]. vvIBDV causes an acute form of IBD with a high mortality spike, characterized by a sudden onset and short duration of illness, lasting 2–4 days after infection [1,2].

The target cells of vvIBDV are actively proliferating B cells and macrophages in various lymphoid tissues and organs, and the massive destruction of B cells by vvIBDV infection is demonstrated by severe atrophy of the bursa of Fabricius (BF) [1,2]. vvIBDV infection is characterized by a sudden onset of depression with ruffled feathers and, frequently, watery or white diarrhea. The common lesions of IBD involve bursal atrophy and hemorrhage in the thigh and pectoral muscles [1].

The primary intervention used in the poultry industry to control IBD has been immunization [1]. However, interventions to increase the survival rate of vvIBDV-infected chickens have not

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yet been studied. Here, we report preliminary findings on the effects of 3 pharmacological agents tested in vvIBDV-infected chickens.

MATERIALS AND METHODS

Pharmacological agents

The drugs tested were dexamethasone, a steroidal anti-inflammatory and immunosuppressive agent used as a therapeutic drug for sepsis [3,4]; aspirin, a non-steroidal anti-inflammatory drug that has been used for its beneficial effect in chickens under heat stress [5] and in *Escherichia coli* infection [6]; and vitamin E and selenium, which are known to improve resistance to oxidative and heat stress [7].

Experimental animals and virus

We used 8- to 9-wk-old brown layer chickens (Hy-Line brown; HLB) without IBDV-specific antibodies. A vvIBDV strain, SNU1030, was identified as a vvIBDV on the basis of the VP2 nucleotide sequence and virulence in specific-pathogen-free (SPF) chickens [8]. SNU1030 was propagated from the BF of SPF chickens that died within 5 days after inoculation of the virus. BF specimens were pooled, homogenized, and diluted to a 20% (w/v) solution in phosphate-buffered saline (pH 7.2), which was then filtered through a 0.45 μm syringe filter and stored at −70°C until use. To verify the absence of IBDV-specific antibodies, serum samples were collected from experimental chickens, and antibodies were measured using a virus neutralization test, as previously explained [9].

Animal experiments

At 11:00, on day 0 post-inoculation (DPI), BF homogenate (50 μL) was inoculated to the chickens via the intraocular route. Dexamethasone (2 mg/kg, daily intramuscular injection at 12:00) [10] and aspirin (1.25 g/L in feeding water) [11] were administered to the chickens from 0 to 6 DPI. Vitamin E and selenium (300 and 0.2 mg/kg) [6,7] were administered as feed additives from −7 to 6 DPI. All chemicals except dexamethasone (Eagle Vet Tech, Korea) were purchased from Sigma-Aldrich (USA).

In trial I, a total of 48 chickens (8-wk-old) were allocated among 5 experimental groups: normal chickens (control group, n = 9), vvIBDV-inoculated chickens (virus group, n = 10), vvIBDV-inoculated chickens treated with dexamethasone (dexamethasone group, n = 10), aspirin (aspirin group), or vitamin E and selenium (VE-S group, n = 9). In trial II, a total of 44 chickens (8-wk-old) were allocated among 3 groups: 10, 17 and 17 chickens to the control, virus, and dexamethasone groups, respectively. Mortality was checked at 11:00 daily at 0–6 DPI, and blood was sampled from a wing vein. The BF and total body weight were measured daily at 2–4 DPI (dead chickens), and at 6 DPI (surviving chickens).

Plasma NO levels were measured by the Griess reaction using total NO detection kits (Stressgen, Canada) according to the recommended procedures [12]. Rectal temperature was measured daily using a digital thermometer.

