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ABSTRACT

Glucocorticoids are widely used in treatment of different inflammatory diseases. This study was performed to investigate the effect of dexamethasone (Dex) on acute respiratory distress syndrome (ARDS) induced by H5N1 viral infection in mice.

Six- to eight-wk-old BALB/c mice were divided into 3 groups with 80 mice each. Infected-group and Dex-treated infected-group were inoculated intranasally with 1×10^2 MID_{50} of A/Chicken/Hebei/108/2002(H5N1) viruses, with daily intraperitoneal injection of PBS, or 2.5 mg/kg Dex at days 3 to 14 postinoculation, respectively. Control group received noninfectious allantoic fluid and daily intraperitoneal injection of PBS.

In H5N1-infected mice, Dex treatment could not improve the mortality (17/20 vs. 16/20, Dex-treated infected-group vs. infected-group), and did not alleviate the clinical signs, including weight loss, decreased food intake and inactivity. There was no significant amelioration of the hypoxemia and ARDS-associated pathological changes in Dex-treated infected-mice, as assessed by blood gas analysis and histological score. Furthermore, Dex therapy could not inhibit the inflammatory cellular infiltration and the cytokine release (IL-6 and TNF-α) in BALF induced by H5N1 infection.

In conclusion, Dex treatment (2.5 mg/kg) from days 3 to 14 postinoculation has no beneficial effect on ARDS caused by H5N1 infection in mice.

Keywords: dexamethasone; acute respiratory distress syndrome; avian influenza A H5N1 virus; cytokine

INTRODUCTION

H5N1 avian influenza virus has constituted huge health problems to human beings, largely because it might cause a pandemic influenza which leads to catastrophic consequences (1, 2). Although neuraminidase inhibitors are effective in treating avian influenza, high mortality (nearly 63.2%) still was found in confirmed H5N1 patients, based on 240 deaths among 380 confirmed cases reported to the WHO since December 2003 (3). Previous studies demonstrated that the acute respiratory distress syndrome (ARDS), incriminated as a possible pathogenic mechanism of human H5N1 disease, might be one of the most important reasons of patient death (4). Therefore, it is urgent to study the therapeutic intervention in the ARDS of human H5N1 disease.

Glucocorticoids have been used for therapy of the ARDS induced by different mechanisms. In recent years, infectious agents have become the most important factors of ARDS, especially the avian influenza virus and severe acute respiratory syndrome coronavirus (SARS-CoV) (5–8). Chen *et al.* (9) investigated the efficacy and safety profiles of corticosteroid therapy in SARS patients, and reported that proper use of corticosteroid in confirmed critical SARS resulted in lower mortality and shorter hospital stay. Meduri *et al.* (10, 11) reported that prolonged administration of methylprednisolone in patients with unresolving ARDS significantly improved the pulmonary and extrapulmonary organ dysfunction and reduced mortality. Although glucocorticoids have been used clinically in the management of ARDS in H5N1 patients in Hong Kong, Vietnam and Thailand, there is no evidence showing a beneficial role of glucocorticoids in management of severe H5N1 infection (12–14). Therefore, the therapeutic effects of glucocorticoids should be further evaluated in therapy of the ARDS caused by H5N1 viral infection. An H5N1 virus-induced mouse model of ARDS has been described recently by our groups (15). The model could recapitulate most of clinical and pathological changes observed in human ARDS induced by H5N1 virus infection. In this study, we investigated the

efficacy of dexamethasone (Dex), a potent and long-lasting glucocorticoid, in the treatment of the H5N1 virus-induced ARDS in mice in order to assess whether the Dex can be used as a potential therapy of H5N1-induced ARDS in humans.

METHODS

Virus

The virus was isolated from chicken in the Hebei Province of China in January 2002, and identified as avian influenza A H5N1 virus by means of hemagglutination inhibition and neuraminidase inhibition tests. The isolated strain was designated as A/Chicken/Hebei/108/2002 (H5N1) (Chicken/HB/108). The complete genome sequences (DQ343152, DQ349116, DQ351860, DQ351861, DQ351866, DQ351867, DQ351872 and DQ351873) of the virus can be obtained from GenBank. The virus caused 100% (8/8) mortality of 4-week-old specific pathogen-free (SPF) chicken within 2 days after intravenous infection with 0.2 ml of infectious allantoic fluid at 1:10 dilution (data not shown). On the basis of the criteria of viral virulence (16), this virus is a highly pathogenic avian influenza virus. Our previous studies (15) showed that this H5N1 virus infection in mice resulted in the typical ARDS, which was characterized by about 80% mortality, progressive hypoxemia, pulmonary inflammatory cellular infiltration, and alveolar edema and hemorrhage.

Virus was propagated in 10-day-old embryonated hen eggs at 37 °C for 32 h. Third-passage virus was gradient purified and stored at –80 °C until use.

Animals and Inoculation Protocol

Six to eight-wk-old SPF BALB/c mice (Beijing Laboratory Animal Research Centre, Beijing) were housed in microisolator cages ventilated under negative pressure with HEPA-filtered air. During the experiment, animals had access to food and water *ad libitum*. The mice were lightly anesthetized with diethyl ether and then inoculated intranasally (50 μl) with 1×10^2 MID_{50} of Chicken/HB/108 H5N1 virus diluted in sterile saline. Mock-infected control animals were

inoculated intranasally (50 µl) with an equivalent dilution of noninfectious allantoic fluid. All manipulations were performed under biosafety level 3+ (BSL-3+) laboratory conditions. All procedures were carried out under the guidelines for animal experiments at China Agricultural University.

Determination of proper treatment dose of Dex in H5N1 infected mice

We tested three different therapeutic doses (0.5, 2.5 or 5 mg/kg) of Dex to determine the proper treatment dosage. The choice of these concentrations was based on the therapeutic range used in patients with ARDS and experimental dosage used in animal studies (17–19). Intraperitoneal injection of Dex was frequently used in mice with the adequate absorption from the peritoneal cavity (18, 19). In this study, three dose groups of mice (20 each) were daily given the different dose of Dex (Sigma, dissolved in 100 µl PBS) by intraperitoneal injection at days 3 to 14 after H5N1 infection, respectively. The control mice received a daily intraperitoneal injection of 100 µl PBS after H5N1 infection. Our observation showed that the high dose of Dex (5 mg/kg) resulted in an increased mortality rate compared to that observed in control mice (data not shown). This result indicated that 2.5 mg/kg may be the proper treatment dosage, which will be used in the following experimental procedure.

