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Effect of dexamethasone on acute respiratory distress syndrome induced by the H5N1 virus in mice

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

BALB/c mice, 6–8 weeks old, were divided into three groups with 80 mice in each. The infected group and the DEX-treated infected group were inoculated intranasally with 1×10^2 50% mouse infectious dose of A/Chicken/Hebei/108/2002 (H5N1) viruses, with daily intraperitoneal injections of PBS, or 2.5 mg·kg^{-1} DEX at days 3–14 post inoculation, respectively. The control group received noninfectious allantoic fluid and a daily intraperitoneal injection of PBS.

In H5N1-infected mice, DEX treatment did not improve the mortality (17 out of 20 *versus* 16 out of 20 deaths in the DEX-treated infected group *versus* the infected group), and did not alleviate clinical signs, including weight loss, decreased food intake and inactivity. There was no significant amelioration of the hypoxaemia and ARDS-associated pathological changes in DEX-treated infected mice, as assessed by blood gas analysis and histological score. Furthermore, DEX therapy did not inhibit inflammatory cellular infiltration and cytokine release (interleukin-6 and tumour necrosis factor-α) in bronchoalveolar lavage fluid induced by the H5N1 infection.

In conclusion, dexamethasone treatment (2.5 mg·kg^{-1}) from days 3–14 post inoculation has no beneficial effect on acute respiratory distress syndrome caused by the H5N1 infection in mice.

KEYWORDS: Acute respiratory distress syndrome, cytokine, dexamethasone, H5N1 avian influenza A virus

The H5N1 avian influenza virus poses 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 for treating avian influenza, high mortality (nearly 63.2%) was still found in confirmed H5N1 patients, based on 240 deaths among 380 confirmed cases reported to the World Health Organization since December 2003 [3]. Previous studies demonstrated that acute respiratory distress syndrome (ARDS), incriminated as a possible pathogenic mechanism of human H5N1 disease, might be one of the most important reasons for patient death [4]. Therefore, there is an urgent need to study the therapeutic intervention in ARDS of human H5N1 disease.

Glucocorticoids have been used for the therapy of ARDS induced by different mechanisms. In recent years, infectious agents have become the most important factors in ARDS, especially the avian influenza virus and severe acute respiratory syndrome (SARS) coronavirus [5–8]. CHEN *et al.* [9] investigated the efficacy and safety profiles of corticosteroid therapy in SARS patients, and reported that the proper use of corticosteroids in confirmed critical SARS resulted in lower mortality and shorter hospital stay. MEDURI and co-workers [10, 11] reported that prolonged administration of methylprednisolone in patients with unresolving ARDS significantly improved 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 the management of severe H5N1 infection [12–14]. Therefore, the therapeutic effects of glucocorticoids should be further evaluated in therapy for ARDS caused by the H5N1 viral infection.

A H5N1 virus-induced mouse model of ARDS has been described recently by research groups [15]. The model could recapitulate most clinical

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and pathological changes observed in human ARDS induced by the H5N1 virus infection. In the current study, the efficacy of dexamethasone (DEX), a potent and long-lasting glucocorticoid, was investigated in the treatment of H5N1 virus-induced ARDS in mice, in order to assess whether DEX can be used as a potential therapy of H5N1-induced ARDS in humans.

METHODS

Virus

The virus was isolated from chickens in the Hebei Province of China in January 2002, and identified as H5N1 avian influenza A virus by means of haemagglutination 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 (National Center for Biotechnology Information, Bethesda, MD, USA). The virus caused 100% (eight out of eight) mortality of 4-week-old specific pathogen-free (SPF) chickens within 2 days of *i.v.* 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. Previous studies [15] have shown that, in mice, this H5N1 virus infection resulted in typical ARDS, which was characterised by ~80% mortality, progressive hypoxaemia, pulmonary inflammatory cellular infiltration and alveolar oedema and haemorrhage.

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

Animals and inoculation protocol

SPF 6–8 week old BALB/c mice (Beijing Laboratory Animal Research Centre, Beijing, China) were housed in microisolator cages ventilated under negative pressure with high-efficiency particulate air filtered air. During the experiment, animals had access to food and water *ad libitum*. The mice were lightly anaesthetised with diethyl ether and then inoculated intranasally (50 μL) with 1×10^2 50% mouse infectious dose (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+ laboratory conditions. All procedures were carried out under the guidelines for animal experiments at the China Agricultural University (Beijing).

