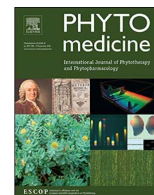
Phytomedicine 34 (2017) 127–135

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed



Original Article

Intranasal co-administration of 1,8-cineole with influenza vaccine provide cross-protection against influenza virus infection

Yun Li[a], Yu-ling Xu[b], Yan-ni Lai[a], Shang-hui Liao[a], Ni Liu[a], Pei-ping Xu[a,*]

[a] Institute of Tropical Medicine, Guangzhou University of Chinese Medicine, 12 Ji chang Rd., San Yuan li St., Bai Yun Dist., Guangzhou, PR China
[b] Guangzhou Health Vocational and Technical College, Guangzhou, PR China

ARTICLE INFO

Keywords:
1,8-cineole
Adjuvant
Influenza virus vaccine
Cross-protection
Mice

ABSTRACT

Background: Vaccination is the most efficient means for protection against influenza. However, the various vaccines have low efficacy to protect against pandemic strains because of antigenic drift and recombination of influenza virus. Adjuvant therapy is one of the attempts to improve influenza vaccine effective cross-protection against influenza virus infection. Our previous study confirmed that 1,8-cineole inhibits the NF-κB, reduces pro-inflammatory cytokines, and relieves the pathological changes of viral pneumonia in mice infected with influenza virus.

Hypothesis/Purpose: 1,8-cineole, administered via intranasal (i.n.) route, may also have the capacity to be an adjuvant of the influenza vaccine. This study was designed to investigate the potential use of i.n. co-administration of 1,8-cineole, a major component of the *Eucalyptus* essential oils, with influenza vaccine and whether could provide cross-protection against influenza virus infection in a mouse model.

Study design: I.n. co-administration of 1,8-cineole in two doses (6.25 and 12.5 mg/kg) with influenza vaccine was investigated in a mouse model in order to see whether it could provide cross-protection against influenza virus infection.

Methods: The mice were intranasally immunized three times at the 0, 7 and 14 day with vaccine containing 0.2 µg hemagglutinin (HA) and/or without 1,8-cineole. Seven days after the 3rd immunization dose, the mice were infected with 50 µl of 15 LD_{50} (50% mouse lethal dose) influenza virus A/FM/1/47 (H1N1). On day 6 post-infection, 10 mice *per* group were sacrificed to collect samples, to take the body weight and lung, and detect the viral load, pathological changes in the lungs and antibody, etc. The collected samples included blood serum and nasal lavage fluids. In addition, the survival experiments were carried out to investigate the survival of mice.

Results: Mice i.n. inoculated with influenza vaccine and 12.5 mg/kg 1,8-cineole increased the production of influenza-specific serum immunoglobulin (Ig) G2a antibodies, stimulated mucosal secretive IgA (s-IgA) responses at the nasal cavity, improved the expression of respiratory tract intraepithelial lymphocytes (IELs) in the upper respiratory tract, and promoted dendritic cell (DC) maturation and the expression of co-stimulatory molecules cluster of differentiation (CD)40, CD80 and CD86 in peripheral blood. Importantly, mice that had received 1,8-cineole-supplemented influenza vaccine showed longer survival time, milder inflammation, less weight loss and mortality rate and lower lung index and viral titers compared to that of mice immunized a non-1,8-cineole-adjuvanted split vaccine. Thus, i.n. immunization with 1,8-cineole-adjuvanted vaccine induces a superior cross-protective immunity against infection with influenza than an inactivated vaccine only.

Conclusion: These results suggest that 1,8-cineole (12.5 mg/kg) has a cross-protection against influenza virus, co-administered with inactivated influenza viral antigen in a mouse model.

Abbreviations: IFV, influenza virus; i.n., intranasal; IELs, intraepithelial lymphocytes; Ig, immunoglobulin; DC, dendritic cell; CT, cholera toxin; LT, heat-labile toxin; NF-κB, nuclear factor-kappa B; HPLC, high performance liquid chromatography; NC, normal control; IFV, influenza virus; IFV-C, IFV control group; HA, hemagglutinin; LD_{50}, 50% mouse lethal does; HE, hematoxylin and eosin; qRT-PCR, real-time RT-PCR; s-IgA, secretive IgA; OD, optical density; PBMC, peripheral blood mononuclear cells; CD, cluster of differentiation; CIA, corrected immune positive values; ELISA, enzyme linked immunosorbent assay; NK, natural killer; TLR, toll-like receptor

* Corresponding author.
E-mail address: xupeiping@gzucm.edu.cn (P.-p. Xu).

http://dx.doi.org/10.1016/j.phymed.2017.08.014
Received 29 January 2017; Received in revised form 5 July 2017; Accepted 15 August 2017
0944-7113/ © 2017 Elsevier GmbH. All rights reserved.

Introduction

Vaccination is the most efficient means for protection against the influenza. Influenza vaccines included inactivated vaccine and subunit vaccine in use, which are usually administered *via* subcutaneous (s.c.) inoculation (Treanor, 2015). However, the various vaccines show low effectiveness to protect against pandemic influenza virus strains. Antigenic drift and recombination have major impact on the vaccine effectiveness, especially when an outbreak occurs (Ludwig, 2009). Inactivated viral vaccines, for example, are often effective in fighting infections caused by homologous virus types, but have no effect on different subtypes of the virus. Natural infection usually induces cross protection against different drift variants and of different subtypes, so it is the hotspot to study a vaccine that can induce protection against different influenza viruses (Tamura et al., 1992). Some studies have shown that the method for providing the highest cross protection against infection of various virus is to inoculate the mice with i.n. immunization rather than with subcutaneous immunization. Importantly, the use of an adjuvant is one of the attempts to improve influenza vaccine cross-protection against virus infection, e.g. cholera toxin (CT) (e.g. CT B subunits) or the *Escherichia coli* heat-labile toxin (LT) (e.g. LT B subunits) (Tamura et al., 1992), but the use of them in humans are excluded because of their high toxicity.