Experimental Design and Dex Administration

Two experiments were conducted to observe the effects of Dex treatment on the development of ARDS. The first experiment was designed to investigate the effects of Dex on mortality and clinical features of ARDS in H5N1-infected mice. Six- to 8-week-old female SPF BALB/c mice were divided randomly into 3 groups (infected group, Dex-treated infected group and uninfected control group) with 20 mice each. Infected group and Dex-treated infected group were inoculated with H5N1 virus as described in the inoculation protocol, followed by daily intraperitoneal injection of 100 µl PBS, or 2.5 mg/kg Dex dissolved in 100 µl PBS at days 3 to 14 after H5N1 infection, respectively. Uninfected control group received the noninfectious

allantoic fluid (Mock infection) and a daily injection of 100 μl PBS at days 3 to 14. Since we found that daily administration of 2.5 mg/kg Dex to normal mice for 12 days had no obvious effect on the parameters observed in this study, the Dex-treated group without infection was excluded from experimental procedures (data not shown). In order to observe the therapeutic effect, the food intake, body weight, inactivity, anal temperature (measurement with an infrared thermometer) and mortality were monitored daily in each group. To observe the food intake, the mice were housed individually, on grids in boxes which were changed twice/week. They were weighed daily and their food intake was measured by offering daily known weights of food and separating and weighing any leftover food in the box at each change. Since the infected mice exhibit the development, progression and early recovery of ARDS over a 14-day time course, the mice were observed for 14 days postinoculation.

In the second experiment, the effect of Dex on the development of ARDS induced by H5N1 virus was studied. Six- to 8-week-old female SPF BALB/c mice were also divided randomly into 3 groups with 80 mice each, as described in experiment 1. Since about 80% animals died between day 6 and day 8 postinoculation, larger groups (80 per group) of mice were used. Virus inoculation and Dex administration were same as those used in experiment 1. Ten mice of each group were weighed and euthanized on days 3, 5, 6, 8, and 14 postinoculation, and the following parameters were characterized. Lung injury was assessed by testing lung water content and histopathology. Arterial blood gas, the white blood cell counts and tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels in bronchoalveolar lavage fluid (BALF) and the viral titers in the lungs were measured at indicated time.

Lung Histopathology

Four mice of each group were weighed and euthanized. Left lobes of lungs were fixed in buffered 10% formalin and then embedded in paraffin. Five-micrometer-thick sections were stained with hematoxylin-eosin for light microscopy. The histopathological changes in the lung

tissue were examined at different time points. The histopathology was reviewed in a blinded manner with respect to which group or mouse was being reviewed, using a modified histological scoring system as previously described by Belperio *et al.* (20) and Germann *et al.* (21). Briefly, four easily identifiable pathologic processes were chosen to grade semi-quantitatively on a scale of 0–4: (a) alveolar and interstitial edema, (b) hemorrhage, (c) margination and infiltration of inflammatory cells, and (d) formation of bronchiolitis. A score of 0 represented normal lungs; 1, mild; 2, moderate; 3, severe; and 4, very severe histopathological change. The results for the time course of histopathological changes were expressed as means ± SE (three sections from each lung, four lungs per group) at each time point.

Assessment of Lung Water Content and **Arterial Blood Gas Analysis**

The right lung was weighed before and after oven-desiccation at 80°C to determine the lung wet-dry weight ratios and wet lung-body weight ratios, which were taken as indicators of lung edema (22).

Blood gas analysis was performed as described by Fagan *et al.* (23). Four mice in each group were anesthetized with pentobarbital sodium and arterial blood sample (0.3 ml), which was drawn into a heparinized syringe by percutaneous left ventricular sampling from lightly anesthetized mouse spontaneously breathing room air. Blood gas analysis was immediately performed with an IL1740 pH/blood gas/electrolytes analyzer (Instrumentation Laboratory, Lexington, MA).

Cell Counts and Measurement of TNF-α and IL-6 in BALF

After collection of the samples of blood, four animals of each group were used for bronchoalveolar lavage. BALF was collected from each animal as described by Majeski *et al.* (24) and Nick *et al.* (25). In brief, the lungs were lavaged twice with a total 1.0 ml saline (4°C) through the endotracheal tube. The recovery rate of BALF was higher than 90% for all the

animals tested. After the amount of fluid recovered was recorded, an aliquot of lavage fluid was diluted 1:1 with 0.01% crystal violet dye and 2.7% acetic acid for leukocyte staining and erythrocyte hemolysis. The number of leukocytes in BALF was counted by a hemacytometer under a microscope. The remainder of BALF was centrifuged for ten minutes (300 × *g*). Cell differential counts were determined by Wright staining of a spun sample using morphological criteria under a light microscope with evaluation of not less than 200 cells per slide. All slides were counted twice by different observers blinded to the status of the animal. The supernatant was stored at –70°C until the measurement of TNF-α and IL-6 using ELISA kits (Sigma).

Virus Titration

Virus titration was performed as previously described (26). Whole lungs were collected and homogenized in cold phosphate-buffered saline. Clarified homogenates were titrated for viral infectivity in embryonated chicken eggs from initial dilutions of 1:10. The virus titers were expressed as Mean of $\log_{10}$ EID_{50} in per milliliter ± standard deviation (1 MID_{50} is about 1×10^4 EID_{50}).

Statistics

All data are expressed as means ± SD except the score of histopathology. Statistical analysis was performed using the SPSS-statistical software package for Windows, version 13.0 (SPSS Inc. Chicago, IL, USA). Differences between groups were examined for statistical significance using a two-tailed Student *t* test. A *p* value less than 0.05 was considered statistically significant differences. A power analysis was performed to test the effect sizes using power and precision v3.