Determination of proper treatment dose of DEX in H5N1-infected mice

Three different therapeutic doses (0.5, 2.5 and 5 $mg \cdot kg^{-1}$) of DEX were tested in order 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 has been frequently used in mice with adequate absorption from the peritoneal cavity [18, 19]. In the current study, three dose groups of mice (20 each) were given the different doses of DEX (Sigma, St Louis, MO, USA; dissolved in 100 μL PBS) daily by *i.p.* injection at days 3–14 after H5N1 infection. The control mice received a daily *i.p.* injection of 100 μL PBS after H5N1 infection. The present authors observed that the high dose of DEX (5 $mg \cdot kg^{-1}$) resulted in an increased mortality rate compared with that observed in control mice (data not shown). This result indicated that 2.5 $mg \cdot kg^{-1}$ may be the proper treatment dosage, which was used in the following experimental procedure.

Experimental design and DEX administration

Two experiments were conducted in order to observe the effect of DEX treatment on the development of ARDS. The first experiment was designed to investigate the effect of DEX on mortality and clinical features of ARDS in H5N1-infected mice. SPF 6–8 week old BALB/c female mice were divided randomly into three groups (infected group, DEX-treated infected group and uninfected control group) with 20 mice in each. The infected group and the DEX-treated infected group were inoculated with the H5N1 virus as described in the inoculation protocol, followed by daily *i.p.* injections of 100 μL PBS, or 2.5 $mg \cdot kg^{-1}$ DEX dissolved in 100 μL PBS at days 3–14 after H5N1 infection, respectively. The uninfected control group received the noninfectious allantoic fluid (mock infection) and a daily injection of 100 μL PBS at days 3–14. Since daily administration of 2.5 $mg \cdot kg^{-1}$ DEX to normal mice for 12 days had no obvious effect on the parameters observed in the current study, the DEX-treated group without infection was excluded from experimental procedures (data not shown). In order to observe the therapeutic effect, food intake, body weight, inactivity, anal temperature (measurement with an infrared thermometer) and mortality were monitored daily in each group. To observe food intake, mice were housed individually on grids in boxes which were changed twice a 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 post inoculation.

In the second experiment, the effect of DEX on the development of ARDS induced by the H5N1 virus was studied. Female SPF 6–8 week old BALB/c mice were also divided randomly into three groups with 80 mice in each, as described in experiment one. Since ~80% animals died between day 6 and day 8 post inoculation, larger groups (80 per group) of mice were used. Virus inoculation and DEX administration were the same as those used in experiment one. Ten mice from each group were weighed and euthanised on days 3, 5, 6, 8 and 14 post inoculation, and the following parameters were characterised. Lung injury was assessed by testing lung water content and histopathology. Arterial blood gas, white blood cell (WBC) count and tumour necrosis factor (TNF)-α and interleukin (IL)-6 levels in bronchoalveolar lavage fluid (BALF) and the viral titres in the lungs were measured at the indicated time.

Lung histopathology

Four mice from each group were weighed and euthanised. The left lobe of the lungs were fixed in buffered 10% formalin and then embedded in paraffin. Sections were cut into 5-μm slices and were stained with haematoxylin–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 and HAFNER [21]. Briefly, four easily identifiable pathological processes were chosen in order to grade, semi-quantitatively, on a scale of 0–4: alveolar and interstitial oedema; haemorrhage; margination; and infiltration of inflammatory cells and formation of bronchiolitis. A score of 0 represented normal lungs; 1 represented mild; 2 moderate; 3 severe; and 4 very severe histopathological changes. The results for the time course of histopathological changes were expressed as mean±SEM (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 oedema [22].

Blood gas analysis was performed as described by FAGAN *et al.* [23]. Four mice in each group were anaesthetised with pentobarbital sodium and an arterial blood sample was taken (0.3 mL), which was drawn into a heparinised syringe by percutaneous left ventricular sampling from lightly anaesthetised mice spontaneously breathing in room air. Blood gas analysis was immediately performed with an IL1740 pH/blood gas/electrolytes analyser (Instrumentation Laboratory, Lexington, MA, USA).

Cell counts and measurement of TNF-α and IL-6 in BALF

After the collection of blood samples, four animals from each group were used for BALF testing. 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 >90% for all tested animals. 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 haemolysis. The number of leukocytes in BALF was counted using a haemacytometer under a microscope. The remainder of the BALF was centrifuged for 10 min ($300 \times g$). Cell differential counts were determined by Wright staining of a spun sample using morphological criteria under a light microscope with evaluation of >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 homogenised in cold PBS. Clarified homogenates were titrated for viral infectivity in embryonated chicken eggs from initial dilutions of 1:10. The virus titres were expressed as mean±SD of $\log_{10}$ 50% egg infectious dose (EID_{50}) per mL (1 MID_{50} is $\sim 1 \times 10^4$ EID_{50}).

