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Early dexamethasone treatment exacerbates enterovirus 71 infection in mice

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ABSTRACT

Enterovirus 71 (EV71) infection can induce encephalitis. Overt immune responses is suspected to cause severe symptoms, so anti-inflammatory agents, corticosteroids have been recommended for treatment. However, one clinical study reported that treatment with glucocorticoids, dexamethasone (Dex) exacerbates disease severity. Here we investigated Dex treatment on EV71 infection using the murine model and found that both long-term (14-day) and short-term (4-day) Dex treatment starting from 1 or 3 days postinfection increased the mortality and disease severity of infected mice. Dex treatment starting from 4 or 8 days postinfection did not affect mouse mortality and disease severity. Early Dex treatment starting from 1 day postinfection caused atrophy and enhanced apoptosis in lymphoid organs to decrease the numbers of lymphocytes ($CD4^+$ T cells, $CD8^+$ T cells, and $CD19^+$ B cells) and to increase viral loads in infected tissues of mice. Our results demonstrate that Dex treatment has no beneficial effect on EV71 infection.

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Introduction

Enterovirus 71 (EV71) is a member of the family *Picornaviridae*. EV71 infects humans by the fecal-oral route and can induce fever and mild symptoms, such as herpangina or hand-foot-and-mouth disease (Chang et al., 2007; Ho et al., 1999; Huang et al., 1999). It can also infect the central nervous system (CNS) to induce neurological manifestations, including aseptic meningitis, encephalomyelitis, brainstem encephalitis, and acute flaccid paralysis, especially in young children. Brainstem encephalitis combined with pulmonary edema complications often cause death or long-term neurological sequelae (Chang et al., 2007). Widespread and deadly EV71 outbreaks have been reported, particularly in the Asia-Pacific region, which are estimated to have infected millions of children and have caused death and severe neurological sequelae in thousands of children since the last decade (Qiu, 2008; Solomon et al., 2010). As vaccines and antiviral therapies specific for EV71 are not available, EV71 infection is becoming endemic in areas with outbreaks and has massive potential for explosive epidemics.

Currently, there is no effective treatment for EV71-infected patients with fatal symptoms, because the pathogenesis remains elusive. Immature or insufficient immunity has been suspected to associate with increased morbidity and mortality in infected patients, as infants and young children are highly susceptible to fatal infection. The finding of lymphopenia with decreased levels of $CD4^+$ and $CD8^+$ T cells in infected patients with brainstem encephalitis and pulmonary edema supports this notion (Chang et al., 2006; Wang et al., 2003a). However, some clinical studies suggested that elevated cellular immunity with a predominance of lymphocytes may be linked with unfavorable outcomes (Chang et al., 2004; Hsia et al., 2005; Lum et al., 1998; Wang et al., 1999; Yan et al., 2000). In addition, elevated cytokine and chemokine levels are detected in encephalitis patients (Lin et al., 2002, 2003; Wang et al., 2003a, 2006, 2008). Cytokine storm has therefore been proposed to contribute to severe complications in infected patients (Lin et al., 2002, 2003), so anti-inflammatory therapy is recommended for treatment (Lin et al., 2002). Moreover, one

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report indicates that immunopathology may induce neurological diseases and showed that late corticosteroid treatment about three weeks after infection improved the neurological illness (opso-myoclonus syndrome) of one patient (McMinn et al., 2001). Later, another report of patients with pulmonary edema and respiratory failure demonstrated that corticosteroid treatment improves the long-term neurological deficits of three patients (Nolan et al., 2003). Nevertheless, one recent report with 134 cases finds that early use of glucocorticoids, such as dexamethasone (Dex), by rural practitioners to treat fever in outpatients is associated with increased risk of severe disease or death in China during 2008 outbreak (Ma et al., 2010). Steroid treatment is also suspected to link with a significant number of fatal cases in Cambodia during 2012 outbreak (Seiff, 2012). Clinical reports regarding the effect of corticosteroid treatment on EV71 disease progression remains unclear. The present study was therefore designed to examine the effect of Dex treatment in vivo because of the paucity of reports on this issue. Using a murine infection model, our results showed that early Dex treatment increases EV71 lethality in mice, which may explain the increased mortality of infected patients in China during 2008 outbreak and in Cambodia during 2012 outbreak (Ma et al., 2010; Seiff, 2012).

Results

Early Dex treatment increases the mortality and tissue viral loads of EV71-infected mice