RESULTS

Effect of Dex on Clinical Feature and Mortality

Table 1 shows the effect of 2.5 mg/kg Dex treatment on the clinical feature in H5N1-infected mice. It was found that the mice in infected group and Dex-treated infected group showed a

similar time course of clinical signs with similar severity. The onset and duration of clinical signs were as follows: on day 3 postinoculation, slight altered gait, inactivity, ruffled fur, inappetence and weight loss; by day 6 postinoculation, the more severe inappetence, emaciation, and the visual signs of labored respirations and respiratory distress; on days 9 to 14 postinoculation, the gradual resolution of clinical signs. The onset of inappetence and inactivity was correlated with loss of body weight, which continued to decline until death. The body temperature slightly declined during the H5N1 infection, and dramatically declined before the mice died. The mice in two groups began to die from day 6 postinoculation, and had the similar total mortality rates (17/20 vs. 16/20, Dex-treated infected group vs. infected group) over a 14-day time course, with death peak between days 7 and 8 postinoculation. There was no significant difference in the survival period of dead mice between two groups (7.38 ± 0.62 vs. 7.35 ± 0.70 d, infected group vs. Dex-treated infected group). None of the uninfected control mice showed any clinical signs or died. Power analysis was performed to test effect sizes, and the results showed that the values of the power were more than 0.85. Moreover, most of them were more than 0.9 when the sample size was forty. The data mentioned above suggested that the daily treatment with Dex at a concentration of 2.5 mg/kg has no effect on the development and outcome of H5N1 viral infection in mice.

Effect of DEX on Lung Histopathology

Two groups of infected mice displayed a similar histopathological pattern, including an initial peribronchiolar patchy pneumonia at days 3 to 5 postinoculation (Figure 1A and 1C) and the predominately peribronchiolar lesions, the hemorrhage, fully developed bronchiolitis (Figure 1B and 1D) and bronchopneumonia by day 8 postinoculation. The pathological lesions in lungs were characterized by the inflammatory cellular infiltration, the interstitial and alveolar edema and the hemorrhage. The time courses of histopathological changes in lungs, based on histological scoring system, were presented in Figure 2. As shown in the grading of the lung

histopathology, Dex treatment did not alleviate any of the lung histopathological lesions associated with the ARDS, including the development of the alveolar and interstitial edema, hemorrhage, a significant margination and infiltration of inflammatory cells, and the formation of severe bronchiolitis.

Effect of Dex on Pulmonary Edema and Arterial Blood Gases

In infected group, the lung wet-dry weight ratios and wet lung-body weight ratios, as the indicators of pulmonary edema, did not change obviously within 3 days postinoculation, but dramatically elevated on days 5 to 8 postinoculation. There were no statistically significant differences in these two parameters at different time points between infected group and Dex-treated infected group ($p > 0.05$), indicating two groups of infected mice developed the similar severity of lung edema on days 5 to 8 postinoculation, shown in Figure 3.

Table 2 shows the time courses of arterial blood gas parameters in mice. Two groups of infected mice showed the slightly decreased partial pressure of arterial oxygen (PaO_2), and the slightly increased partial pressure of arterial carbon dioxide ($PaCO_2$) after 3 days postinoculation. Most of them presented apparent clinical signs of respiratory distress from day 6 postinoculation, and blood gas analysis also showed that PaO_2 dramatically decreased in two groups of infected mice as compared to the uninfected control mice ($p < 0.05$). No statistically significant differences were observed in PaO_2 and $PaCO_2$ in different time points between infected group and Dex-treated infected group ($p > 0.05$).

Effect of Dex on Inflammatory Cell and Cytokines (TNF-α and IL-6) in BALF

Figure 4 shows the time course of white blood cell (WBC) sum and differential counts in BALF on days 3, 5, 6, 8, and 14 postinoculation. The number of WBC in the infected mice increased gradually from day 3 postinoculation, and reached its peak by day 6 postinoculation. The phenotypes of the inflammatory cellular infiltrate were mainly polymorphonuclear leukocyte (PMN) and lymphocytes. The Dex-treated infected mice also developed the infiltration of

WBC into BALF, but the number of lymphocytes in lung and BALF of Dex-treated infected mice slightly reduced compared to those in infected mice ($p > 0.05$).

Figure 5 shows that the TNF-α and IL-6 levels in two group of infected mice dramatically increased on days 3 to 8 postinoculation in BALF compared with uninfected control mice ($p < 0.05$). There were no statistically significant differences in the levels of TNF-α and IL-6 in different time points between infected group and Dex-treated infected group ($p > 0.05$).

Effect of Dex on Viral Infection of the Lungs

The viral titers in lungs of infected mice increased on days 3 to 8 postinoculation, and the peak virus titer reached to 7.8 $\log_{10}$ EID_{50}. As shown in Figure 6, Dex treatment didn't affect the amount of virus recovered from the infected mouse lungs.

DISCUSSION

In this study, experiments were performed to investigate the effect of Dex treatment on the development of ARDS induced by H5N1 virus in mice. The results showed that 2.5 mg/kg/d Dex administration from days 3 to 14 after infection did not alter the time course of development of ARDS induced by H5N1 viral infection. First, it was found that Dex treatment could not alleviate the clinical signs of H5N1-infected mice, such as altered gait, inactivity, ruffled fur, weight loss, and labored respirations. Both Dex-treated and Dex-untreated H5N1-infected BALB/c mice showed the same time course of clinical signs with similar severity. Second, Dex treatment could not decrease the total morality, or prolong the survival period of the mice infected by H5N1 over a 14-day time course. In addition, Dex treatment did not alleviate the lung histopathological lesion associated with the ARDS, such as the development of the alveolar and interstitial edema, hemorrhage and a significant margination and infiltration of inflammatory cells, and the formation of severe bronchiolitis. Furthermore, lung edema and hypoxemia, as assessed by lung wet-dry weight ratios and blood gas analysis, were not modulated after Dex treatment in H5N1 virus-infected mice. Besides, daily treatment

with Dex did not obviously affect the amount of viruses recovered from infected mouse lungs. Therefore, our data demonstrated that administration of Dex at 2.5 mg/kg from days 3 to 14 after H5N1 infection does not affect the development and outcome of ARDS induced by H5N1 virus in mice.