Statistics

All data are expressed as mean±SD, except the histopathology score. Differences between groups were examined for statistical

TABLE 1 Effects of dexamethasone (DEX) on the clinical features and mortality in H5N1-infected mice

	Days after inoculation						
	3	5	6	7	8	9	14
Food intake g							
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 g							
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 °C							
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

Data are presented as mean±SD, unless otherwise stated. There were no statistically significant differences in food intake, body weight and temperature at different time points between the infected group and the DEX-treated infected group ($p>0.05$). Cumulative mortality was calculated as the percentage of cumulative mice deaths relative to total number treated at each time point. The two infected groups had similar total mortality rates (17 out of 20 *versus* 16 out of 20 for the DEX-treated infection *versus* the infection) over a 14-day time course. #: mice inoculated with H5N1 virus, and treated with PBS from days 3–14 post inoculation; ¶: mice inoculated with H5N1 virus, and treated with DEX (2.5 $mg \cdot kg^{-1} \cdot day^{-1}$) from days 3–14 post inoculation; +: mice inoculated with noninfectious allantoic fluid, and treated with PBS from day 3–14 post inoculation.

significance using an unpaired two-tailed t-test. A p-value <0.05 was considered statistically significant.

RESULTS

Effect of DEX on clinical features and mortality

Table 1 shows the effect of 2.5 $mg \cdot kg^{-1}$ DEX treatment on clinical features in H5N1-infected mice. It was found that mice in the 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 post inoculation, slightly altered gait, inactivity, ruffled fur, inappetence and weight loss; by day 6 post inoculation, more severe inappetence, emaciation, and the visual signs of laboured respirations and respiratory distress; on days 9–14 post inoculation, 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 the two infected groups began to die from day 6 post inoculation, and had similar total mortality rates (17 out of 20 *versus* 16 out of 20 deaths for the DEX-treated infected group *versus* the

