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EXPERIMENTAL STUDY

Combination of ribavirin and reduning protects mice against severe pneumonia induced by H1N1 influenza a virus

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Abstract

OBJECTIVE: To investigate the effects of ribavirin administration combined with Reduning in a mouse model of influenza A (H1N1)-induced severe pneumonia.

METHODS: Influenza A/Beijing/501/2009 (H1N1)-infected C57BL/6 mice were randomly divided into four experimental groups treated with either a mock injection of phosphate-buffered saline (PBS), ribavirin (66.6 mg/kg daily) or Reduning (86.6 mg/kg daily), or a combination of both, for 7 days. Mice were monitored for clinical signs and survival, and body weight was measured daily for 14 days. Virus titer, lung wet-to-dry ratios, pathology and cytokines including interleukin (IL)-6, IL-10, and interferon (IFN)-γ were assayed on different days.

RESULTS: In the untreated group injected with phosphate buffer saline, all the mice died of the infection. The survival rate of mice treated with Reduning was only 10%, whereas 100% of the ribavirin- and the combination-treated mice survived. Low lung viral loads indicated that ribavirin significantly inhibited virus replication, whereas Reduning did not. Lung wet-to-dry ratios demonstrated that both ribavirin and Reduning, administered together or separately, reduced acute lung edema compared with results in the untreated group. Pathology analyses also showed that treatment with a combination of both drugs relieved pathological lesions, whereas the single drug treatment did not. Levels of IL-6, IL-10 and IFN-γ in mice treated with ribavirin or the combination of both ribavirin and Reduning were all significantly lower than in the untreated group, especially in the combination-treated group. In addition, Reduning administration significantly decreased both IL-6 and IL-10 production but had no effect on IFN-γ.

CONCLUSION: Due to the synergistic effect of antiviral and antiinflammation, the combination of ribavirin and Reduning could be an effective treatment for severe H1N1 which was considered to be significant to delayed antiviral and drug resistant.

Key words: Influenza, Human; Pneumonia; Ribavirin; Medicine, Chinese Traditional; Reduning injection

INTRODUCTION

In April 2009, H1N1 swine flu first emerged in Mexico.[1] On August 10th 2010, the World Health Organization declared influenza A H1N1 (pH1N1) a pandemic. During this period, 214 countries reported confirmed cases of influenza A H1N1; and the disease resulted in 18 449 deaths. Mainland China alone reported a total of 12.8 million cases with 805 deaths and a 0.5% mortality rate. Some severely infected patients developed acute pneumonia, which was the predominant cause of reported deaths. Although the mortality caused by pH1N1 infection was low, 9%-31% of pH1N1 patients required admission to intensive care units, and 14%-46% of these patients ultimately died.[2-5] To this day, the main treatment strategy for severe pneumonia relies on different types of adjunctive therapies, although those treatments are controversial. The clinical treatment of pH1N1 mainly depends on neuraminidase inhibitors such as oseltamivir, and antiviral compounds such as ribavirin. However, antiviral therapy usually involves a delayed response or has low efficacy.[6] Systemic corticosteroids are often used to treat severe influenza, although several studies have indicated that this may increase the risk of mortality.[7]

Chinese medicine has been widely used in the treatment of pH1N1. Some drugs such as Shufengjiedu capsules and Maxingshigan decoctions have shown effectiveness.[8] However, there have been few studies reporting the effects of Chinese medicine on severe influenza. Reduning, one clearing heat and detoxifying Traditional Chinese Medicine (TCM) injection, was maily used for treatment of acute and severe disease in clinical. Many studies had reported that Reduning could reduce the duration of influenza illness which was attributed to the role in inhibiting NA and anti-inflamation. Compared with antiviral injections, formulations of capsules, decoctions, or granules alone have been unable to provide immediate relief for severe influenza in clinical practice. This study investigated the efficacy of antiviral administration combined with injections of the Chinese medicine Reduning for the treatment of severe influenza.

MATERIALS AND METHODS

Virus, cells and animals

Influenza A/Beijing/501/2009 (H1N1) (BJ501) and Madin Darby Canine Kidney cells were obtained from the Beijing Institute of Microbiology and Epidemiology, State Key Laboratory of Pathogen and Biosecurity. Specific pathogen free (SPF) C57BL/6 female mice, 4-6 weeks old (Experimental Animal Production License No. SCXK 2012-003), weighing 14-16 g, were provided by the Laboratory Animal Center of the Academy of Military Medical Science (Beijing, China).

Drugs and reagents

Ribavirin Injection (Batch No. 1003167, 0.1 g /2 mL) was obtained from the Shandong Lu Kang Chen Xin Pharmaceutical Co, Ltd. (Shandong, China). Reduning (Batch No. 1009022, 6 g /10 mL) was purchased from the Kang Yuan Pharmaceutical Co, Ltd. (Hunan, China). The mouse inflammatory cytokines cytometric bead array was obtained from BD Biosciences (Franklin Lakes, NJ, USA).

Therapeutic efficacy of different drugs or combination against pH1N1 in mice

To evaluate the therapeutic role of ribavirin, Reduning or both against BJ501 infection, following intraperitoneal (i.p.) anesthetization with pentobarbital sodium, C57BL/6 mice received 2×10^5 $TCID_{50}$ of BJ501 intranasally (i.n.) on day 0 as shown previously [9-10], followed by i.p. injections of Reduning (86.6 $mg \cdot kg^{-1} \cdot d^{-1}$), ribavirin (66.6 $mg \cdot kg^{-1} \cdot d^{-1}$), a combination of both, or a mock injection phosphate buffer saline (PBS) once a day for 7 days post infection (in order to achieving theraputic drug concentration once infection, each group were administered 12 h before infection. Mice in each group (n = 10) were monitored daily for survival, clinical signs and body weight. Mice from each group were euthanized on different days, and the lungs were collected as described below.

The viral load in the lung tissue of mice

Lung tissues from five mice selected from each group were collected at 4 days post-infection (DPI). The titers of viruses in the lung tissue were determined by the cell culture infective dose 50% (CCID50) assay in Madin Darby canine kidney cells.[9,10]

Acute pulmonary edema (wet-to-dry ratio)

Assessment of acute pulmonary edema was performed at 4 DPI by calculating the lungs' wet-to-dry ratios from the weights of wet lungs and the dry weight obtained after heating the tissues at 68 ℃ for 24 h.

Histological examination

Following pentobarbital sodium anesthesia, two to four mice from each group were sacrificed at 5 DPI. Lungs were fixed in formalin and embedded in paraffin. Ultrathin sections were obtained and stained with hematoxylin-eosin. Lung histopathology was determined by light microscopy (Olympus, Japan).

Cytokine and chemokine measurement

For cytokine measurements, five mice from each group were euthanized and bronchoalveolar lavage fluids (BALFs) were collected at 2 and 4 DPI. BALFs were assessed for interleukin (IL)-6, IL-10 and interferon (IFN)-γ using the cytometric bead array. Array analysis was performed using FCAP Array software.