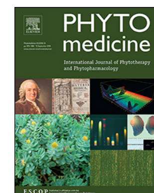
Phytomedicine 48 (2018) 43–50

Contents lists available at ScienceDirect

Phytomedicine

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



The anti-anaphylactoid effects of hydroxysafflor yellow A on the suppression of mast cell Ca^{2+} influx and degranulation

Rui Liu[a], Tingting Zhao[a], Delu Che[a], Jiao Cao[a], Jue Wang[a], Yanni Lv[a], Pengyu Ma[a], Yuanyuan Ding[a], Nana Wang[a], Xiaoyang Wang[a], Nan Wang[a], Jianli Wang[a,c], Zijun Gao[b,*], Tao Zhang[a,*]

[a] School of Pharmacy, Xi'an Jiaotong University, Xi'an 710061 China
[b] Department of Anesthesiology, Xi'an Honghui Hospital, Xi'an Jiaotong University, Xi'an 710054 China
[c] Tianjin Chasesun Pharmaceutical Co., Ltd, Tianjin 301700, China

A R T I C L E I N F O

Keywords:
Hydroxysafflor yellow A
Anti-anaphylactoid effects
Mast cells
Calcium activation
Degranulation

A B S T R A C T

Background: Anaphylaxis is a type of potentially fatal hypersensitivity reaction resulting from the activation of mast cell mediators, especially histamine and lipid mediators. Non-IgE-mediated anaphylaxis can occur because of the direct activation of mast cells. Hydroxysafflor yellow A (HSYA) is the main chemical component of safflower (*Carthamus tinctorius*) and has been reported to have pharmacological activities. However, the anti-anaphylactoid effect of HSYA has not yet been investigated.
Purpose: The aims of this study were to evaluate the anti-anaphylactoid activity of HSYA *in vivo* and to investigate the underlying mechanism *in vitro*.
Methods: The anti-anaphylactoid activity of HSYA was evaluated in a mouse model of hindpaw extravasation. Calcium imaging was used to assess intracellular Ca^{2+} mobilization. The levels of cytokines and chemokines released by stimulated mast cells were measured using enzyme immunoassay kits. Western blotting was used to explore the related molecular signaling pathways.
Results: HSYA markedly inhibited mast cell degranulation by suppressing the activation of intracellular Ca^{2+} mobilization and preventing the release of cytokines and chemokines from mast cells in a dose-dependent manner via the PKC-PLCγ-IP3R signaling pathway.
Conclusion: In summary, HSYA has anti-anaphylactoid pharmacological activity, which makes it a potential candidate for the development of a novel agent to suppress drug-induced anaphylactoid reactions.

Introduction

Anaphylaxis is an acute, generalized, potentially life-threatening, systemic hypersensitivity reaction resulting from the sudden degranulation of mast cells and basophils (Sampson et al., 2006; Simons, 2008), which can occur via immunologic or non-immunologic mechanisms (Greenberger and Ditto, 2012; Simons, 2010). Immunologic anaphylaxis can be divided into IgE-dependent and IgE-independent pathways (Williams and Sharma, 2015). Non-immunologic anaphylaxis usually occurs due to direct stimulation of mast cells, leading to degranulation (Lieberman and Garvey, 2016; McNeil et al., 2015; Subramanian et al., 2016), by some drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) (Aun et al., 2014; Jares et al., 2015), beta-lactam antibiotics (Jares et al., 2015), and others (Montanez et al., 2017). Medications such as quinolones, vancomycin, and opioids have been found to directly stimulate mast cells (Atkinson and Kaliner, 1992; Subramanian et al., 2016). MAS-related G protein-coupled receptor-X2 (MRGPRX2) has been found to mediate this process (Lieberman and Garvey, 2016; McNeil et al., 2015; Subramanian et al., 2016). The activation of mast cell receptors by certain drugs can promote Ca^{2+} mobilization (Holowka et al., 2016) and induce the release of cytokines and chemokines, such as β-hexosaminidase, histamine (HA), tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, and PGD2. All of these mediators play important roles in allergic inflammation and can lead to non-allergic anaphylactic reactions (Galli and Tsai, 2012). Mast cells are well recognized as both effector

Abbreviations: HSYA, Hydroxysafflor yellow A; C48/80, Compound 48/80; HA, Histamine; TNF, Tumor necrosis factor; MCP, Monocyte chemotactic protein; IL, Interleukin; MPMCs, Mouse peritoneal mast cells; DMSO, Dimethyl sulfoxide; LAD2, Laboratory of allergic disease 2; MCDM, Mast cell dissociation media

* Corresponding authors.
E-mail addresses: kobe84629@163.com (Z. Gao), taozhang@mail.xjtu.edu.cn (T. Zhang).

https://doi.org/10.1016/j.phymed.2018.05.009
Received 5 October 2017; Received in revised form 21 March 2018; Accepted 12 May 2018
0944-7113/ © 2018 Elsevier GmbH. All rights reserved.