In patients with ARDS who were conformed H5N1 viral infection, the elevated pro-inflammatory cytokines (mainly TNF-α) were found in the plasma and appeared to play a key role in inflammation and the conformation of ARDS (27, 28). In this paper, we observed an about 30-folds increase in amount of neutrophils and a significant elevation in IL-6 and TNF-α levels in BALF of H5N1 virus-infected mice compared to the control mice at day 8 postinoculation, which were consistent with the authors' previous report (15). Further observation showed that Dex treatment could not modulate the phenotype of the inflammatory cellular infiltrate, and did not inhibit the release of IL-6 and TNF-α in BALF of H5N1 virus-infected mice. These results, together with the histopathological observation in lung, suggested that Dex could not inhibit the development of the inflammatory responses associated with ARDS.

So far, the therapies of ARDS are mainly supportive therapeutic methods, such as intubation and ventilatory support. Currently, there are no established effective treatments to halt the progression of ARDS (29, 30). Corticosteroids modulate the host defense response at virtually all levels, protecting the host from immune system overreaction (31, 32). However, the results obtained from corticosteroid therapy in ARDS patients and animal models have been controversial (33). For example, Ottolini *et al.* demonstrated that high dose triamcinolone effectively reduced pulmonary lesions in rats infected with a strain of H3N2 (A/Wuhan/359/95) (31). In contrast, London L *et al.* demonstrated that methylprednisolone treatment did not attenuate the infiltration of inflammatory leukocytes, and could not inhibit the development of pathological changes in the lungs of mice infected with reovirus 1/L (32). Reports of the use of

corticosteroids in human H5N1 influenza treatment are limited, with only a few case reports in literature. Marissa J. Carter reviewed relevant articles, and found that steroid treatment had not appeared to alter mortality rates (33). Our current data, in consistent with other evidence, could not support a beneficial role of corticosteroids in the management of severe H5N1 infections. Corticosteroid type, administration dose and length of therapy may affect the therapeutic efficacy on ARDS associated H5N1 infection in mice. It is well known that the glucocorticoid of choice for pulmonary treatment is hydrocortisone (for sepsis related syndromes) or metylprednisolone (used in most of the ALI/ARDS studies).Our choice of low-dose Dex for therapy of H5N1 viral ARDS in mice is based on the following considerations. Low-dose Dex was used experimentally in ARDS therapy in mice (18□19). Van Woensel J B M *et al.* demonstrated that Dex (0.6 mg/kg/day) had a beneficial effect in patients with bronchiolitis caused by respiratory syncytial virus infection (34). Furthermore, low-dose Dex (for example, 0.4 mg Dex kg^{-1} for 5 days) was used in treating ARDS induced by H5N1 patients in some countries including Vietnam (33□35). More recently, it has been suggested that lower doses of steroid (1–2 mg/kg/day) for a more prolonged period might benefit the lung while reducing the potential for systemic side-effects (11). Notably, low-dose Dex treatment on day 3 postinoculation was carried out in our laboratories. Other glucocorticoids as well as various administration routes and schedules (early and delayed therapy) should also be studied in future.

In summary, our data show that daily administration of 2.5 mg/kg Dex on days 3 to 14 after infection is not effective at inhibiting the development of ARDS associated H5N1 infection in mice.

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Figure Legends

Figure 1 The lung histopathology as shown by H&E staining after therapy with Dex. The histopathology in H5N1-infected mice was shown in Figure 1A and 1B. At day 5 after infection, there were the interstitial edema about small blood vessel, the thickened alveolar walls and the initial peribronchiolar patchy pneumonia, shown in Figure 1A. At day 8 after infection, Figure 1B showed the predominately peribronchiolar lesions, fully developed bronchiolitis and bronchopneumonia. The severe hemorrhage and inflammatory cellular infiltration were also found in alveolar space, especially adjacent to bronchioles. As shown in Figure 1C (at day 5 after infection) and Figure 1D (at day 8 after infection), the histopathological pattern observed in Dex-treated H5N1-infected mice was similar to that in H5N1-infected mice. H, hemorrhage; Br, bronchiolitis. Objective magnification, 10 (A and C) and 20 (B and D).

Figure 2 Lung histological grading of H5N1-infected mice after therapy with Dex. The histopathological changes in lungs, based on histological scoring system, are expressed as means ± SE (three sections from each lung, four lungs per group, therefore, n = 12) at each time point. *Open bars*, uninfected control mice; *Dotted bars*, Dex-treated infected mice; *Solid bars*, infected mice.

Figure 3 Lung wet-dry weight ratios (A) and wet lung-body weight ratios (B) after therapy with Dex. The means ± SD (n = 4) at each time point are shown. *Open bars*, uninfected control mice; *Dotted bars*, Dex-treated infected mice; *Solid bars*, infected mice. *$P < 0.05$ compared with uninfected control mice.

Figure 4 Kinetic analysis of the total WBC counts (A) and differential counts (B, C and D) in BALF after therapy with Dex. The means ± SD (n = 4) at each time point are shown. B (macrophages), C (lymphocytes) and D (PMNs). *Solid square*, Dex-treated infected mice;

Open triangle, infected mice; *Solid triangles*, uninfected control mice. *p < 0.05 and **p < 0.01 compared with those in uninfected control mice.

Figure 5 TNF-α and IL-6 levels in BALF after therapy with Dex. TNF-α (5A) and IL-6 (5B) in BALF. The means ± SD (n = 4) at each time point are shown. *Solid square*, Dex-treated infected mice; *Open triangl*e, infected mice; *Solid triangles*, uninfected control mice. *p < 0.05 and **p < 0.01 compared with those in uninfected control mice.

Figure 6 Replication of H5N1 viruses in lungs of mice after therapy with Dex. Mice were infected with 1×10^2 MID_{50} of Chicken/HB/108 virus, tissues were collected on different days postinoculation, and the virus was titrated in embryonated eggs. The mean virus titers from three mice per group were expressed as $\log_{10}$ EID_{50} per milliliter ± SD. The limit of virus detection was $10^{1.2}$ EID_{50}/ml for lungs. S*olid bars*, Dex-treated infected mice; *Open bars*, infected mice.

