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High effectiveness of triptolide, an active diterpenoid triepoxide, in suppressing Kir-channel currents from human glioma cells

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ABSTRACT

Triptolide (Trip), a diterpene triepoxide isolated from medicinal vine *Trypterygium wilfordii* Hook. F. possessed multiple biological activities including antineoplastic actions. However, no report concerning its effects on ion currents has been published. In this study, we attempted to determine whether this compound has any effects on ion currents in malignant glioma cells. The mRNA expression of KCNJ10 (Kir4.1) was detected in U373 glioma cells. The inwardly rectifying K^+ currents ($I_{K(IR)}$) in U373 cells were almost fully blocked by $BaCl_2$ (1 mM). Trip (30 nM–10 μM) effectively decreased the amplitude of $I_{K(IR)}$ in a concentration-dependent manner with an IC_{50} value of 0.72 μM. In chlorotoxin-treated U373 cells, Trip-mediated block of $I_{K(IR)}$ remained effective. Addition of Trip (3 μM) slightly inhibited the amplitude of Ca^{2+}-activated K^+ current and sustained K^+ outward current in U373 cells. In cell-attached configuration, when Trip was added to the bath, the activity of inwardly rectifying K^+ (Kir) channels diminished with no change in single-channel conductance. Its suppression of Kir channels was accompanied by a reduction in the slow component of mean open time. Under current-clamp conditions, addition of Trip depolarized the membrane along with changes in frequency histogram of resting potential. Block by this component of Kir4.1 channels may be an important mechanism underlying its actions on the functional activity of glioma cells. Targeting at Kir4.1 channels may be clinically useful as an adjunctive regimen to anti-cancer drugs.

1. Introduction

Triptolide (Trip) is a diterpene triepoxide isolated from traditional Chinese medicinal vine *Trypterygium wilfordii* Hook. F. It possesses multiple biological activities, such as antitumor, immunosuppression, and antifertility (Zheng et al., 2013). Trip can protect neurons in the CNS and promote axon growth of dopaminergic neurons (Chen et al., 2007). It also promotes spinal cord repair through down-regulation of astrogliosis and inflammation in animal model of spinal cord injury (Su et al., 2010). This compound has been reported to inhibit proliferation and invasion of malignant glioma cells (Zhang et al., 2012) and induce apoptotic changes in malignant glioma cells (Lin et al., 2007). Because of its small size and high lipid solubility, Trip can cross the blood–brain barrier and produce significant effects on glial or glioma cells (Zheng et al., 2013).

Glioblastoma multiforme is a cancer with dismal prognosis and the relative short life expectancy for its victim was disappointing despite recent new treatment. Ion currents reported in malignant glioma cells are thought to influence activity of these cells (Hibino et al., 2004; Higashimori and Sontheimer, 2007; Labrakakis et al., 1997; Ullrich et al., 1996; Weaver et al., 2006). It has also been reported that the magnitude of functional expression in inwardly rectifying K^+ (Kir) channels enriched in glioma cells may interfere with progression of malignant tumors (Olsen and Sontheimer, 2008). Whether Trip and other related compounds can interact with ion currents in these cells were poorly understood and recent evidence showed its ability to produce multiple biological activities (Hsu et al., 2012; Zheng et al., 2013).

Glial cell membrane was found to be exclusively permeable to K^+ ions and that glial cells functionally express K^+ channels at

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high density (Olsen and Sontheimer, 2008). Among them, the activity of Kir4.1 (KCNJ10) channels was previously identified to be crucial in establishing and maintaining a negative resting potential (V_{rest}) in glial cells (Djukic et al., 2007; Hibino et al., 2004; Higashimori and Sontheimer, 2007; Olsen and Sontheimer, 2008). Furthermore, owing to the role of astrocytes in "spatial potassium buffering", Kir4.1-channel activity is also thought to be necessary in the maintenance of neuronal excitability (Haj-Yasein et al., 2011; Kofuji et al., 2002; Olsen and Sontheimer, 2008; Su et al., 2007). Because any changes in the activity of Kir4.1 channels can perturb cell growth and invasion in glioma cells (Higashimori and Sontheimer, 2007), these channels can be important anti-neoplastic targets. However, whether Trip exerts any effects on the activity of Kir channels is largely unknown.

Here, we attempted to characterize biophysical and pharmacological properties of ion currents in human glioma cells (e.g., U373 cells), to explore the effects of Trip on ion currents in these cells, and investigate whether this compound or other related compounds have any effects on ion currents, in particular inwardly rectifying K^+ current ($I_{K(IR)}$). Our results revealed for the first time that Trip can interact with Kir channels to reduce the amplitude of $I_{K(IR)}$ via a manner not entirely limited to changes in the activity of matrix metalloproteinases (MMPs).

