Biomedicine & Pharmacotherapy 101 (2018) 414–421

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

A silyl andrographolide analogue suppresses Wnt/β-catenin signaling pathway in colon cancer

Somrudee Reabroi[a], Arthit Chairoungdua[a,b], Rungnapha Saeeng[c], Teerapich Kasemsuk[d], Witchuda Saengsawang[a,b], Weiming Zhu[e], Pawinee Piyachaturawat[a,*]

[a] Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
[b] Excellent Center for Drug Discovery (ECDD), Mahidol University, Bangkok 10400, Thailand
[c] Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Burapha University, Chonburi 20131, Thailand
[d] Department of Chemistry, Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chanthaburi 22000, Thailand
[e] Key Laboratory of Marine Drugs, Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China

ARTICLE INFO

Keywords:
Andrographolide analogue
Anti-cancer
Colorectal cancer
Wnt/β-catenin
GSK-3β

ABSTRACT

Hyperactivation of Wnt/β-catenin signaling implicated in oncogenesis of colorectal cancer (CRC) is a potential molecular target for chemotherapy. An andrographolide analogue, 3A.1 (19-*tert*-butyldiphenylsilyl-8, 17-epoxy andrographolide) has previously been reported to be potently cytotoxic toward cancer cells by unknown molecular mechanisms. The present study explored the anti-cancer activity of analogue 3A.1 on Wnt/β-catenin signaling in colon cancer cells (HT29 cells) which were more sensitive to the others (HCT116 and SW480 cells). Analogue 3A.1 inhibited viability of HT29 cells with IC_{50} value of 11.1 ± 1.4 μM at 24 h, which was more potent than that of the parent andrographolide. Analogue 3A.1 also suppressed the proliferation of HT29 cells and induced cell apoptosis in a dose-dependent manner. Its apoptotic activity was accompanied with increased expressions of proteins related to DNA damages; PARP-1 and γ-H2AX. In addition, analogue 3A.1 significantly inhibited T-cell factor and lymphoid enhancer factor (TCF/LEF) promoter activity of Wnt/β-catenin signaling. Accordingly, the expressions of Wnt target genes and β-catenin protein were suppressed. Moreover, analogue 3A.1 increased the activity of GSK-3β kinase, which is a negative regulator responsible for degradation of intracellular β-catenin. This mode of action was further supported by the absence of the effects after treatment with a GSK-3β inhibitor, and over-expression of a mutant β-catenin (S33Y). Our findings reveal, for the first time, an insight into the molecular mechanism of the anti-cancer activity of analogue 3A.1 through the inhibition of Wnt/β-catenin/GSK-3β pathway and provide a therapeutic potential of the andrographolide analogue 3A.1 in CRC treatment.

1. Introduction

Colorectal cancer (CRC) is one of the most common deadly gastrointestinal cancers found in both men and women in the United States [1]. The pathogenesis of CRC is complicated with the involvement of multiple signaling pathways [2,3]. Inappropriate activation of a canonical Wnt/β-catenin signaling is one of the key pathways driven for initiation and progression of CRC, and this pathway has been considered as one of the attractive targets for CRC therapy [2]. The mutations of Wnt components; APC, and β-catenin, are reported in colon cancer by 85%, and 15%, respectively [4,5]. These genetic alterations cause intracellular β-catenin to escape from degradation, subsequently enable the constitutive Wnt transcription to promote oncogenic events [6] and hence limits the treatments [7].

The key player of Wnt/β-catenin signaling pathway is β-catenin protein [8]. In the absence of Wnt ligands, β-catenin is phosphorylated at residues Ser45/Ser33/Ser37/Ser41 by the destruction complex components including adenomatous polyposis coli (APC), axin2, casein kinase 1 (CK1) and glycogen synthase kinase-3β (GSK-3β) [9]. This marks cytosolic β-catenin for ubiquitination and degradation in a proteasome to keep β-catenin in low level. Binding of Wnt ligands with its receptor Frizzed (Fz) and a co-receptor low-density lipoprotein receptor related protein 5/6 (LRP5/6) triggers the dissociation of the destruction complex [10] and subsequently causes an accumulation of non-