Footnotes

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Table 1 Effects of Dex on the clinical features and mortality in H5N1-infected mice

	Groups	Time (*d*) after Inoculation						
		3	5	6	7	8	9	14
Food Intake	Infection†	4.2 ± 0.6	3.3 ± 0.3	2.7 ± 0.7	2.3 ± 0.6	2.4 ± 0.7	3.1 ± 0.5	4.6 ± 0.2
	Infection+Dex‡	4.1 ± 0.4	3.2 ± 0.5	2.8 ± 0.4	2.5 ± 0.3	2.5 ± 0.6	3.3 ± 0.2	4.7 ± 0.5
	Control§	4.5 ± 0.7	4.3 ± 0.5	4.7 ± 0.4	4.5 ± 0.7	4.6 ± 0.4	4.4 ± 0.8	4.5 ± 0.6
Body Weight	Infection†	19.3 ± 0.8	18.2 ± 1.2	17.7 ± 1.6	15.1 ± 2.3	14.2 ± 1.8	15.2 ± 0.8	19.2 ± 1.6
	Infection+Dex‡	19.6 ± 0.8	18.5 ± 0.7	17.4 ± 1.4	16.2 ± 1.8	14.7 ± 1.8	15.9 ± 2.2	20.7 ± 0.5
	Control§	20.1 ± 0.4	20.3 ± 0.5	20.8 ± 0.5	21.3 ± 0.3	21.5 ± 0.8	21.8 ± 0.7	22.4 ± 0.7
Temperature	Infection†	37.3 ± 0.7	36.5 ± 0.9	35.2 ± 1.2	34.6 ± 2.2	35.1 ± 1.7	36.8 ± 1.5	37.2 ± 0.9
	Infection+Dex‡	37.4 ± 0.8	36.3 ± 1.1	35.1 ± 1.4	34.3 ± 2.1	35.3 ± 1.9	36.7 ± 1.3	37.5 ± 0.6
	Control§	37.6 ± 0.5	37.7 ± 0.6	37.5 ± 0.4	38.1 ± 0.3	37.7 ± 0.4	37.8 ± 0.3	37.6 ± 0.5
Cumulative Mortality	Infection†	0/20	0/20	1/20	9/20	16/20	16/20	16/20
	Infection+Dex‡	0/20	0/20	2/20	9/20	17/20	17/20	17/20
	Control§	0/20	0/20	0/20	0/20	0/20	0/20	0/20

Values except morality were expressed as means ± SD. There were no statistically significant differences in food intake, body weight and temperature at different time points between infected group and Dex-treated infected group ($p > 0.05$). Cumulative mortality was calculated as the percentage of cumulative mice dead relative to total number treated at each time point. Two group had the similar total mortality rates (17/21 vs. 16/21, Dex-treated infection vs. infection) over a 14-day time course.

† Mice inoculated with H5N1 virus, and treated with PBS from days 3 to 14 postinoculation.

‡ Mice inoculated with H5N1 virus, and treated with 2.5 mg/kg/d Dex from days 3 to14 postinoculation.

§ Mice inoculated with noninfectious allantoic fluid, and treated with PBS from days 3 to postinoculation.

Table 2 Effect of Dex treatment on arterial blood gases

	Groups	Time (*d*) after Inoculation				
		3	5	6	8	14
PO_2	Control[§]	12.37 ± 1.46	12.29 ± 1.34	12.36 ± 0.96	12.38 ± 1.23	12.39 ± 1.05
	Infection[†]	12.20 ± 1.06	9.12 ± 1.27	6.83 ± 1.46	7.15 ± 1.83	11.70 ± 1.83
	Infection + Dex[‡]	11.92 ± 1.21	9.21 ± 1.34	6.76 ± 0.96	7.28 ± 1.23	11.92 ± 1.35
PCO_2	Control[§]	5.39 ± 0.59	5.27 ± 0.43	5.23 ± 0.77	5.22 ± 0.46	5.26 ± 0.71
	Infection[†]	5.41 ± 0.21	5.93 ± 0.18	7.17 ± 0.61	7.31 ± 0.46	5.47 ± 0.52
	Infection + Dex[‡]	5.43 ± 0.32	6.17 ± 0.27	7.23 ± 0.77	7.43 ± 0. 65	5.82 ± 0.86
pH	Control[§]	7.36 ± 0.062	7.36 ± 0.049	7.35 ± 0.045	7.37 ± 0.043	7.36 ± 0.078
	Infection[†]	7.38 ± 0.051	7.24 ± 0.067	7.19 ± 0.072	7.18 ± 0.058	7.32 ± 0.053
	Infection + Dex[‡]	7.36 ± 0.071	7.28 ± 0.035	7.21 ± 0.067	7.20 ± 0.049	7.34 ± 0.025

Values (n = 4) were expressed as means ± SD. There were no statistically significant differences in PO_2, PCO_2 and pH at different time points between infected group and Dex-treated infected group ($p > 0.05$).

[†] Mice inoculated with H5N1 virus, and treated with PBS from days 3 to 14 postinoculation.

[‡] Mice inoculated with H5N1 virus, and treated with 2.5 mg/kg/d Dex from days 3 to 14 postinoculation.

[§] Mice inoculated with noninfectious allantoic fluid, and treated with PBS from days 3 to 14 postinoculation.

