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Effect of matrine on the expression of substance P receptor and inflammatory cytokines production in human skin keratinocytes and fibroblasts

Ji-Yong Liu, Jin-Hong Hu *, Quan-Gang Zhu, Feng-Qian Li, Jing Wang, Hua-Jun Sun

Department of Pharmacy, Changhai Hospital, Second Military Medical University, 174 Changhai Road, Shanghai 200433, China

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Abstract

Matrine is a kind of alkaloid found in certain *Sophora* plants, which has been extensively used in China for the treatment of viral hepatitis, cancer, cardiac diseases and skin diseases (such as atopic dermatitis and eczema). It also has been confirmed that substance P (SP) and its receptor (neurokinin-1 receptor, NK-1R) are involved in the pathogenesis of inflammatory skin disorders. So the present study was designed to investigate the effect of matrine on the expression of NK-1R and cytokines production induced by SP in HaCaT cells (a human epidermal keratinocyte cell line) and dermal fibroblasts. In addition, cell viability was also evaluated. The results showed that matrine inhibited the expression of NK-1R in HaCaT cells and fibroblasts. SP induced the production of interleukin (IL)-1β, IL-8, interferon (IFN)-γ, and monocyte chemotactic protein (MCP)-1 in both cell types. Matrine 5–100 μg/mL had little effect on cell viability. It inhibited SP-induced IL-1β, IL-8 and MCP-1 production in HaCaT cells and fibroblasts, while it increased the production of IFN-γ in HaCaT cells. Both SP and matrine had no effect on the secretion of IL-6. These findings suggest that matrine may have potential treatment function on SP related cutaneous inflammation by inhibition of the expression of substance P receptor and regulation of the production of inflammatory cytokines.

Keywords: Matrine; Substance P receptor; Cytokines; HaCaT cell line; Fibroblast; Cutaneous inflammation

1. Introduction

Recently, it has been well established that a dynamic interaction between the skin and the nervous system plays an important role in both skin homeostasis and cutaneous disease. Neuropeptides released from the network of nerve fibers in the skin can interact with specific receptors on cutaneous cells including epidermal keratinocytes, Langerhans cells, dermal microvascular endothelial cells, fibroblasts, and mast cells to mediate a cascade of inflammatory and proliferative activities [1,2]. Substance P (SP) is a member of tachykinin peptide family and is widely distributed in the central and peripheral nervous system. It has been proved to be involved in the pathogenesis of inflammatory skin disorders such as atopic dermatitis and psoriasis [3–5]. SP modulates important cellular

* Corresponding author. Tel.: +86 21 2507 0665; fax: +86 21 2507 0668.

E-mail addresses: liujiyong999@yahoo.com.cn (J.-Y. Liu), hujh@smmu.edu.cn (J.-H. Hu).

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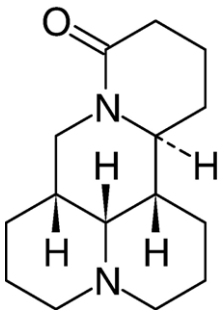

Fig. 1. Chemical structure of matrine ($C_{15}H_{24}N_2O$, MW=248.37).

functions in various cells by preferentially activating a specific receptor, the SP receptor (SPR) (also designated neurokinin-1 receptor, NK-1R). The NK-1R is a G-protein-coupled receptor for bearing a seven-transmembrane domain, which has been shown to associate with different Gα protein isotypes [6]. The expression of NK-1R is found in T cells, B cells [7], monocytes/macrophages [8], and mast cells [9]. Marriott and Bost [10] investigated the gene expression of NK-1R in murine and human dendritic cells. Lai et al. [11] have reported the expression of SP and its receptor in human lymphocytes. Our previous study demonstrated the expression of NK-1R in human epidermal HaCaT cell and dermal fibroblasts [12].

