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A novel pentapeptide originated from calf thymus named TIPP shows an inhibitory effect on lung allergic inflammation

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ABSTRACT

Thymic immunosuppressive pentapeptide (TIPP) is a novel pentapeptide originally obtained from calf thymic immunosuppressive extract. In this study we aimed to investigate the anti-inflammatory effect and mechanisms of TIPP *in vivo* with an ovalbumin-induced mouse allergic asthma model. We investigated the effects of TIPP on the infiltration of inflammation cells, immune cell subtypes, Th2 cytokines in BALF and IgE in serum, mRNA levels of IL-4, IL-10, TNF-α and eotaxin-1, expression of MCP-1, VCAM-1 and COX-2, and activation of MAP kinases and NF-κB. Our results showed that TIPP significantly inhibited the increase in Th2 cytokines and OVA-specific IgE production, mRNA levels of IL-4, TNF-α and eotaxin-1 and the expression of MCP-1, VCAM-1 and COX-2 in lung tissues, as well effectively resisting the balance changes of cells in BALF. In addition, it was found that the administration of TIPP attenuated the activation of MAP kinases and NF-κB in the lung tissues of the allergic mice. Our data suggest that TIPP effectively suppresses the allergic and inflammatory responses in allergic mice *via* blocking MAP kinases/NF-κB signalling pathway. The investigation indicated that TIPP may become an anti-allergic and anti-inflammatory drug.

1. Introduction

Asthma, an allergic inflammatory disease, is prevalent worldwide both in adults and children [1,2]. Airway inflammation, resulting from interactions between T lymphocytes, B lymphocytes, mast cells, granulocytes, dendritic cells and macrophages, is fundamental to asthma pathogenesis [3,4]. A series of cytokines and chemokines are involved in this disorder process. Th2 cytokines (IL-4, 5, 13) are thought to be in a dominant position. IL-4 and IL-13 are crucial for IgE production by B cells and mucus hypersecretion. In addition, they can induce the expression of macrophage chemoattractant proteins (MCPs), eotaxins and vascular cell adhesion molecule 1 (VCAM-1), which promote the infiltration of inflammatory cells into lung tissues [5,6]. IL-5 plays an essential role in eosinophil differentiation and accumulation, and enhances eosinophil cytotoxicity and the release of pro-inflammatory mediators [7]. Tumour necrosis factor-α (TNF-α), a pleiotropic inflammatory cytokine secreted from T cells and mast cells, presents higher levels in bronchoalveolar fluid from asthma patients [8,9]. As an immunosuppressive cytokine, IL-10, along with its secreting cells, $CD4^+CD25^+$ regulatory cells, is reported to play an inhibitory effect in pulmonary inflammation and asthma [10].

The transcription factor, nuclear factor-κB (NF-κB), which exists in almost all cell types, plays an essential role in immunity and inflammation. Evidence has shown that there was a close connection between NF-κB activation and allergic asthma [11,12]. The activation of NF-κB induces the expression of cytokines (*e.g.*, IL-4, IL-5, and TNF-α), chemokines (*e.g.*, eotaxin and MCP-1), VCAM-1 and cyclooxygenase-2 (COX-2), which facilitates the development of asthma [13]. Therefore, a lot of studies have focused on the NF-κB signalling pathway in asthma, and the results have demonstrated that this pathway is a target for the treatment of asthma.

In the 1970s, thymic extracts containing non-cytotoxic-specific inhibitors termed as 'thymic chalones' were isolated from the thymus of animals [14–16]. Experimental results showed that these crude extracts had a negative regulating function in the synthesis of DNA and the proliferation of lymphocytes [17,18]. Our lab began to study thymic immunosuppressive extract (TISE) in the 1980s and developed a new method for TISE preparation. Our prior research has shown that TISE inhibits the immune and allergic responses effectively both *in vitro* and *in vivo* [19–21]. For further investigation of the immunosuppressive activity and mechanism, low molecular weight immunosuppressive factors

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derived from calf thymus were fractionated with micrococcal nuclease digestion, acid hydrolysis and RP-HPLC separation, through tracking the immunosuppressive activity. After further isolation and purification, a novel pentapeptide with the sequence of Ala-Glu-Trp-Cys-Pro was obtained from TISE. We found that this pentapeptide had an inhibitory effect on splenocyte proliferation caused by Con A stimulation and named it thymic immunosuppressive pentapeptide (TIPP, Fig. 1). In view of the apparent effects of TISE against allergic responses, we speculated that TIPP may have therapeutic actions in allergic inflammation. In order to confirm our speculation, we investigated the anti-inflammatory effects and molecular mechanism of TIPP on lung allergic inflammation in an ovalbumin (OVA)-induced mouse model of allergic asthma.