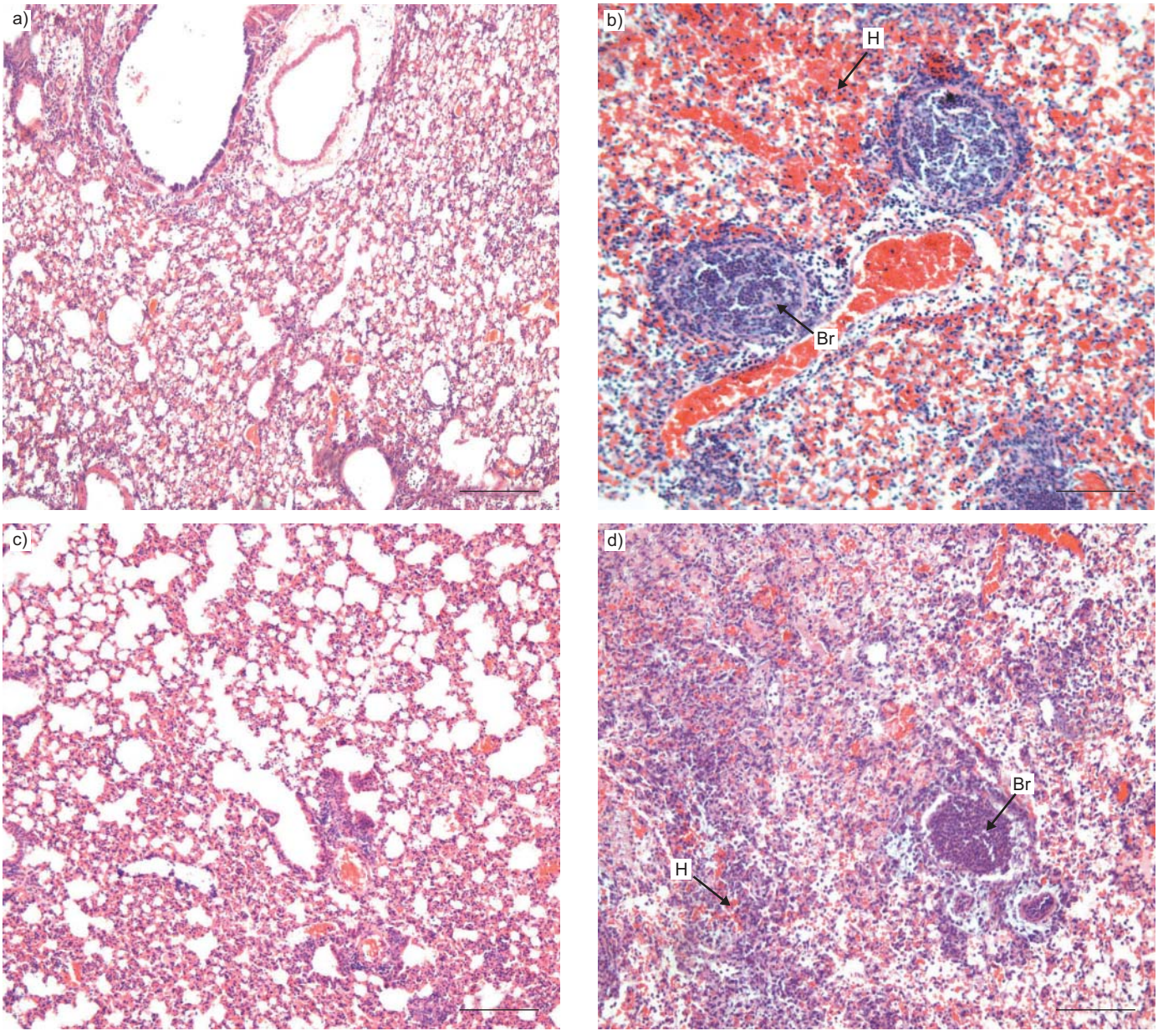

FIGURE 1. The lung histopathology as shown by haematoxylin–eosin staining after therapy with dexamethasone (DEX). a and b) Histopathology in H5N1-infected mice. a) At day 5 after infection, there was interstitial oedema around small blood vessels, thickened alveolar walls and initial peribronchiolar patchy pneumonia. b) At day 8 after infection, there were predominately peribronchiolar lesions, fully developed bronchiolitis (Br) and bronchopneumonia. Severe haemorrhage (H) and inflammatory cellular infiltration were also found in alveolar space, especially adjacent to the bronchioles. c) At day 5 and d) day 8 after infection, the histopathological pattern observed in DEX-treated H5N1-infected mice was similar to that in H5N1-infected mice. a) and c) Scale bars=200 μm. b) and d) Scale bars=100 μm.

infected group) over a 14-day time course, with a peak in mortality between days 7 and 8 post inoculation. There was no significant difference in the survival period of mice between the two groups (7.38 ± 0.62 *versus* 7.35 ± 0.70 days for the infected group *versus* the 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 >0.85. Moreover, most of them were >0.9 when the sample size was 40. The data mentioned above suggested that daily treatment with DEX at a concentration of 2.5 $mg \cdot kg^{-1}$ has no effect on the development and outcome of the H5N1 viral infection in mice.

Effect of DEX on lung histopathology

The two infected groups displayed a similar histopathological pattern, including initial peribronchiolar patchy pneumonia at days 3–5 post inoculation (fig. 1a and c) and predominately peribronchiolar lesions, haemorrhage, fully developed bronchiolitis (fig. 1b and d) and bronchopneumonia by day 8 post inoculation. The pathological lesions in the lungs were characterised by inflammatory cellular infiltration, interstitial and alveolar oedema and haemorrhage. The time courses of histopathological changes in the lungs, based on a histological scoring system, are 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 ARDS, including the development of alveolar and interstitial oedema, haemorrhage, a significant margination and infiltration of inflammatory cells, and the formation of severe bronchiolitis.

Effect of DEX on pulmonary oedema and arterial blood gases

In the infected group, the lung wet/dry weight ratios and wet lung/body weight ratios, as the indicators of pulmonary oedema, did not change significantly within 3 days post inoculation, but were dramatically elevated on days 5–8 post inoculation. There were no statistically significant differences in these two parameters at different time points between the infected group and the DEX-treated infected group ($p>0.05$), indicating that the two groups of infected mice developed a similar severity of lung oedema on days 5–8 post inoculation (fig. 3).

Table 2 shows the time courses of arterial blood gas parameters in mice. The two infected mice groups showed a slight decrease in arterial oxygen tension (P_{a,O_2}), and a slight increase in arterial carbon dioxide tension (P_{a,CO_2}) 3 days post inoculation. Most of the infected mice presented with apparent clinical signs of respiratory distress from day 6 post inoculation, and blood gas analysis also showed that P_{a,O_2}