Figure 1 The lung histopathology as shown by H&E staining after therapy with Dex

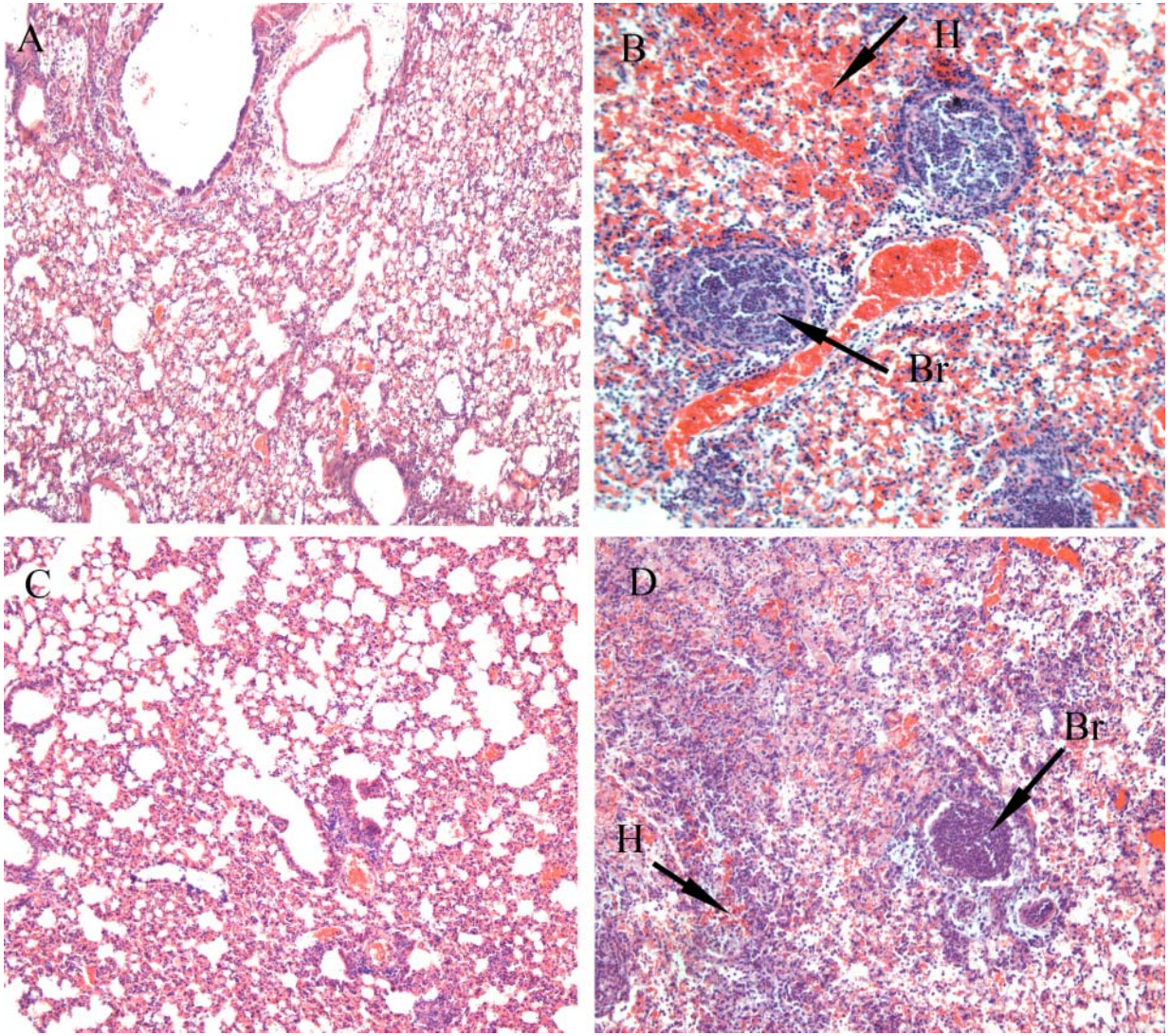


Figure 2 Lung histological grading of H5N1-infected mice after therapy with Dex

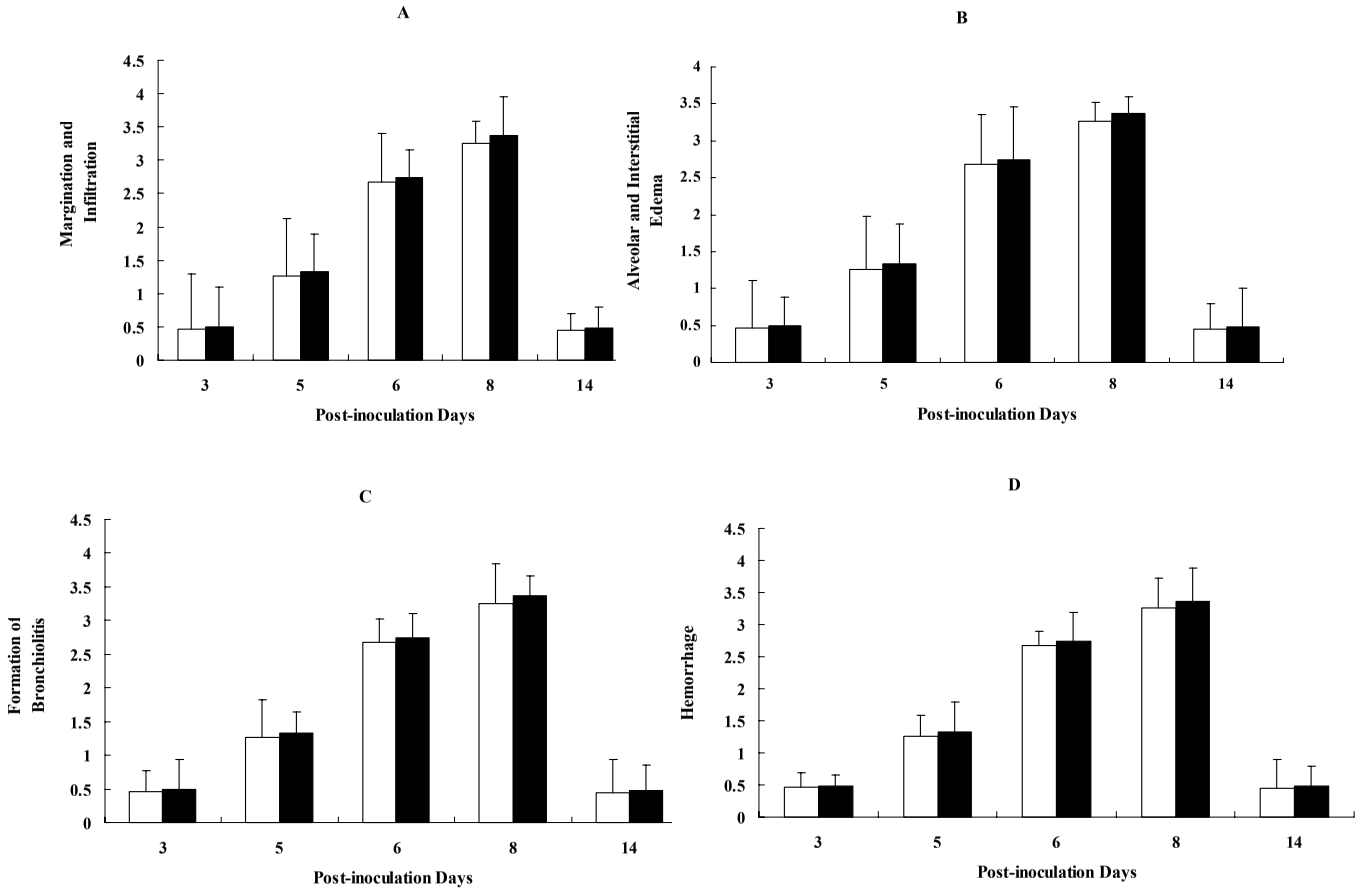


