Phytomedicine 34 (2017) 136–142

Contents lists available at ScienceDirect

Phytomedicine

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

Cytotoxic activities of *Telectadium dongnaiense* and its constituents by inhibition of the Wnt/β-catenin signaling pathway

Won Kyung Kim[a], Duc-Hiep Bach[a], Hyung Won Ryu[b], Jedo Oh[a], Hyen Joo Park[a], Ji-Young Hong[a], Hyuk-Hwan Song[b], Sangmi Eum[c], Tran The Bach[d], Sang Kook Lee[a,*]

[a] College of Pharmacy, Natural Products Research Institute, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul, 08826, Republic of Korea
[b] Natural Medicine Research Center, Korea Research Institute of Bioscience & Biotechnology, 30 Yeongudanji-ro, Ochang-eup, Cheongju-si, Chungbuk, 28116, Republic of Korea
[c] International Biological Material Research Center, Korea Research Institute of Bioscience & Biotechnology, 30 Yeongudanji-ro, Ochang-eup, Cheongju-si, Chungbuk, 28116, Republic of Korea
[d] Institute of Ecology and Biological Resources, Vietnam Academy of Sciences and Technology, 18 Hoang Quoc Viet, Caugiay, Hanoi, Vietnam

ARTICLE INFO

Keywords:
Wnt/β-catenin signaling
Cytotoxicity
Colon cancer cells
Asclepiadaceae
Telectadium dongnaiense Pierre ex Costantin
Periplocin

ABSTRACT

Background: Wnt/β-catenin signaling pathway is a potential target for the treatment of human colon cancer. Thus, the inhibitory effects of various plant extracts on cell proliferation and Wnt signal transduction were evaluated to discover a Wnt signaling inhibitor.

Purpose: The present study aimed to investigate the cytotoxicity involved in Wnt pathway of the MeOH extract from *Telectadium dongnaiense* bark (TDB) and to identify its bioactive constituents by bioassay-guided fractionation.

Methods: The sulforhodamine B-based proliferation assay and the β-catenin/TCF-responsive reporter gene assay were employed as screening systems. The isolation and identification of compounds were elucidated on the basis of spectroscopic methods. Inhibitory effects on the expression levels of Wnt target genes were determined by real-time PCR and western blotting.

Results: The extract of TDB most strongly inhibited cell proliferation and TOPflash activity (IC_{50} = 1.5 and 2.0 μg/ml), which was correlated with its inhibitory effects on the expression of Wnt target genes. Three major compounds were isolated from bioactive fractions and were identified as 1,4-dicaffeoylquinic acid (**1**), quercetin 3-rutinoside (**2**), and periplocin (**3**). Only compound **3** showed anti-proliferative activity (IC_{50} = 0.06 μM) and exhibited Wnt signaling inhibitory effects in HCT116 colon cancer cells.

Conclusions: This study contributes to understanding the cytotoxic properties of TDB extract and its constituents and provides a potent strategy for its further application.

Introduction

The Wnt/β-catenin signaling pathway is a key pathway that controls various biological processes, including cell proliferation, cell motility, stem cell differentiation, and cell fate determination (Clevers, 2006; Huelsken and Birchmeier, 2001). However, aberrantly activated Wnt signaling can lead to tumorigenesis, cancer progression, and tumor cell metastasis (MacDonald et al., 2009). β-Catenin stabilization is a well characterized feature in the regulation of the canonical Wnt signal transduction (Gao et al., 2014). In the absence of Wnt ligands, cytosolic β-catenin binds to a multiprotein destruction complex composed of axin, casein kinase 1α (CK1α), adenomatous polyposis coli (APC), and glycogen synthase kinase 3β (GSK3β), which leads to the phosphorylation of β-catenin (Liu et al., 2002). Phosphorylated β-catenin subsequently undergoes poly-ubiquitination and proteasomal degradation by the F-box protein β-transducin-repeat-containing protein and ubiquitin E3 ligase (Kitagawa et al., 1999). However, β-catenin is conserved when Wnt proteins bind to the Frizzled receptor and its co-receptors, low-density lipoprotein-related receptors 5 and 6, causing the dissociation of the β-catenin destruction complex (Wong et al., 2003).

Abbreviations: TDB, *Telectadium dongnaiense* Pierre ex Costantin bark; CK1α, casein kinase 1α; APC, adenomatous polyposis coli; GSK3β, glycogen synthase kinase 3β; TCF, T-cell transcription factor; LEF, lymphoid enhancer-binding factor; SRB, sulforhodamine B; HPLC, high-performance liquid chromatography; NMR, Nuclear magnetic resonance spectroscopy; RPMI, Roswell Park Memorial Institute; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; DMSO, Dimethyl sulfoxide; TCA, trichloroacetic acid; BSA, bovine serum albumin; PCR, polymerase chain reaction; CT, threshold cycle; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; PBST, phosphate-buffered saline containing 0.1% Tween-20

* Corresponding author.
E-mail address: sklee61@snu.ac.kr (S.K. Lee).

http://dx.doi.org/10.1016/j.phymed.2017.08.008
Received 25 March 2017; Received in revised form 13 June 2017; Accepted 9 August 2017