Abbreviations: CRC, colorectal cancer; TBDPS, *tert*-butyldiphenylsilyl; GSK-3β, glycogen synthase kinase-3β; TCF/LEF, T-cell factor and lymphoid enhancer factor; PARP-1, poly (ADP-ribose) polymerase-1

* Corresponding author: Department of Physiology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok, 10400, Thailand.
E-mail address: pawinee.pia@mahidol.ac.th (P. Piyachaturawat).

https://doi.org/10.1016/j.biopha.2018.02.119
Received 10 January 2018; Received in revised form 16 February 2018; Accepted 23 February 2018

phosphorylated β-catenin, which in turn translocate into the nucleus. The formation of the translocated β-catenin and TCF/LEF transcription factors inside the nucleus leads to transactivation of Wnt-responsive genes, including c-myc, cyclin D1, survivin, and MMP-7 that participate in promoting cancer proliferation, survival, and metastasis [11–14]. In addition to TCF4 in the nucleus, a cross-link of Wnt signaling to the expression of topoisomerase IIα (Topo-IIα) has been reported, and the overexpression of Topo-IIα enhances TCF/LEF transcription of Wnt signaling [15]. Topo-IIα is a nuclear enzyme that catalyzes topological conversion of DNA double strands, facilitating proper DNA replication and transcription [16,17]. High expression of Topo-IIα in colon tumor has been correlated to resistant to therapy with Topo-IIα inhibitors [18], suggesting that multiple targets of an anti-cancer agent rather than a single one is required for improving therapeutic effectiveness. As a complexity of colon tumorigenesis involves multiple pathways, it is, therefore, crucial to find more molecular targets for anti-cancer agents to effectively improve the responsiveness of patients to therapy.

Andrographolide is a major diterpenoid isolated from *Andrographis paniculata* which has been widely studied because of its intrinsic cytotoxic property to cancer cells [19,20,21]. One of the mechanisms underlying apoptotic-promoting effect of andrographolide has been reported to mediate through induction of endoplasmic reticulum (ER) stress by increasing ROS levels [22] which then activates the activity of IRE-1, a proximal sensor related to unfolded protein response (UPR) of ER [23]. The accumulation of unfolded proteins caused by andrographolide-induced ER stress activates different signaling cascades and cellular responses such as caspase-mediated apoptosis, induction of cell cycle arrest, and inhibition of cell survival [22,23]. Based on accumulating evidence in anticancer activity of andrographolide, it is considered as a promising chemotherapeutic agent. Unfortunately, its potential use in clinic is limited by low potency and poor oral availability [24,25]. Structural modifications of andrographolide to improve the potency and efficacy for anti-cancer activities have received much interest. Recently, we have demonstrated that an andrographolide analogue 3A.1 (19-*tert*-butyldiphenylsilyl-8,17-epoxy andrographolide) was highly cytotoxic to a panel of mammalian cancer cell lines (CHO, HepG2, UISO-BCA1, Hela) including cholangiocarcinoma by inhibiting the activity of Topo-IIα [26,27,28]. However, other intracellular anticancer mechanisms of analogue 3A.1 on gastrointestinal cancer cells are largely unknown. As Wnt/β-catenin signaling is a critical pathway in regulating CRC proliferation, the present study investigated more specific molecular effects of analogue 3A.1 in HT29 (colorectal cancer) cells by targeting on Wnt/β-catenin signaling pathway. Herein, we demonstrated for the first time that the anti-cancer effect of analogue 3A.1 was associated with inhibition of Wnt/β-catenin signaling in HT29 cells and was essentially dependent on the kinase function of GSK-3β, a Wnt negative regulator.

2. Materials and methods

2.1. Cell culture

Colorectal cancer (CRC); HT29, HCT116, and SW480, HEK293T (human embryonic kidney), and Chang liver (human normal liver) cells were obtained from the American Type Culture Collection (ATCC, USA). HT29 cells were maintained in Dulbecco's modified Eagle's medium: Nutrient Mix F-12 (DMEM/F12) (Sigma, St. Louis, MO, USA). HCT116 and SW480 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA). HEK293T and Chang liver cells were maintained in Minimum Essential Media (MEM) (Invitrogen, Carlsbad, CA, USA). All growth media were supplemented with 10% fetal bovine serum (FBS) (Hyclone, Perbio, UT, USA), 100 μg/ml penicillin, and 100 μg/ml streptomycin (Invitrogen). All cells were cultured at 37 °C in a humidified incubator with 5% CO_2.