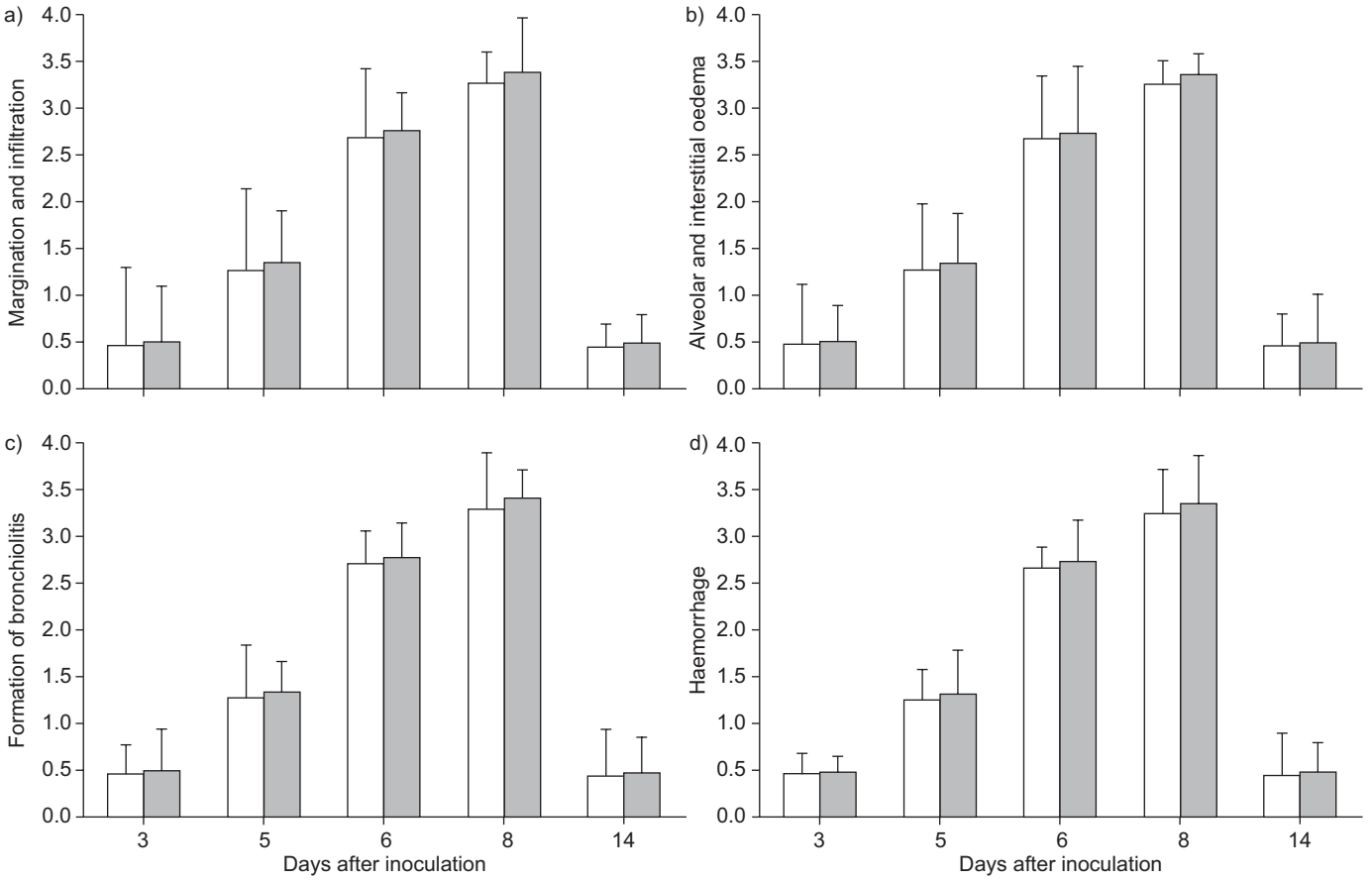

FIGURE 2. Lung histological grading of H5N1-infected mice after therapy with dexamethasone (DEX). The histopathological changes in lungs, based on the histological scoring system, are expressed as mean ± SEM (three sections from each lung, four lungs per group; n=12) at each time point. □: infected mice; ■: DEX-treated infected mice.

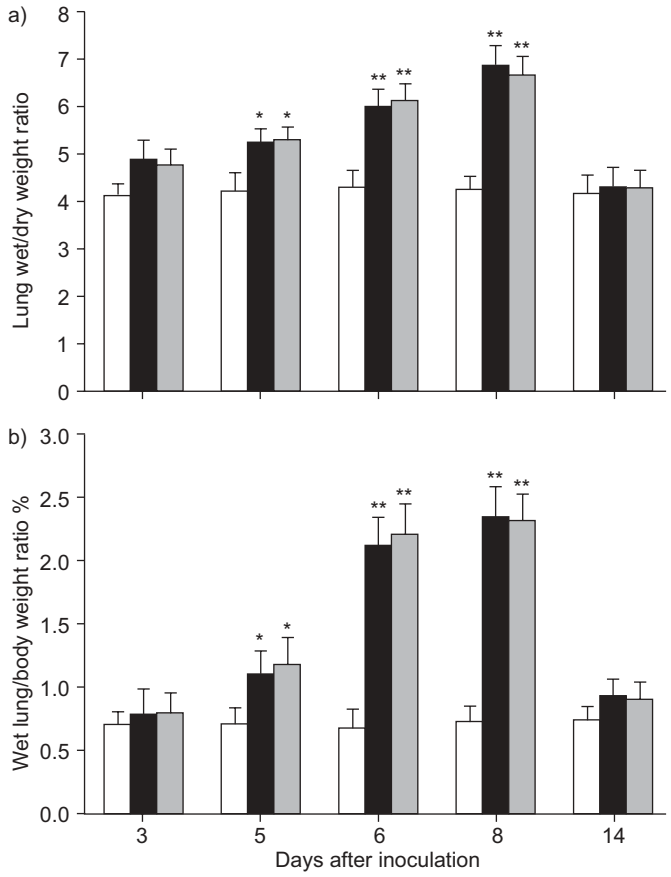

FIGURE 3. a) Lung wet/dry weight ratios and b) wet lung/body weight ratios after therapy with dexamethasone (DEX). The mean±SD (n=4) at each time point is shown. □: uninfected control mice; ■: DEX-treated infected mice; ■: infected mice. *: $p<0.05$; **: $p<0.01$ compared with uninfected control mice.

