Fitoterapia 99 (2014) 284–291

Contents lists available at ScienceDirect

Fitoterapia

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

Stimulation effect of wide type CFTR chloride channel by the naturally occurring flavonoid tangeretin

Yu Jiang [a], Bo Yu [a], Xue Wang [a], Yujie Sui [b], Yaofang Zhang [a], Shuang Yang [a], Hong Yang [a,*], Tonghui Ma [a]

[a] School of Life Sciences, Liaoning Provincial Key Laboratory of Biotechnology and Drug Discovery, Liaoning Normal University, Dalian 116029, PR China
[b] Central Research Laboratory, Jilin University Bethune Second Hospital, Changchun 130041, PR China

ARTICLE INFO

Article history:
Received 30 August 2014
Accepted in revised form 15 October 2014
Available online 22 October 2014

Keywords:
CFTR chloride channel
Tangeretin
Activator
Fluorescence quenching tests
Patch clamp
Short-circuit current

ABSTRACT

Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated chloride channel expressed in the apical membrane of serous epithelial cells. Both deficiency and overactivation of CFTR may cause fluid and salt secretion related diseases. In the present study, we identified tangeretin from *Pericarpium Citri Reticulatae Viride* as a CFTR activator using high-throughput screening based on FRT cell-based fluorescence assay. The activation effect of tangeretin on CFTR chloride channel and the possible underlying mechanisms were investigated. Fluorescence quenching tests showed that tangeretin dose- and time-dependently activated CFTR chloride channel, the activity had rapid and reversible characteristics and the activation effect could be completely reversed by the CFTR specific blocker $CFTR_{inh}$-172. Primary mechanism studies indicated that the activation effect of tangeretin on CFTR chloride channel was FSK dependent as well as had additional effect with FSK and IBMX suggesting that tangeretin activates CFTR by direct interacting with the protein. Ex-vivo tests revealed that tangeretin could accelerate the speed of the submucosal gland fluid secretion. Short-circuit current measurement demonstrated that tangeretin activated rat colonic mucosa chloride current. Thus, CFTR Cl^- channel is a molecular target of natural compound tangeretin. Tangeretin may have potential use for the treatment of CFTR-related diseases like cystic fibrosis, bronchiectasis and habitual constipation.

1. Introduction

Epithelial fluid secretion is composed by water and ion secretion. Sodium and potassium are the main cation channels while HCO_3^- and Cl^- are mainly involved in the anion transport in which CFTR (cystic fibrosis transmembrane conductance regulator), CaCC (calcium-activated chloride channel) and ClC (voltage-gated channel) take most responsibility for chloride channel transport function. CFTR is a cAMP-dependent chloride channel permeable to Cl^- and HCO_3^- [1] which belongs to the ATP-binding cassette super-family, abundantly expressed in apical membrane of serous epithelial cells. Mutations in CFTR gene result in cystic fibrosis (CF), the most common lethal genetic disease among Caucasians with hallmark defects in electrolytes and fluid transport. Besides, CFTR expresses in uterine, airway, pancreas, intestinal tract and corneal epithelia in which lacking of fluid secretion function caused by CFTR defect leads to infertility, bronchiectasis [2], chronic pancreatitis [3], habitual constipation [4] as well as xerophthalmia [5]. About one fifth of CF babies are born with meconium ileuses [6,7] which may be a consequence of lacking CFTR-mediated Cl^- secretion by the intestinal epithelium or from pancreatic dysfunction [8–11]. Therefore, CFTR potentiators may be of great value worldwide as a molecular therapeutic target for diseases mentioned above.

* Corresponding author.
E-mail address: hyanglnnu@gmail.com (H. Yang).

http://dx.doi.org/10.1016/j.fitote.2014.10.013
0367-326X/

Current therapies or early examples of stratified medicine for the treatment of CF and accompanying ileuses and airway obstruction are mostly from oncology [12] and combinatorial compounds or through surgery which may also come with various side effects. Natural compounds have been regarded to be more beneficial in comparison with combinatorial compounds in drug discoveries. Notwithstanding the positive effects of traditional Chinese medicine confirmed by practice, the exact effective constituents remain unclear. Unveiling active ingredients and mechanisms are needed for transforming herbal practices into evidence-based medicine.

In previous studies, we set up a high-throughput screening-based natural compound discovery strategy to systematically identify active natural compounds that are against different molecular targets. By screening the fraction library constructed from 500 herbal plants, we found large amounts of active fractions that had effect on CFTR chloride channel activity, among which three active fractions from *Pericarpium Citri Reticulatae Viride* were confirmed to have CFTR activation activity. The aim of the present study was to isolate active compound from the active fractions of *Pericarpium Citri Reticulatae Viride* and investigate mechanisms involved in the activation of CFTR chloride channel activity.