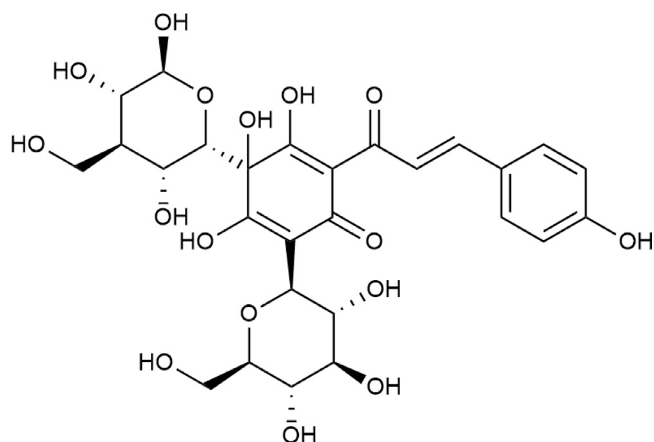


Fig. 1. Chemical structure of hydroxysafflor yellow A (HSYA).

and immunoregulatory cells that play a significant role in allergy-related disease (Metcalfe et al., 1997). It is important to inhibit the activity of mast cells to prevent anaphylaxis.

Hydroxysafflor yellow A (HSYA) (Fig. 1) is a highly water-soluble monomer of *Flos Carthami*, which is a type of traditional Chinese herbal medicine used in the treatment of cardiocerebrovascular diseases (Kelly et al., 1997; Sun et al., 2009). HSYA has been demonstrated to have neuroprotective effects, and it has the potential to be used as a neuroprotective agent for the treatment of focal cerebral ischemia (Zhu et al., 2003). Xinbing et al. demonstrated that HSYA can exert a neuroprotective effect against cerebral ischemia-reperfusion injury in rats (Wei et al., 2005). The inhibitory effect of HSYA on pulmonary inflammation in mice (Sun et al., 2010) and its ability to attenuate lipopolysaccharide-induced inflammatory signal transduction in human alveolar epithelial A549 cells (Song et al., 2013) have also been recently reported. HSYA also exerts beneficial effects on oxygen free radical-scavenging, anti-inflammatory actions and anti-apoptotic mechanisms (Ji et al., 2009; Tian et al., 2008; Wu et al., 2012). In addition, studies have also shown that HSYA can significantly decrease rat mast cell degranulation and reduce the release of inflammatory mediators *in vitro* (Han et al., 2015). However, the anti-anaphylactoid effect and the underlying therapeutic mechanisms of HSYA remain poorly understood.

In recent years, there has been important progress in the discovery of several mast cell-regulated inhibitory mechanisms. It is important to find more effective drugs to inhibit mast cell responses during anaphylactoid reactions. Basic secretagogues can induce mast cell degranulation, of which compound 48/80 (C48/80) is the most potent [15]. C48/80 acts directly on G-proteins rather than on high-affinity IgE receptors to promote mast cell degranulation (McNeil et al., 2015). The anti-anaphylactoid effects of any molecule can be validated by its ability to prevent C48/80-induced degranulation in mast cells. In this study, C48/80 was used to promote degranulation in mast cells and thereby to initiate anaphylactoid reactions. Mouse peritoneal mast cells (MPMCs) and human LAD2 mast cells were used to study the inhibitory effect of HSYA on mast cells via the suppression of intracellular Ca^{2+} mobilization and degranulation. HSYA also inhibited the release of β-hexosaminidase and HA triggered by ciprofloxacin and LL-37, which further confirmed its inhibitory effect on mast cells. Results from Western blotting demonstrated that HSYA inhibits the activation of mast cells via the PKC-PLCγ-IP3R signaling pathway in LAD2 cells. HSYA can suppress a C48/80-induced cutaneous flare in a dose-dependent manner. These findings demonstrate the anti-allergic activity of HSYA and its underlying mechanism, which indicates the potential of HSYA to be used as a novel therapeutic agent for the treatment of drug-induced anaphylactoid reactions.