TABLE 2 Effect of dexamethasone (DEX) treatment on arterial blood gases

	Days after inoculation				
	3	5	6	8	14
P_{a,O_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
P_{a,CO_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

Data are presented as mean± SD. There were no statistically significant differences in arterial oxygen tension (P_{a,O_2}), arterial carbon dioxide tension (P_{a,CO_2}) and pH at different time points between the infected group and the DEX-treated infected group ($p>0.05$; n=4). #: mice inoculated with non-infectious allantoic fluid, and treated with PBS days 3–14 post inoculation; ¶: mice inoculated with H5N1 virus, and treated with PBS days 3–14 post inoculation; +: mice inoculated with H5N1 virus, and treated with DEX (2.5 $mg \cdot kg^{-1} \cdot day^{-1}$) from days 3 to 14 post inoculation.

dramatically decreased in the two infected groups compared with the uninfected control mice ($p<0.05$). No statistically significant differences were observed in P_{a,O_2} and P_{a,CO_2} at different time points between the infected group and the DEX-treated infected group ($p>0.05$).

Effect of DEX on inflammatory cell and cytokine levels in BALF

Figure 4 shows the time course of total WBC count and differential counts in BALF on days 3, 5, 6, 8 and 14 post inoculation. The total WBC count in infected mice increased gradually from day 3 post inoculation, and reached its peak by day 6 post inoculation. The phenotypes of the inflammatory cellular infiltrate were mainly polymorphonuclear leukocytes and lymphocytes. The DEX-treated infected mice also developed infiltration of WBCs into BALF, but the number of lymphocytes in the lung and BALF of the DEX-treated infected mice was slightly reduced compared with those in infected mice ($p>0.05$).

Figure 5 shows that TNF-α and IL-6 levels in the two infected groups dramatically increased from days 3 to 8 post inoculation 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 the infected group and the DEX-treated infected group ($p>0.05$).

Effect of DEX on viral infection of the lungs

The viral titres in the lungs of infected mice increased on days 3–8 post inoculation; the peak virus titre reached 7.8 log_{10} EID50. DEX treatment did not affect the amount of virus recovered from the infected mouse lungs (fig. 6).

DISCUSSION

In the present study, experiments were performed in order to investigate the effect of DEX treatment on the development of ARDS induced by the H5N1 virus in mice. The results showed that the administration of 2.5 $mg \cdot kg^{-1} \cdot day^{-1}$ DEX from days 3 to 14 after infection did not alter the time course of the development of ARDS induced by the H5N1 viral infection. First, it was found that DEX treatment did not alleviate the clinical signs of the H5N1-infected mice, such as altered gait, inactivity, ruffled fur, weight loss and laboured respirations. Both DEX-treated and DEX-untreated H5N1-infected BALB/c mice showed the same time course of clinical signs with similar severity. Secondly, DEX treatment did 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 lung histopathological lesions associated with ARDS, such as the development of alveolar and interstitial oedema, haemorrhage and a significant