Figure 3 Lung wet-dry weight ratios (A) and wet lung-body weight ratios (B) after therapy with Dex

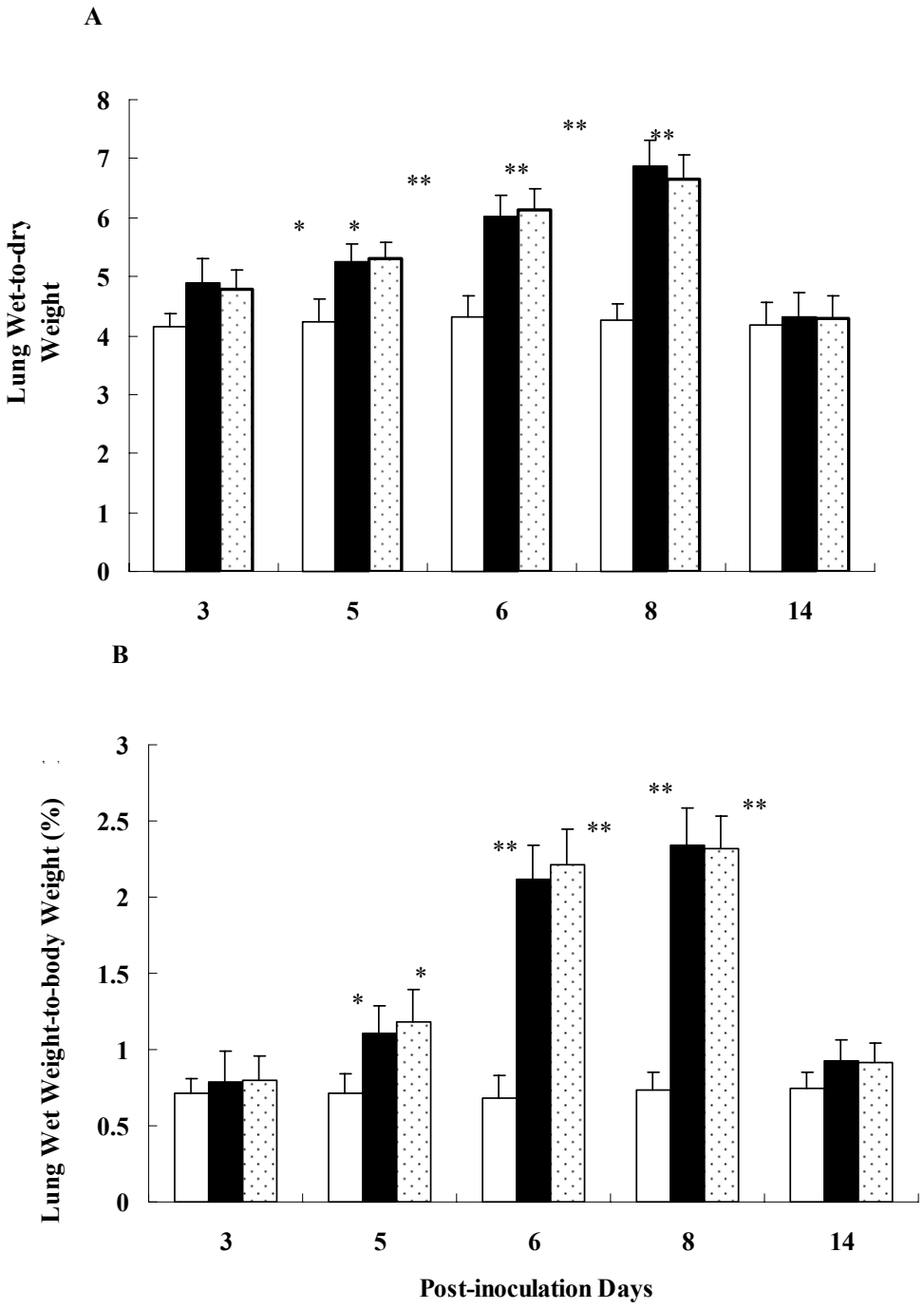


Figure 4 Kinetic analysis of the total WBC counts (A) and differential counts (B, C and D) in BALF after therapy with Dex