Materials and methods

Drugs and reagents

C48/80 (a classic mast cell activator and canonical basic secretagogue), dimethyl sulfoxide (DMSO), MTT, Evans blue and p-nitrophenyl N-acetyl-β-D-glucosamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). HSYA was obtained from Chengdu Pufei De Biotech Co., LTD (Chengdu, Sichuan, China) and the purity is ≥98% based on HPLC analysis. Saline was purchased from Shandong Qidu Pharmaceutical Co., Ltd. A Human IL-8 ELISA Kit, Human MCP-1 ELISA Kit and Human TNF-α ELISA Kit were purchased from ExCell Biology, Inc. (Shanghai, China). Fluo-3 AM was procured from Thermo Fisher Scientific (Waltham, MA, USA). Pluronic F-127 gel was procured from Biotium (Fremont, CA, USA). The anti-PLCγ1 (#5690,), anti-phosphorylated-PLCγ1 (P-PLCγ1, Ser1248) (#8713), anti-IP3R (#3763), anti-phosphorylated-IP3R (P-IP3R, Ser1756) (#8548), anti-phosphorylated PKC (P-PKC, Ser1756) (#9371), anti-P38 (#8690), anti-phosphorylated-P38 (P-P38, Thr180/Tyr182) (#4511), anti-Akt (#4691), anti-phosphorylated-Akt (P-Akt, Ser473), (#4060), anti-Erk1/2 (p44/42 MAPK (Erk1/2), #9102), anti-phosphorylated-Erk1/2 (P-Erk1/2, P-p44/42 MAPK (Erk1/2) [Thr202/Tyr204], #9101) and anti-GAPDH (#2118) were procured from Cell Signaling Technology (Danvers, MA, USA). The anti-PKC (#GR257247-4) were procured from Abcam (Cambridge, MA, USA).

Cell lines

Laboratory of Allergic Disease 2 (LAD2) human mast cells were kindly provided by A. Kirshenbaum and D. Metcalfe (NIH, USA). Cells were maintained in StemPro-34 medium supplemented with StemPro nutrient supplement, 100 U penicillin, 100 U streptomycin, 2 mM L-glutamine and 100 ng/ml human stem cell factor in an incubator with 5% CO_2 at 37 °C. The culture medium was replaced every 7 days, and the cells were maintained at a density of 2×10^6 cells/ml.

Purification of mouse peritoneal mast cells (MPMCs)

Two- to five-month-old adult mice were sacrificed through CO_2 inhalation. A total of 12 ml ice-cold mast cell dissociation media (MCDM; HBSS with 3% fetal bovine serum and 10 mM HEPES, pH 7.2) was used to obtain two to three sequential peritoneal lavages, which were then centrifuged at 200 g and 4 °C for 5 min. The cells from each mouse were resuspended in 2 ml MCDM, layered over 4 ml of an isotonic 70% Percoll suspension (2.8 ml Percoll, 320 µl 10 × HBSS, 40 µl 1 M HEPES, and 830 µl MCDM) and centrifuged at 500 g and 4 °C for 20 min. Mast cells were collected from the pellet, and their purity was confirmed at >95% by morphological analysis and toluidine blue staining. The mast cells were resuspended at 5×10^5 to 1×10^6 cells/ml in Dulbecco's Modified Eagle's Medium (DMEM) with 25 ng/ml recombinant mouse stem cell factor (SCF) and seeded in 96-well plates.

Intracellular Ca^{2+} mobilization assay

LAD2 cells or MPMCs were incubated with 0, 50 µM, 100 µM or 200 µM HSYA diluted in calcium imaging buffer (CIB; 125 mM NaCl, 3 mM KCl, 2.5 mM $CaCl_2$, 0.6 mM $MgCl_2$, 10 mM HEPES, 20 mM glucose, 1.2 mM $NaHCO_3$, and 20 mM sucrose, adjusted to pH 7.4 with NaOH) for 30 min. The incubation buffer consisted of 4 µM Fluo-3 AM with 0.1% Pluronic F-127. For imaging, cells were washed twice in CIB. Fluo-3,AM-loaded cells were imaged with excitation at 488 nm. Unless otherwise specified, 30 µg/ml of C48/80 was slowly added to each well beginning from 10 s, and responses were monitored at 3 s intervals for an additional 120 s.

Download English Version:

https://daneshyari.com/en/article/8517816

Download Persian Version:

https://daneshyari.com/article/8517816

Daneshyari.com