2. Materials and methods

2.1. Drugs and solutions

Triptolide (PG 490 or NSC 163062; CAS-No. 38748-32-2; $C_{20}H_{24}O_6$) and tetraethylammonium chloride (TEA) were obtained from Sigma-Aldrich (St. Louis, MO), isobavachalcone was purchased from Enzo (Plymouth Meeting, PA), iberiotoxin and verruculogen were from Alomone (Jerusalem, Israel), PF573228 and BMS191011 were from Tocris (Bristol, UK), and ketamine was from Cerilliant Corp. (Round Rock, TX). Chlorotoxin was kindly provided by Professor Dr. Woei-Jer Chuang (Department of Biochemistry, National Cheng Kung University Medical College, Tainan, Taiwan). Tissue culture media, fetal bovine serum (FBS), L-glutamine, penicillin-streptomycin, fungizone and trypsin were obtained from Invitrogen (Carlsbad, CA). All other chemicals including $MgCl_2$ and $BaCl_2$ were obtained from regular commercial chemicals and of reagent grade. Reagent water was deionized using a Milli-Q water purification system (Millipore, Bedford, MA).

The composition of bathing solution (i.e., normal Tyrode's solution) was 136.5 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.53 mM $MgCl_2$, 5.5 mM glucose, and 5.5 mM HEPES–NaOH buffer, pH 7.4. To measure K^+ currents or membrane potential, the patch pipette was filled with a solution consisting of 140 mM KCl, 1 mM $MgCl_2$, 3 mM Na_2ATP, 0.1 mM Na_2GTP, 0.1 mM EGTA, and 5 mM HEPES–KOH buffer, pH 7.2. For the recordings of large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels or for the purpose of increasing $I_{K(IR)}$ magnitude, high K^+-bathing solution was used and its composition was 145 mM KCl, 0.53 mM $MgCl_2$, and 5 mM HEPES–KOH, pH 7.2, and the pipette solution contained 145 mM KCl, 2 mM $MgCl_2$, and 5 mM HEPES–KOH, pH 7.2. The pipette solution was filtered on the day of use with a 0.22 μm pore size syringe filter (Millipore).

Table 1
Target gene primer sequences and expected sizes of RT-PCR products for KCNJ10 and β-actin.

Target gene	Primer sequence	Expected size (bp)	GenBank Accession no.
KCNJ10 (Kir4.1)			
Forward	5′-CCT TGA AAG ATC TCC CTC TT-3′	205	NM_002241.4
Reverse	5′-TGG TCA AAA AGG CTA AAG TC-3′		
β-actin			
Forward	5′-CAT TAA GGA GAA GCT GTG CT-3′	208	NM_001101.3
Reverse	5′-GTT GAA GGT AGT TTC GTG GA-3′		

2.2. Cell preparations

The glioblastoma multiforme cell line (U373) was obtained from American Type Culture Collection (ATCC, Manassas, VA). They were routinely grown and maintained at a density of 10^6/ml in DMEM/F12 nutrient media (Invitrogen, Carlsbad, CA) supplemented with 10% FBS. All cell lines were grown at 37 °C in a 5% CO_2 incubator as monolayer cultures and sub-cultured weekly. Fresh media was added every 2–3 days to maintain a healthy cell population. To observe cell growth, a Nikon Eclipse Ti-E inverted microscope (Li Trading Co., Taipei, Taiwan) equipped with a 5-megapixel cooled digital camera was used. The camera was connected to a personal computer controlled by NIS-Elements BR 3.0 software (Nikon, Kanagawa, Japan). In a separate set of experiments, U373 cells were incubated with chlorotoxin (1 μM) at 37 °C for 6 h prior to electrophysiological recordings.

2.3. Assay of cell proliferation

U373 cells (2×10^4/ml) were cultured at 37 °C in a 96-well microplate and treated with different concentrations of Trip. After exposure to Trip for 24, 48 or 72 h, 20 μl of methyl tetrazolium (MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (5 mg/ml) was added to each well and cells were incubated for another 4 h. The formazan crystals were dissolved in 150 μl dimethylsulfoxide and the absorbance of samples was measured at 490 nm by a microplate reader (model 3550; Bio-Rad Laboratories, CA) (Wu et al., 1998).

2.4. RNA Isolation and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

To detect the expression of KCNJ10 (Kir4.1) mRNA in U373 cells, a semi-quantitative RT-PCR assay was performed. Total RNA samples were extracted from these cells according to TRIzol reagent (Invitrogen) and reverse-transcribed into complementary DNA using Superscript II reverse-transcriptase (Invitrogen). The sequences of forward and reverse primers used for human KCNJ10 and β-actin are illustrated in Table 1. The PCR cycling conditions were 35 cycles of 95 °C for 2 min, 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 10 min. The PCR products were analyzed on 1.5% (w/v) agarose gel containing ethidium bromide and then visualized by ultraviolet transillumination. Optical densities of DNA bands were scanned and subsequently quantified by Alphalmager 2200 (ProteinSimple; Santa Clara, CA).

2.5. Electrophysiological measurements

U373 cells were harvested with 1% trypsin/EDTA solution prior to the experiments and an aliquot of cell suspension was subsequently transferred to a recording chamber which was mounted on the mechanical stage of an inverted fluorescent microscope (CKX-41; Olympus, Tokyo, Japan) coupled to a digital video system (DCR-TRV30; Sony, Japan) with a magnification of up to 1500 ×. Cells were immersed at room temperature (20–25 °C) in normal Tyrode's solution containing 1.8 mM $CaCl_2$. The electrodes were pulled from Kimax-51 capillaries (#34500; Kimble Glass, Vineland, NJ) in a PP-830 puller (Narishige, Tokyo, Japan) or a P-97 Flaming/Brown micropipette puller (Sutter, Novato, CA), and their tips were fire-polished with an MF-83 microforge (Narishige). These