Data are presented as mean ± SEM. The difference of means between groups was determined by the χ^2 or Student's t-test. Statistical significance of differences between means was defined as a p value < 0.05.
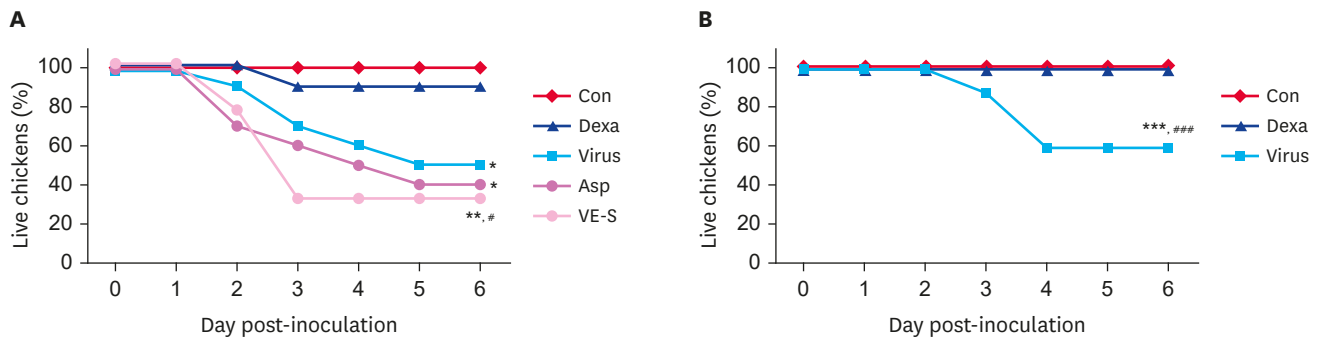
Fig. 1. The effects of dexamethasone, aspirin, and vitamin E with selenium on the mortality of vvIBDV-infected chickens. Note the low mortality of dexamethasone groups in both trial I (A) and trial II (B) compared to the control and virus groups; p = 0.051 between virus and dexamethasone groups by the χ^2 test. The chicken numbers were 10 in trial I except the VE-S group (n = 9), and 17 in trial II except the control group (n = 10).
vvIBDV, very virulent infectious bursal disease virus; Con, control; Dexa, dexamethasone; Asp, aspirin; VE-S, vitamin E + selenium.
*p < 0.05; **p < 0.01; ***p < 0.001 vs. control group by the χ^2 test. #p < 0.05 and ###p < 0.001 vs. the dexamethasone group by the χ^2 test.

RESULTS

Fig. 1 shows the mortality rates of chickens during 6 days after vvIBDV inoculation in two separate trials. In trial I, no chickens died in the control group during the experiment, whereas the chickens in the virus group died between 2 and 4 DPI, but not at 5 and 6 DPI (**Fig. 1A**). The proportion of dead chickens in the virus group was much larger than in the control group (n = 10; 50% vs. 0%; p = 0.013 by the χ^2 test). Interestingly, only one chicken died in the dexamethasone group in the same experiment, the mortality of the dexamethasone group was similar to that of the control group (p = 0.305 by the χ^2 test). In contrast, the mortality of the vvIBDV-inoculated chickens in the aspirin or VE-S group did not significantly differ from that of the chickens in the virus group. In the second trial (**Fig. 1B**), the mortality in the virus group was 35% (6 deaths in 17 chickens), whereas that of the dexamethasone group (n = 17) was 0%. The mortality of the dexamethasone group was significantly smaller than that of the virus group (n = 17; 0% vs. 35%, p < 0.001 by the χ^2 test). In the virus and control groups, the overall mortality was 40.7% and 3.7%, respectively.

Plasma NO levels in the surviving chickens (trial I) increased at 2 and 3 DPI and then returned to the control level after 4 DPI (**Fig. 2**). Some vvIBDV-challenged chickens showed a sharp NO surge at 2 DPI and were found dead at 3 DPI (1, 1, and 2 animals in the dexamethasone, aspirin, and VE-S groups, respectively; **Fig. 2C-E**). Other chickens infected died without such a surge at 2–5 DPI. The overall time course of plasma NO levels corresponded to that of IBDV-induced mortality, but no evident correlation was found between NO levels and death in individual animals.

Body temperature (BT) in the virus group (n = 10) was elevated by 1.7°C at 3 DPI from baseline (42 ± 0.17°C at 0 DPI), and then returned to baseline at 4 DPI. Similar change was observed in the dexamethasone group, but not in the aspirin and VE-S groups. There was no surge in BT in the moribund animals.