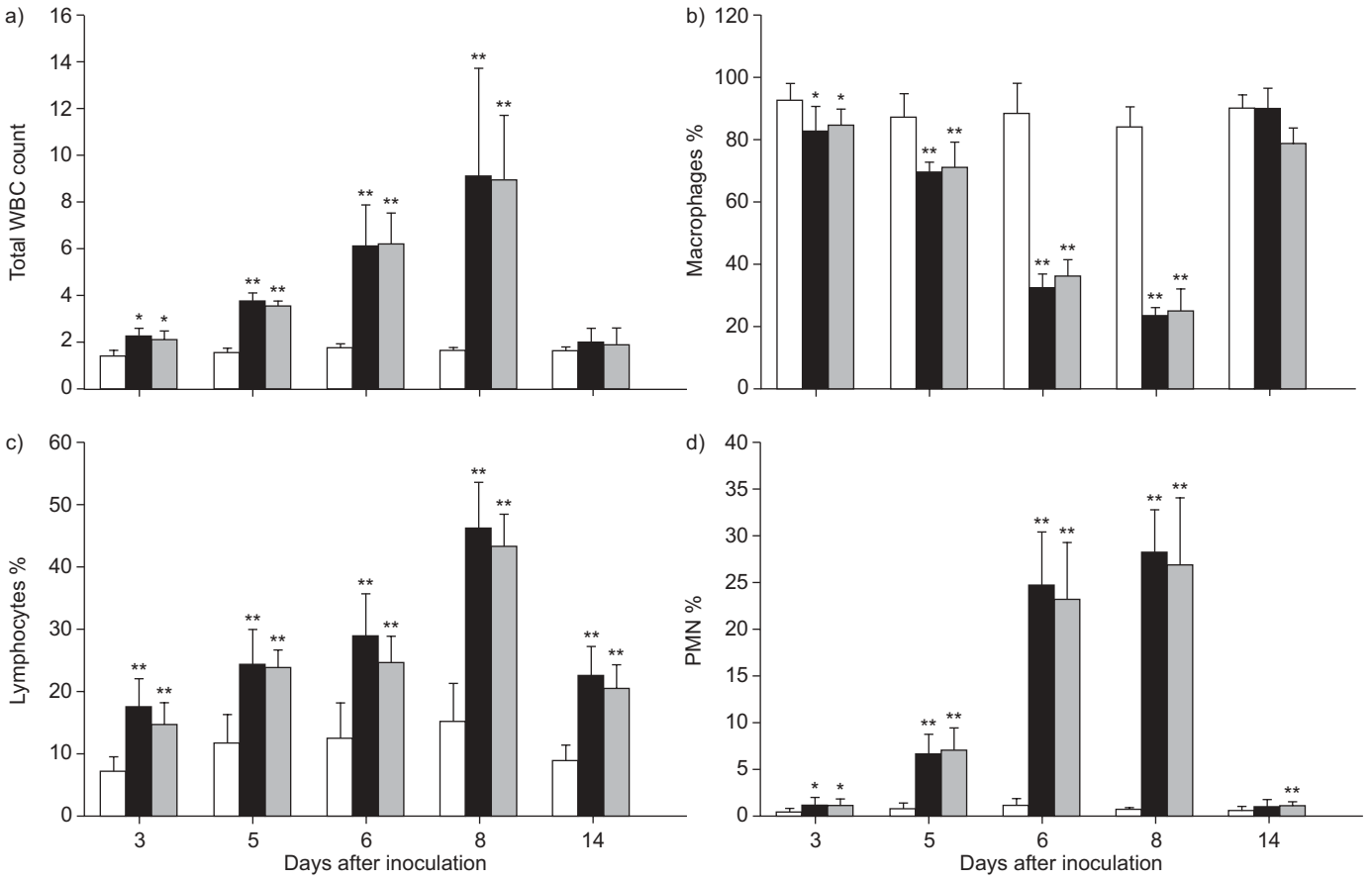

FIGURE 4. Kinetic analysis of a) total white blood cell (WBC) count and differential counts of b) macrophages, c) lymphocytes and d) polymorphonuclear leukocytes (PMNs) in bronchoalveolar lavage fluid after therapy with dexamethasone (DEX). The mean±SD (n=4) at each time point is shown. □: uninfected control mice; ■: DEX-treated infected mice; ■: infected mice. *: $p<0.05$; **: $p<0.01$ compared with uninfected control mice.

margination and infiltration of inflammatory cells, and the formation of severe bronchiolitis. Furthermore, lung oedema and hypoxaemia, 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 virus recovered from infected mouse lungs. Therefore, the present data demonstrate that the administration of DEX at 2.5 $mg \cdot kg^{-1}$ from days 3 to 14 after H5N1 infection does not affect the development and outcome of ARDS induced by the H5N1 virus in mice.

In patients with ARDS confirmed to be caused by the H5N1 viral infection, 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 the current study, a ~30-fold increase in the number of neutrophils and a significant elevation in IL-6 and TNF-α levels in BALF was observed in H5N1 virus-infected mice compared with control mice at day 8 post inoculation, which is consistent with a 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 of the lung, suggested that DEX could not inhibit the development of inflammatory responses associated with ARDS.

To date, ARDS treatments 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 defence 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.* [31] demonstrated that high-dose triamcinolone effectively reduced pulmonary lesions in rats infected with a strain of H3N2 (A/Wuhan/359/95). In contrast, LONDON *et al.* [32] 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. Reports of the use of corticosteroids in human H5N1 influenza treatment are limited, with only a few case reports in the literature. CARTER [33] has reviewed relevant articles, and found that steroid treatment does not appear to alter mortality rates. The current data, consistent with other evidence, could not support a beneficial role of corticosteroids in the management of severe H5N1 infections.