2. Materials and methods

2.1. Materials

TIPP (>95% purity, endotoxin free) was synthesised by ChinaPeptides Co., Ltd. (Shanghai, China). RPMI 1640 Medium and foetal bovine serum (FBS) were purchased from Gibco (Paisley, UK). Ovalbumin (OVA, Grade V), concanavalin A (Con A), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), lipopolysaccharides (LPS) and dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, USA). Alu-Gel-S suspension was provided by SERVA Electrophoresis GmbH (Heidelberg, Germany). Enzyme-linked

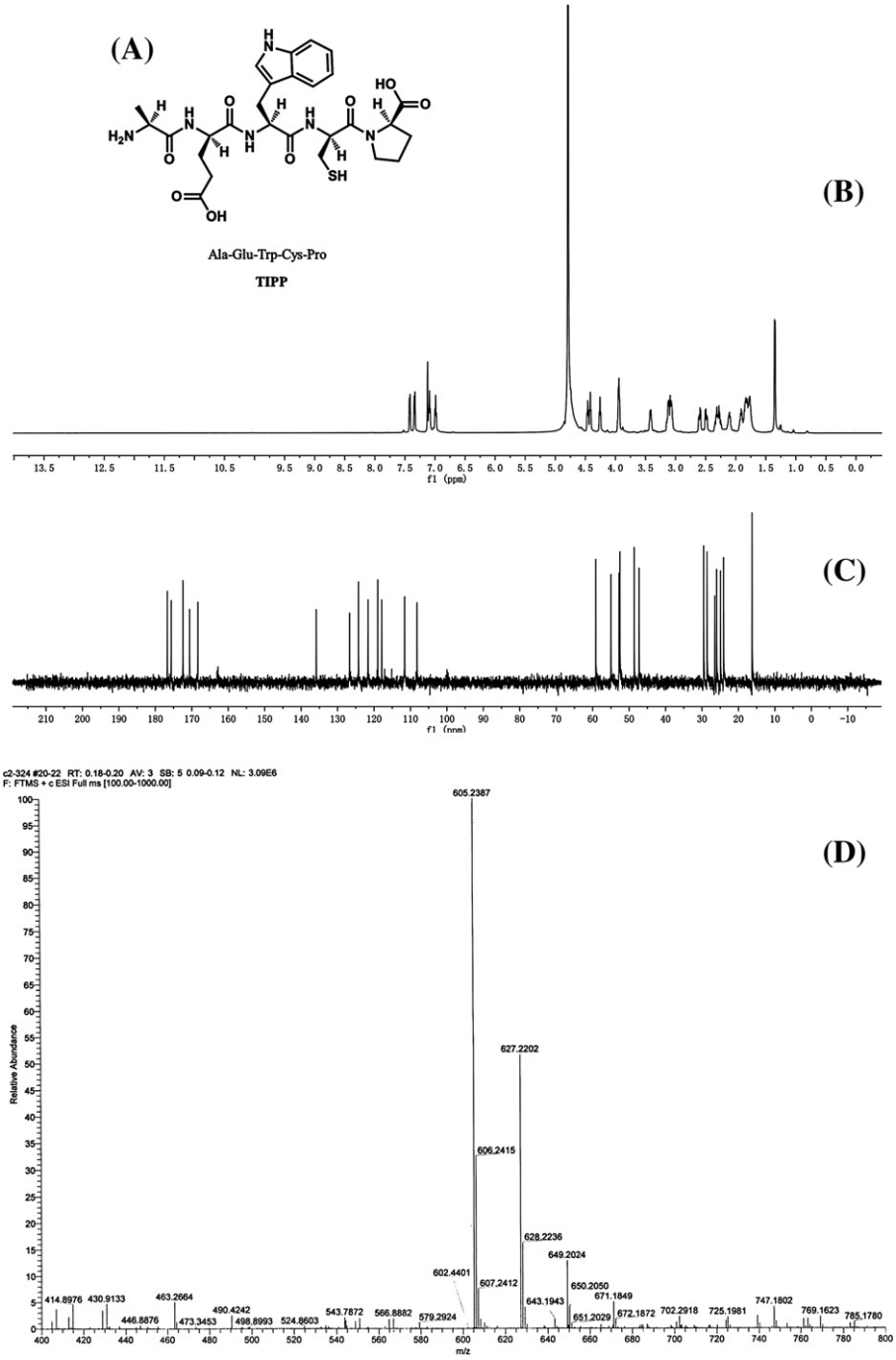

Fig. 1. (A) Chemical structure of TIPP; (B) 600 MHz ^{1}H NMR spectra of TIPP in D_2O; (C) 150 MHz ^{13}C NMR spectra of TIPP in D_2O; (D) HRMS spectra of TIPP.