The BF-to-body weight (B/B) ratio of vvIBDV-infected chickens was smaller than that of the control group in both trials I and II (p < 0.01, **Table 1**). Furthermore, the B/B ratio in the dexamethasone group was significantly smaller than that in the virus group in surviving chickens, which indicates that the BF shrank more in the dexamethasone group than in the other vvIBDV-infected groups (virus, aspirin or VE-S groups).
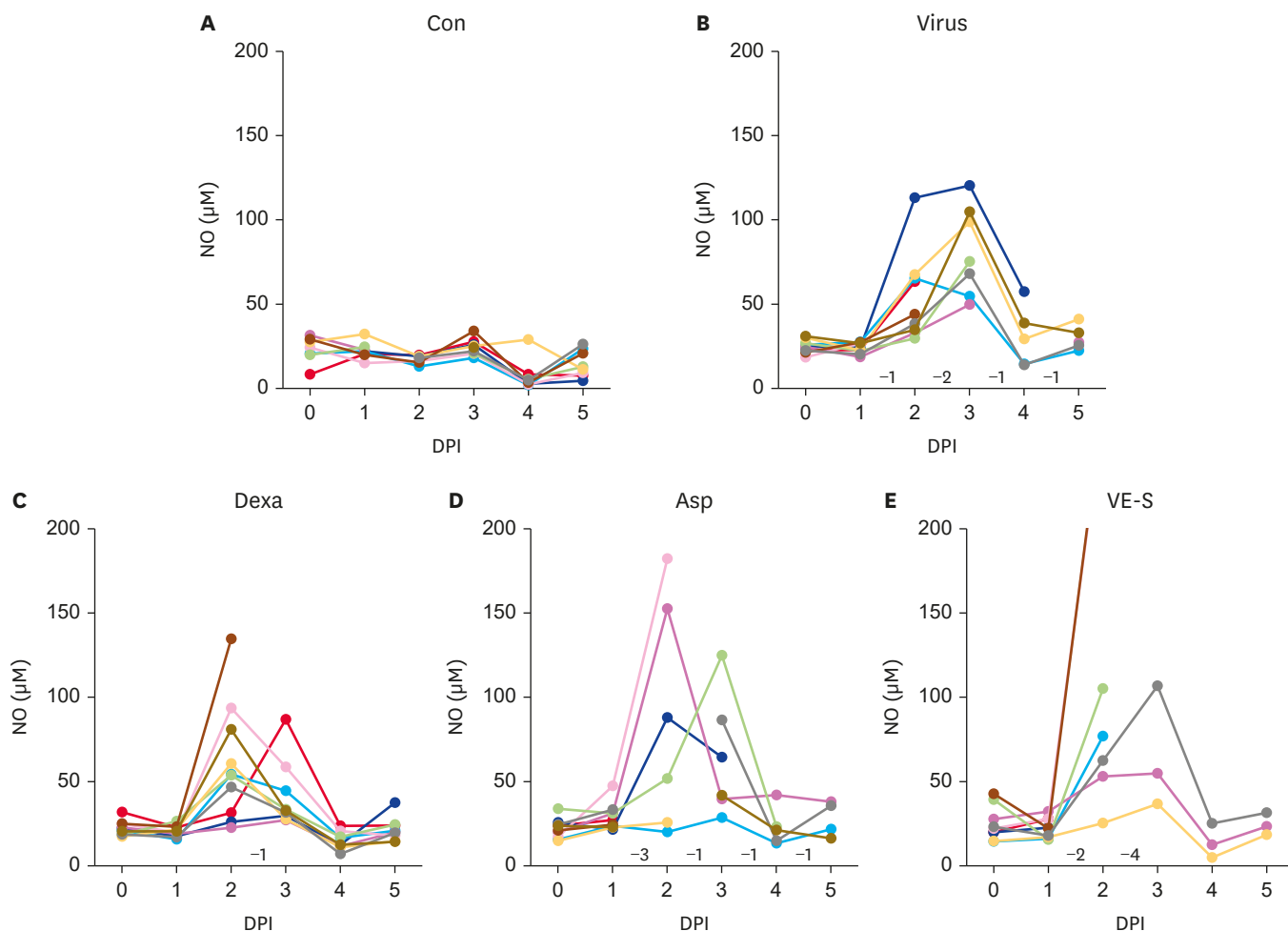
Fig. 2. Plasma NO levels of live chickens in the 5 experimental groups of trial I. Each symbol represents the plasma NO level of an individual chicken. Note the NO surge 1 day before dying in some chickens. The number of chickens in each group at 0 DPI was 10 except the VE-S group (n = 9) and the number of dead animals found each day is shown over the horizontal axis with minus sign (−).
Con, control; Dexa, dexamethasone; Asp, aspirin; VE-S, vitamin E + selenium; DPI, day post-inoculation.