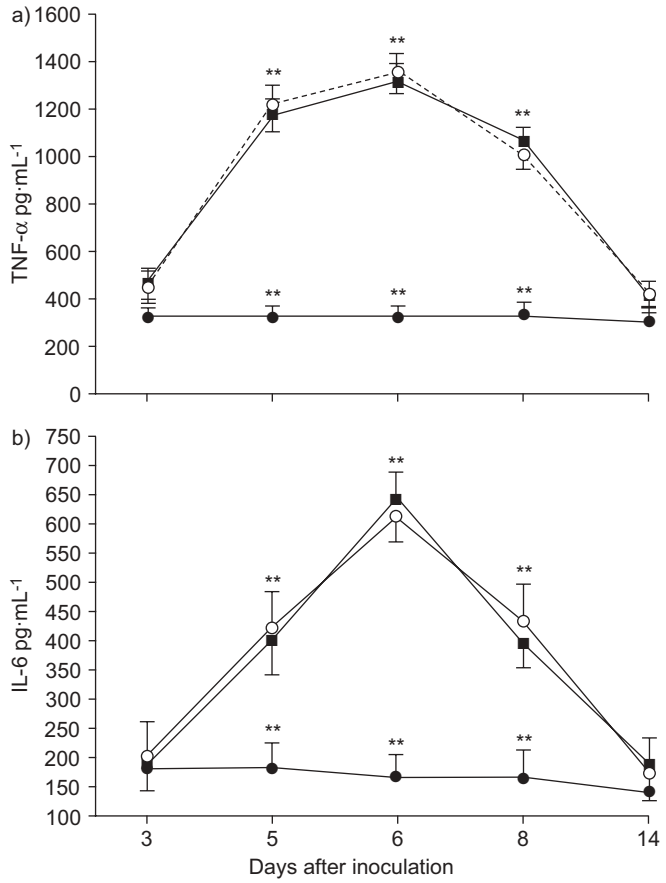

FIGURE 5. a) Tumour necrosis factor (TNF)-α and b) interleukin (IL)-6 levels in bronchoalveolar lavage fluid after therapy with dexamethsone (DEX). The mean±SD (n=4) at each time point is shown. ■: DEX-treated infected mice; ○: infected mice; ●: uninfected control mice. *: $p<0.05$; **: $p<0.01$ compared with uninfected control mice.

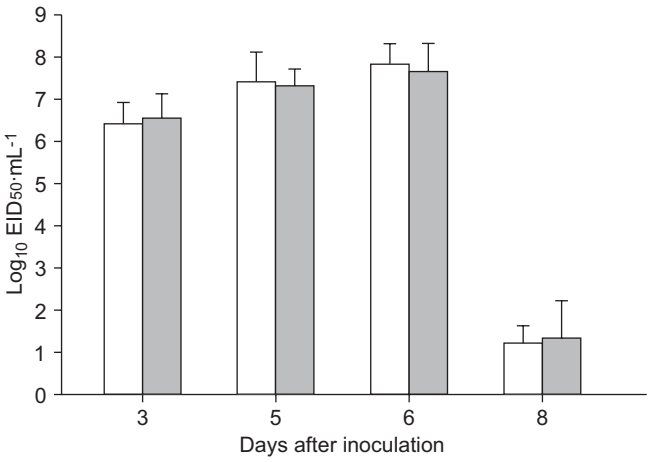

FIGURE 6. Replication of H5N1 viruses in the lungs of mice after therapy with dexamethasone (DEX). Mice were infected with 1×10^2 50% mouse infectious dose of Chicken/HB/108 virus, tissues were collected on different days post inoculation, and the virus was titrated in embryonated eggs. The mean±SD virus titres from three mice per group were expressed as $\log_{10}$ 50% egg infectious dose (EID_{50}) per mL. The limit of virus detection was $10^{1.2}$ EID_{50} per mL for lungs. ■: DEX-treated infected mice; □: infected mice.

Corticosteroid type, administration dose and length of therapy may affect the therapeutic efficacy of corticosteroids in treating ARDS associated with H5N1 infection in mice. It is well known that the glucocorticoid of choice for pulmonary treatment is hydrocortisone (for sepsis-related syndromes) or methylprednisolone (used in most acute lung injury/ARDS studies). The present authors' choice of low-dose DEX for the therapy of H5N1 viral ARDS in mice is based on the following considerations. Low-dose DEX has been used experimentally in ARDS therapy in mice [18, 19]. VAN WOENSEL *et al.* [34] have demonstrated that DEX (0.6 $mg\cdot kg^{-1}\cdot day^{-1}$) had a beneficial effect in patients with bronchiolitis caused by a respiratory syncytial virus infection. Furthermore, low-dose DEX (*e.g.* 0.4 $mg\cdot kg^{-1}$ for 5 days) has been 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 $mg\cdot kg^{-1}\cdot day^{-1}$) 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 post inoculation was carried out in the present authors' laboratories. Other glucocorticoids, as well as various administration routes and schedules (early and delayed therapy), should also be studied in the future.

In summary, the current data show that daily administration of 2.5 $mg\cdot kg^{-1}$ dexamethasone on days 3–14 after infection is not effective at inhibiting the development of acute respiratory distress syndrome associated with H5N1 infection in mice.

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