It has been reported that the rural practitioners in village clinics of China prescribed Dex to treat fever in child outpatients during EV71 outbreak (Ma et al., 2010). We therefore tested Dex treatment on infected mice that developed fever. Virus infection induces viremia or interferon, which can induce fever. As it is difficult to measure temperature in mice, we used the presence of viremia or interferon as the indication of fever in infected mice. In the present study, 14-day-old C57BL/6J mice were infected with 8×10^4 PFU/mouse of EV71. Infectious virus was detected in the mouse blood with 4×10^3, 1×10^3, and 2×10^2 PFU/ml at days 1, 4, and 6 postinfection (p.i.), respectively. Additionally, our previous report detected interferon-α in sera of infected mice from 10 min to 3 days p.i. (Liu et al., 2005). Thus, we started Dex treatment from day 1 p.i. A previous study showed that the effects of Dex on herpes simplex virus-infected mice were depended on the timing of the treatment (Sergerie et al., 2007). Dex treatment starting from day 1 p.i. increased the mortality of infected mice, while Dex treatment starting from day 3 p.i. reduced the mortality of infected mice. This prompted us to treat mice with Dex (6.5 mg/kg/day in one shot) at different time points after infection (from days 1, 3, 4, or 8 to 14 p.i.) or with phosphate-buffered saline (PBS) from days 1 to 14 p.i. (Fig. 1A). There were eight to nice mice in each group. The survival rates of infected mice treated with Dex starting from days 4 or 8 p.i. or with PBS were comparable and about 90% (Fig. 1B). Infected mice treated with Dex starting from day 3 p.i. and, especially from day 1 p.i. displayed signs of encephalitis as manifested by hunched posture, lethargy, hind limb paralysis, and ataxia around days 5 to 6 p.i. (Fig. 1C) with final survival rates of 50% and 12%, respectively, by day 30 p.i. (Fig. 1B). Dex treatment starting from day 1 p.i. significantly increased the mortality and disease severity of infected mice when compared with PBS treatment ($p<0.05$). In separate experiments, tissues of infected mice treated with PBS or Dex from day 1 p.i. ($n=3$ to 6 samples per time point in each group) were harvested to determine viral titers (Fig. 1D). Viral titers in peripheral organs (heart, lung, liver, intestine, kidney, spleen, and thymus), CNS tissues (brain without the brainstem region, brain stem, and spinal cord), and blood of Dex-treated mice were higher than those of PBS-treated mice almost at all the time points (2, 4, 6, or 8 days p.i.) examined.

Short-term Dex treatment increases the mortality and tissue viral loads of EV71-infected mice

Short-term (three- to four-day) Dex treatment was prescribed by the rural practitioners in village clinics of China to treat fever in child outpatients (Ma et al., 2010). We therefore tested the effect of short-term Dex treatment by giving infected mice PBS ($n=9$) or Dex ($n=8$) for 3 days from days 1 to 4 p.i. (Fig. 2A). All infected mice treated with Dex succumbed to death within 14 days p.i. (Fig. 2B) with a final survival rate significantly lower than that of infected mice treated with PBS by 78% ($p<0.001$). The disease scores of infected mice treated with Dex were also significantly higher than those of infected mice treated with PBS (Fig. 2C; $p<0.05$). Viral titers in peripheral organs (heart, lung, liver, intestine, kidney, and spleen) and CNS tissues (brain without the brainstem region, brain stem, and spinal cord) of Dex-treated mice were all higher than those of PBS-treated mice at days 6 and 8 p.i. (Fig. 2D; $n=3$ samples per time point in each group).

Early Dex treatment increases the mortality of severely infected mice

Dex treatment has been recommended for patients suffering from severe symptoms, brainstem encephalitis and pulmonary edema with a survival rate of less than 30% (Chang et al., 1999; Ho et al., 1999). To determine the effect of Dex treatment on severely infected mice with a low survival rate, we infected mice with a high viral dose of 6×10^5 PFU/mouse. Infected mice were treated with Dex from days 1 or 4 to 14 p.i. or with PBS from days 1 to 14 p.i. (Fig. 3A). There were eight, ten, and nine mice in each group, respectively. The survival rates of infected mice treated with Dex from day 4 p.i. or with PBS were comparable and about 30% (Fig. 3B). All infected mice treated with Dex from day 1 p.i. succumbed to death within 10 days p.i., with a survival rate significantly lower than that of infected mice treated with PBS ($p<0.001$).

Dex treatment causes atrophy in lymphoid organs and reduces lymphocyte numbers in infected organs

Previous studies showed that Dex reduced T and B cells (Ashwell et al., 2000; Merino et al., 1994). In the present study, we observed that the sizes of thymus and spleen were reduced in Dex-treated mice. Therefore, we examined the effect of Dex treatment starting from day 1 p.i. on the lymphoid organs of mock-infected and infected mice. The thymus weights of PBS-treated mice infected with or without EV71 were constant and comparable from days 2 to 6 p.i. (at the age of 16–20 days old) (Fig. 4A). Dex treatment significantly decreased the thymus weights of mock-infected and infected mice to a similar degree from days 2 to 6 p.i. when compared with PBS treatment ($p<0.05$). The spleen weights of mock-infected mice treated with PBS continued to increase from days 2 to 6 p.i. (Fig. 4B). EV71 infection did not affect the spleen weights from days 2 to 4 p.i., but slightly decreased the weights at day 6 p.i. ($p>0.05$). Dex treatment significantly decreased the spleen weights of mock-infected and infected mice to a similar degree at days 4 and 6 p.i. when compared with PBS treatment ($p<0.001$).

Our previous report found that all three types of lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells, and B cells are important in protecting mice from EV71 infection, as mice deficient in any of these cells displayed high mortality rates and tissue viral loads (Lin et al., 2009; Wang et al., 2012). Because the spleen has all three types of lymphocytes, we next determined the lymphocyte types and levels in the mouse spleen affected by Dex treatment. Spleens of

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