Table 1. B/B ratio of surviving and dead chickens inoculated with vvIBDV

Experiment	Group	Live at 6 DPI	Dead at 2–4 DPI
Trial I	Control	3.10 ± 0.24 (n = 9)	
	Virus	1.66 ± 0.17** (n = 5)	2.42 ± 0.30 (n = 5)
	Dexamethasone	1.12 ± 0.07**,## (n = 9)	1.65 (n = 1)
	Vitamin E + selenium	1.83 ± 0.15** (n = 4)	2.72 ± 0.13** (n = 6)
	Aspirin	1.46 ± 0.24** (n = 4)	2.33 ± 0.26 (n = 6)
Trial II	Control	4.19 ± 0.31 (n = 10)	
	Virus	2.72 ± 0.15** (n = 11)	3.62 ± 0.28** (n = 6)
	Dexamethasone	2.16 ± 0.17**,# (n = 17)	

B/B ratios of chickens were calculated by dividing bursa weight (mg) by body weight (g) of chickens during 2–4 DPI (dead chickens) or 6 DPI (surviving chickens). Data are presented as mean ± SEM. The treatment details in each trial are described in the Materials and Methods.
B/B, bursa of Fabricius-to-body weight; vvIBDV, very virulent infectious bursal disease virus; DPI, days post-inoculation.
**$p < 0.01$ vs. live chickens of the control group; #$p < 0.05$, ##$p < 0.01$ vs. live chickens of the virus group.

DISCUSSION

The present study shows that dexamethasone, a steroidal anti-inflammatory drug, significantly reduced the mortality of vvIBDV-infected chickens at 2–4 DPI, whereas aspirin,

and vitamin E with selenium did not affect the mortality. Plasma NO levels and BT reversibly increased during 2–3 DPI, the critical period when mortality peaked in vvIBDV-inoculated chicken. There was significant bursal atrophy in the dexamethasone-treated surviving animals. Little correlation was observed between the death of individual chickens and these parameters. The most salient observation was that dexamethasone reduced the mortality of vvIBDV-infected chickens by about 90%. This finding is new in poultry and consistent with previous reports on mammalian in that glucocorticoids can be an effective therapeutic agent for septic shock due to their beneficial effects on the release of inflammatory cytokines [3,4] and the acute respiratory distress syndrome of coronavirus disease 2019 [13].

Supplemental vitamin E and selenium did not affect the mortality of vvIBDV-infected chickens in this study. Thus, the biological pathways affected by aspirin, which inhibits prostaglandin synthesis [6], or vitamin E and selenium, which function as antioxidants [7], may not play important roles in the pathophysiology underlying the high mortality of vvIBDV-infected chickens under our experimental conditions.

Plasma NO is an important factor mediating septic shock [2,14]. In this study, some vvIBDV-inoculated chickens died with an NO surge and others died without such a surge, regardless of treatment with dexamethasone, vitamin E with selenium, or aspirin (**Fig. 2**). BT increased also in Dexamethasone treated surviving chickens. This implies that the dexamethasone-induced protection of vvIBDV-infected chickens is unlikely to be exerted through the mechanisms mediating NO and BT elevation. Considering that the reduced B/B ratio was more prominent in the dexamethasone-treated chicken (**Table 1**), the protective effect of dexamethasone might be also due to its suppressive effect on the BF. This observation is consistent with an earlier report showing that vvIBDV-inoculation did not induce clinical IBD in bursectomized chickens [15]. The results in this study provide limited information on the underlying mechanisms of dexamethasone-induced reduction of mortality in vvIBDV-infected chickens. To further confirm and understand the life-saving mechanisms of dexamethasone, more studies are necessary on a larger scale with a focused experimental design.

In conclusion, this study showed that dexamethasone can reduce the mortality rate in acute vvIBDV infection. In poultry farms treating vvIBDV-infected chickens with dexamethasone is currently unlikely to be cost-effective over mass vaccination, but our findings may help to develop therapeutic measures to save individual birds of interest such as companion, rare, and endangered birds with viral infections.

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