2.2. Reagents and antibodies

Andrographolide and the analogue 3A.1 were prepared by Asst. Prof. Dr. Rungnapha Saeeng as previously described [26]. The following reagents were used: Lipofectamine 2000 and TRIzol reagent (Invitrogen); Doxorubicin (Guanyu Bio-tech, Xi'an, China); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lithium chloride (Sigma); Annexin V-FITC apoptosis detection kit (BD Biosciences, SanJose, CA, USA); iScript™ select cDNA synthesis kit (Bio-Rad, Hercules, CA, USA); SYBR Green kit (Applied Biosystem, Carlsbad, CA, USA); BCA protein assay kit, protease inhibitor cocktail, RIPA buffer, Super Signal West Pico Chemiluminescent Substrate (Thermo Scientific, Cramlington, UK), and phosphatase inhibitor cocktail (Millipore, Darmstadt, Germany). Andrographolide, analogue 3A.1, and doxorubicin were dissolved in DMSO as stock solutions at concentration 100 mM and stored in aliquots at −20 °C until use. The final concentration of DMSO (Sigma) in all treatments was less than 0.1%. The following antibodies were used: anti-β-catenin (Santa Cruz Biotechnology, CA, USA); anti-active-β-catenin (Millipore, Darmstadt, Germany); anti-phospho-GSK-3β (Ser9) and anti-GSK-3β (Cell Signaling Technology, Danvers, MA, USA); anti-β-actin (Sigma). The TCF/ LEF reporter plasmids (TOPflash, FOPflash), β-catenin-FLAG and mutant β-catenin S33Y plasmids were described previously [29].

2.3. Cell viability assay

Cells were plated into 96-well plates (1×10^4 cells/well) and treated with various concentrations of tested compounds for 24, 48 and 72 h. At the indicated periods, cell viability was assessed using MTT assay. The medium was removed and replaced with a serum-free medium containing MTT working solution and incubated for 4 h. Formazan product was dissolved with DMSO and the optical density was measured at 540 nm using a Multiskan™ GO Microplate Spectrophotometer (Thermo Scientific, Cramlington, UK)

2.4. Cell proliferation assay

To investigate the effect on cell proliferation, BrdU incorporation assay was used. Cells were plated into 96-well plates (1×10^4 cells/well) for 24 h, and treated with various concentrations of tested compounds for 24 h. After incubation, cells were added with BrdU labeling solution, fixed, and incubated with anti-BrdU peroxidase conjugated antibody using the cell proliferation ELISA BrdU kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. The reaction was stopped by 1 M H_2SO_4 and the absorbance was measured at a wavelength of 450 nm (reference wavelength: 690 nm) using the Multiskan™ GO Microplate Spectrophotometer (Thermo Scientific).

2.5. Flow cytometry analysis

For apoptosis analysis, cells were treated with tested compounds for 24 h. Then, cells were harvested by trypsinization, centrifuged and washed twice with PBS. Cell pellets were re-suspended in Annexin V-FITC and PI (BD Biosciences) containing solution for 15 min in darkness. The different stages of cell death were analyzed by a BD FACS Calibur™ flow cytometer using BD FACS Diva software version 6.1.1 (BD Bioscience) for data analysis.

2.6. Luciferase reporter assay

HEK293T cells were plated into 96-well plates (1×10^4 cells/well) for 18 h. Then, cells were transiently co-transfected with 0.1 μg pcDNA3.1 or β-catenin-FLAG or S33Y plasmid, 0.05 μg TOPflash or FOPflash reporter plasmid and 0.05 μg *Renilla* luciferase reporter plasmid for 24 h by using lipofectamine 2000 reagent according to the manufacturer's instructions. Then, analogue 3A.1 at various