Matrine is the major quinolizidine alkaloids from the root of *Sophora* plants including *Sophora flavescens*, *Sophora alopecuroides* and *Sophora subprotrata* (chemical structure shown in Fig. 1). It has a wide range of pharmacological actions, such as anti-inflammatory [13,14], analgesic [15], antiarrhythmic [16], antitumor [17], antidiarrhea [18] and immunosuppressive effects [19]. It has been extensively used in China for the treatment of viral hepatitis, cancer and cardiac diseases (such as vital myocarditis). Matrine has been shown to have a protective effect on the lipopolysaccharide-reduced liver injury [20], inhibition of proliferation cells and inducing differentiation in K-562 cells [21]. It can inhibit IL-1, IL-6, and TNF-α production in vitro and in vivo [14,20,22]. In recent years, matrine has been used in the treatment of chronic liver disease and has a significant effect on the inhibition of liver fibrosis [23]. Matrine was also used for inflammatory skin disorders such as atopic dermatitis and eczema in traditional Chinese medicine based on its anti-inflammatory and immunosuppressive effects [24]. However, the mechanism has not been elucidated.

In the present study, the effect of matrine on the expression of NK-1R in HaCaT cells and fibroblasts was investigated by flow cytometry and Western blotting analysis. Cytokines production by human skin keratinocytes and fibroblasts is of major importance in the regulation of immune and inflammatory processes in cutaneous diseases. Among the cytokines, interleukin-1β (IL-1β), IL-6, IL-8, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) are most believed to be important involved in the pathogenesis of cutaneous diseases [25–29]. While monocyte chemotactic protein (MCP)-1, a CC chemokine, is presumed to play a pivotal role in the recruitment and accumulation of monocytes in various diseases including cutaneous inflammation [29,30]. Therefore, we investigated the in vitro effect of SP and matrine on the secretion of IL-1β, IL-6, IL-8, TNF-α, IFN-γ and MCP-1 in HaCaT cells and fibroblasts by ELISA. All these findings provided evidence of the influence of matrine on cutaneous inflammation.

2. Materials and methods

2.1. Cell culture

HaCaT cells, spontaneously immortalized, nontumorigenic human skin keratinocyte cell lines kindly provided by

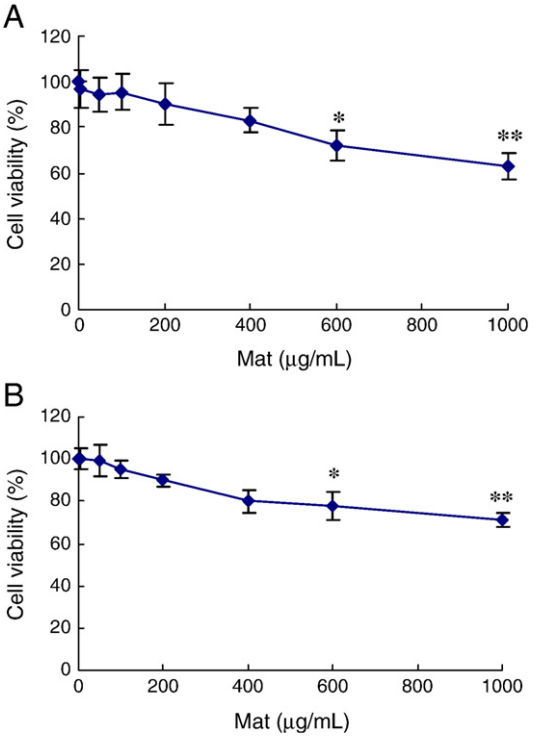

Fig. 2. Effect of matrine on the viability of HaCaT cells (A) and fibroblasts (B). The cells were incubated with various concentrations of matrine for 24 h, DMEM as the negative control. Then cell viability was determined by a MTT assays. Results are expressed as mean±SD of data obtained in three independent experiments. *$P<0.05$, **$P<0.01$ *vs.* control group.