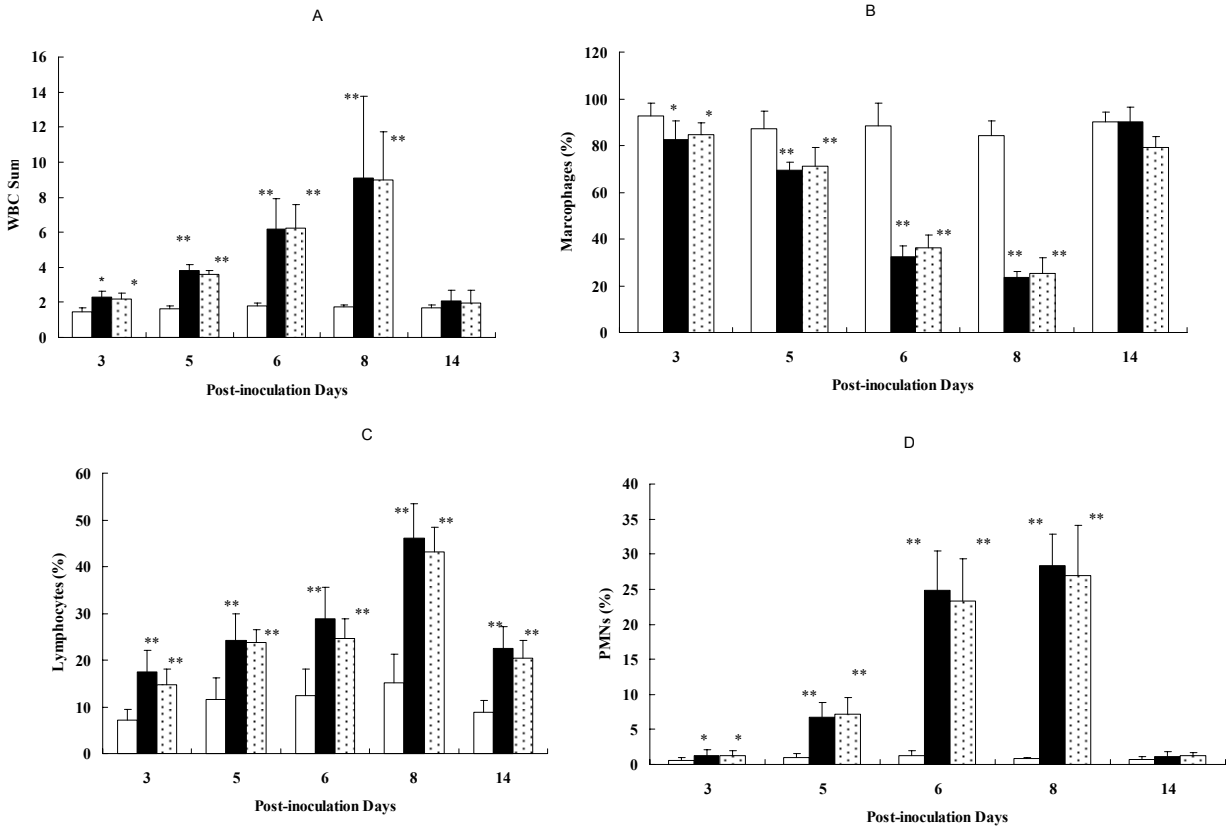
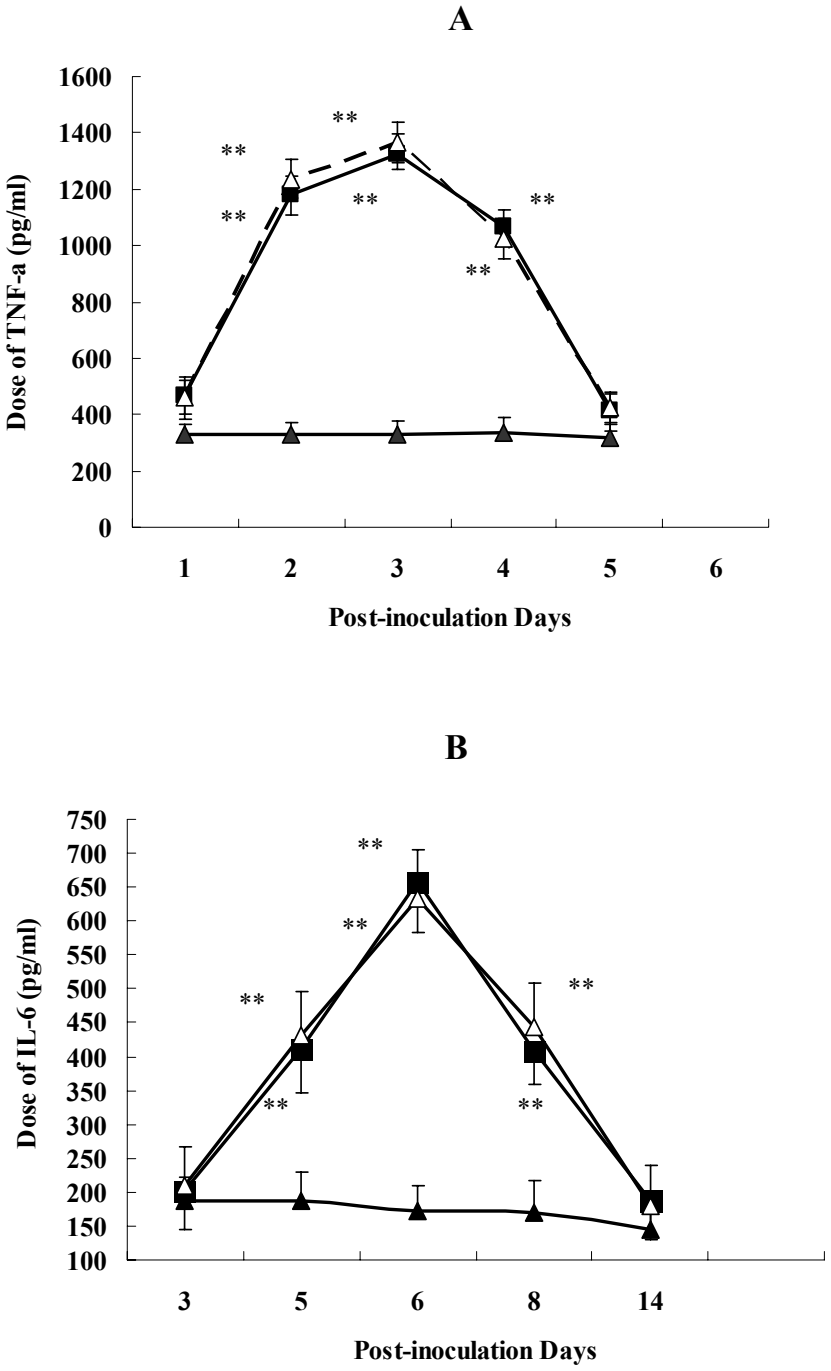


Figure 5 TNF-α and IL-6 levels in BALF after therapy with Dex



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