1,8-Cineole, also known as eucalyptol, is a monoterpene oxide (Fig. 1) that is the principal component of *Eucalyptus* essential oils (49.07 to 83.59%) (Sebei et al., 2015) and is present in many other essential oils (Cimanga et al., 2002). It is used for analgesic and antipyretic remedies for respiratory ailments such as cold, flu, and sinus congestion (Silva et al., 2003) and has demonstrated anti-inflammatory activities (Juergens et al., 2004), including inhibition of nuclear factor-kappa B (NF-κB), a main regulator of cytokine production (Barnes and Karin, 1997). Our previous study confirmed that it inhibits the NF-κB, reduces pro-inflammatory cytokines, and relieves the pathological changes of viral pneumonia in mice infected with influenza virus (IFV) (Li et al., 2016). Since 1,8-cineole is commonly used to treat respiratory diseases via inhalation route (Mondoulet et al., 2012), we hypothesized that 1,8-cineole, administered via i.n. route, may also have the capacity of potentiate the influenza vaccine. In the present study, we investigated the potential use of i.n. co-administration of 1,8-cineole with influenza vaccine and whether could provide cross-protection against influenza virus infection in a mouse model.

Materials and methods

Virus and vaccine

Influenza virus hemagglutinin (HA) vaccine (Split Virion, inactivated) of strain A/California/7/2009 (H1N1) and A/Swiss/9715293/2013 (N3H2) was purchased from Sanofi Pasteur Co. (Shenzhen, China) and Sino Biological Inc. (Beijing, China). Influenza virus strain A/Font Monmouth/47(H1N1, FM1) was acquired from the Chinese Center for Disease Control and Prevention (Beijing, China). The virus was propagated in the embryonated chicken eggs (Li et al., 2016).

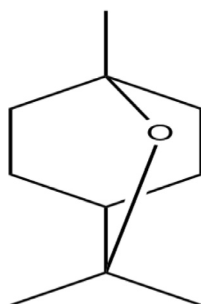


Fig. 1. Structure of 1, 8-cineole.

Adjuvant

1,8-Cineole with purity of ≥98% determined by high performance liquid chromatography (HPLC), was provided by Yuanye Bio-Technology Co. (Shanghai, China) and diluted with sterile saline (suspended in 0.5% Tween 80) to the desired concentration.

Animals

We used specific-pathogen-free, 8-week-old female BALB/c mice (Medical Experience Center of Guangdong Province, Guangzhou, China). The study was approved by the Animal Care and Use Committee of the Guangzhou University of Chinese Medicine (Guangzhou, China, 2015, No. 41). The mice were killed by anesthesia when they developed signs of severe pneumonia, such as extreme thinness (body weight ≤ 11 g).

Immunization and infection

180 mice were divided in 6 groups: normal control (NC, uninfected and unimmunized), IFV control group (+ sterile saline, suspended in 0.5% Tween 80, IFV-C, infected and unimmunized), vaccine control group (vaccine, infected and immunized), 1,8-cineole 12.5 mg/kg group (infected and unimmunized), and two groups of 1,8-cineole + vaccine group (vaccine + 1,8-cineole 6.25 mg/kg and 12.5 mg/kg, infected and immunized). The mice were intranasally immunized three times at 0, 7 and 14 days with vaccine containing 0.2 μg HA and/or without 1,8-cineole in a total volume of 50 μl. Vaccine and 1,8-cineole were admixed just before the immunization (total volume 10 μl per dose). Seven days after the 3rd immunization dose, the mice were challenged with 50 μl of 15 LD_{50} (50% mouse lethal dose) influenza virus A/FM/1/47(H1N1). On day 6 post-infection, 10 mice *per* group were sacrificed to collect related samples, to analyze the body and lung weights and to detect the viral load, pathological changes in the lungs and antibodies, among others. The collected samples included blood for serum and nasal lavage fluids done with 1 ml PBS.

Survival experiments

Seven days after the 3rd immunization dose, the mice were infected with live influenza virus A/FM/1/47(H1N1) (50 μl for *per* nostril, 5 LD_{50}). The animals were followed for 15 days after the infection, by measuring the weight. In addition, survival and clinical symptoms of illness (e.g. ruffled fur, inactivity, and poor appetite) after infection were monitored.

Lung index

The severity of pulmonary edema was assessed by lung index. Mouse body weight was measured, and the lungs were removed and washed with sterile saline. The lung index was calculated following lung weight/body weight ratio multiplied by 100 (Li et al., 2016).

Lung histopathology

Lungs were removed and fixed by 4% paraformaldehyde. Paraffin-embedded fixed sections (5 μm) of lung tissue were stained with hematoxylin and eosin (HE). Slides were read blindly and examined by microscopy for tissue damage such as necrosis and inflammatory cellular infiltration. The histopathology scores were assigned 0 (normal) to 5 (severe) based on the following five criteria: presence of necrotic bronchial and bronchiolar epithelium; serocellular exudates in the bronchial and bronchiolar lumina; inflammatory cells (predominantly neutrophils and lymphocytes) in the bronchiolar, peribronchiolar and

Download English Version:

https://daneshyari.com/en/article/5549315

Download Persian Version:

https://daneshyari.com/article/5549315

Daneshyari.com