2. Materials and methods

2.1. Isolation and purification of tangeretin

Tangeretin was isolated from *Pericarpium Citri Reticulatae Viride* using activity-directed single compound isolation strategy. Briefly, *Pericarpium Citri Reticulatae Viride* was crushed and extracted by 95% ethanol, preparative HPLC and analytical HPLC (Waters 2525, 2695) were used for further fractionation and purification followed by tracking CFTR activation activity. Purity was determined using analytical HPLC, and >99% purity was characterized by MS and the structure was analyzed using NMR.

2.2. Cell lines, animals and compounds

Cell lines used in the present research were fischer rat thyroid epithelial (FRT) cells stably cotransfected with the YFP-H148Q fluorescence protein or YFP-H148Q/I152L fluorescence protein and human wild-type CFTR cDNA [8,9]. Cells were cultured in Nutrient F12 coon's medium (Sigma Chemical Co. St. Louis, MO. U.S.A.) supplemented with 10% fetal bovine serum (HyClone company, USA), 2 mM L-glutamine, 100 u/ml penicillin, and 100 μg/ml streptomycin in a 37 °C incubator (5% CO_2, 95% humidity) before using in iodide influx fluorescence study and patch-clamp experiments.

Male Kunming mice (8–10 weeks) and Wistar rats (200–220 g) were fed a standard chow diet and kept under specific pathogen-free conditions at Dalian Medical University (Permit Number: SCXK liao 2008–0002).

$CFTR_{inh}$-172 was synthesized as described previously [10]. FSK (forskolin), Gen (genistein) and IBMX were all purchased from Sigma (Sigma Chemical Co, St. Louis, MO. U.S.A.). All drugs were dissolved in a concentration of 20 mM in DMSO and diluted in PBS before experiments to ensure that DMSO produces no significant toxicity.

2.3. Iodide influx fluorescence assay

Cells were plated in a 96-well black wall clear bottom plate (Costar, Corning, NY, USA) at a density of 20,000 per well and were incubated until confluence. Cells were washed three times with PBS before 100 nM FSK was added into each well and the cells were incubated for 5 min. Then different concentrations of tangeretin were added into each well and the cells were incubated for another 15 min. YFP fluorescence data were recorded using a FLUOstar Galaxy microplate reader (BMG Lab Technologies, Inc.) equipped with HQ500/20X (500 ± 10 nm) excitation and HQ 535/30M (535 ± 15 nm) emission filters (Chroma Technology Corp.) and syringe pumps. Iodide influx rates (d[I−] / dt) were computed as described in reference [11].

2.4. Patch-clamp study

Patch-clamp experiments were performed at room temperature (25 °C) with an EPC10 amplifier (HEKA, Lamberecht/Pfalz, Germany) as described in reference [12]. Briefly, FRT cells stably transfected with wt-CFTR were plated onto cover glasses for inside-out patch recordings. Patch-clamp electrodes were made from B15024F glass capillaries (VitalSense Scientific Instrument) and fire polished to yield a resistance of 3–5 MΩ in the bath solution. The membrane potential of the excised inside-out membrane patch was held at −50 mV for all experiments. Currents were filtered at 100 Hz with an eight-pole Bessel filter (Warner Instrument) and captured onto a hard disk at a sampling rate of 500 Hz. The pipette solution contained (in mM): 140 N-methyl-D-glucamine chlorides (NMDG-Cl), 2 $MgCl_2$, 5 $CaCl_2$, and 10 HEPES, pH 7.4. During experiments, FRT cells were first incubated in the bath solution containing (in mM): 145 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 5 glucose, and 5 HEPES, pH 7.4, and secondly 20 mM sucrose was added into the bath solution to prevent activation of swelling-induced currents. The membrane patch was excised into the I/O solution containing (in mM): 150 NMDG-Cl, 10 EGTA, 10 HEPES, 8 Tris and 2 $MgCl_2$. CFTR current was activated by exposing the excised patch to the I/O solution containing protein kinase A (PKA, 25 U/ml) and adenosine triphosphate (ATP, 1 mM) while the control trace was obtained in the PKA-free I/O solution. The patch was then exposed to tangeretin in the ATP-containing I/O solution to evaluate the drug effect.

2.5. Submucosal gland fluid secretion stimulation measurement

Kunming mice (8–10 weeks) were anesthetized and the trachea submucosal gland was removed quickly, washed with normal saline and fixed with the mucosal side up on a sponge saturated with KH buffer containing (in mM): 117 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 1.2 KH_2PO_4, 24.8 $NaHCO_3$, 2.5 $CaCl_2$ and 11.1 L-glucose, pH 7.4. Mucus was removed using a cotton swab for several times before 100 μM tangeretin was added to the serosal side of the tissue and incubated for 10 min. After 20 μl water saturated mineral oil was added onto the surface, tissue was observed under a Stereo Microscopy (Olympus,