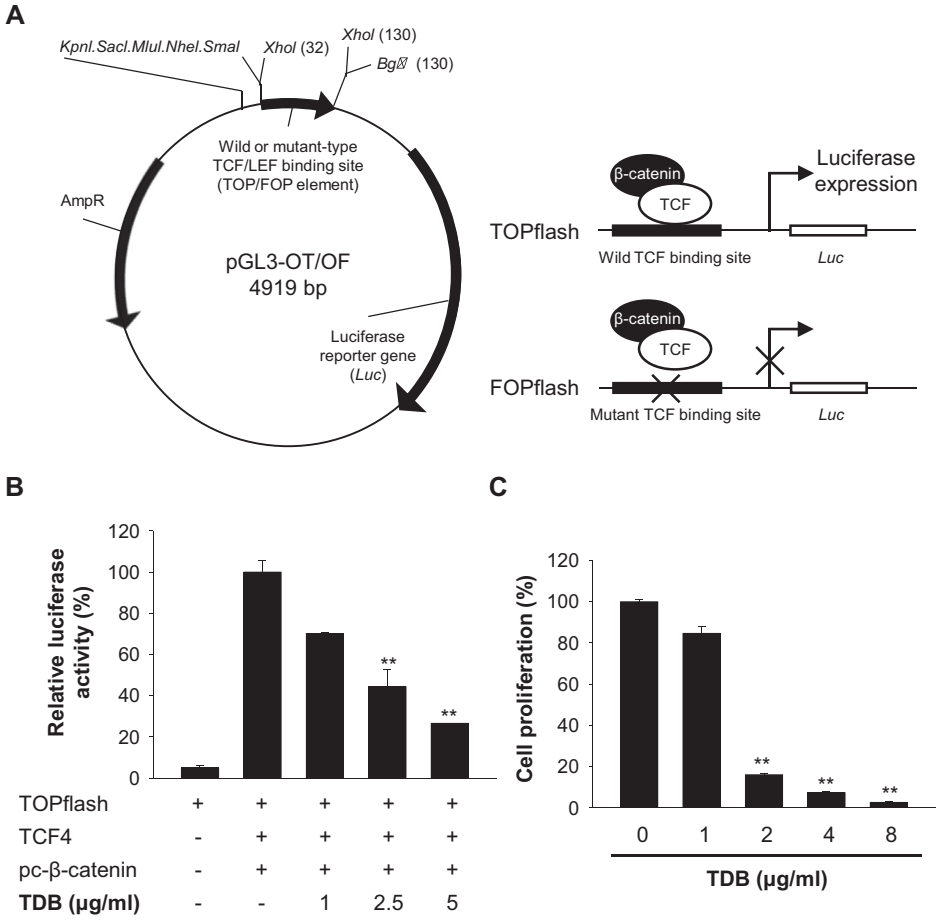

Fig. 1. Inhibitory effects of TDB extract on the β-catenin/TCF transcriptional activity and colon cancer cell growth. (A) Map of the pGL3-OT/OF reporter plasmids (left) with examples of promoter activation (right). The reporter vectors contained the LEF/TCF elements (32–130 bp) as TCF binding sites (B) Levels of TOPflash activity in HEK293 cells that were transiently transfected with the TOPflash and *Renilla* reporter gene vectors and a TCF4-, β-catenin- expression vectors for 24 h and then were treated with the TDB extracts for an additional 24 h Luciferase activities were normalized with the *Renilla* values and expressed as relative luciferase activity (%). (C) Proliferation rate of HCT116 cells treated with the TDB extract for 72 h, determined using the SRB assay. The data were expressed as the mean values ± SD ($n = 3$) and are representative of three separate determinations (**$p < 0.01$).

Consequently, the unphosphorylated β-catenin can accumulate in the cytoplasm, translocate into the nucleus, and bind to transcription factors of the T-cell transcription factor (TCF)/lymphoid enhancer-binding factor (LEF) family, which eventually contribute to the transcription of Wnt target genes such as c-myc, cyclin D1, and survivin (Arend et al., 2013). Recently, APC mutations are revealed in 80–90% cases of sporadic colorectal cancer, and β-catenin mutations were found in the majority of cases, indicating that activated Wnt/β-catenin signaling is markedly associated with the progression of colorectal cancer (Morin et al., 1997). Hence, the discovery of Wnt signaling inhibitors is considered as a potential strategy to develop anticancer agents for the treatment of colorectal cancers.

Primarily to identify Wnt signaling inhibitors from natural products, the anti-proliferative activities of various plant extracts were assessed using sulforhodamine B (SRB) assay in HCT116 human colon cancer cells. In addition, human embryonic kidney cells (HEK293) which transiently transfected with TCF reporter plasmid (TOPflash, pGL3-OT) were employed to evaluate the effects of extracts on TCF transcriptional activity. This TOPflash contains two set of three copies of the binding site upstream of the thymidine kinase minimal promoter and luciferase open reading frame, which can produce luciferase in response to activation of β-catenin mediated TCF/LEF transcription (Miller et al., 1999). Among active extracts, the MeOH extract of *Telectadium dongnaiense* Pierre ex Costantin bark (TDB) was found to have a potent inhibitory effect on the Wnt signaling and anti-proliferative activity against HCT116 cells. TDB, which belongs to the Asclepiadaceae family, is distributed mainly in Dongnai Province in Vietnam (Costantin, 1912). Asclepiadaceae plants have been reported to contain various constituents, including alkaloids and glycosides (Honda et al., 1995; Cheung et al., 1983). Several pharmacological properties, such as antimicrobial and anti-tumor activities, have also been reported (Nenaah, 2013; Xue et al., 2015). However, the biological activities of TDB and its components have not yet been identified.

In the present study, the cytotoxic properties of TDB extract were first determined, a Wnt signaling inhibitory compound was prepared from this extract using an activity-guided isolation process, and whether these compounds exert anti-proliferative activity against colon cancer cells through inhibiting the Wnt/β-catenin signaling pathway was investigated. This study may be the first to demonstrate the cytotoxic activity of TDB and to identify its constituents.

Materials and methods

Plant materials

The TDB was collected in Nam Giang district of Quang Nam province, Vietnam in 2009. The samples were identified by Dr. Tran The Bach at the Institute of Ecology and Biological Resources, Hanoi, Vietnam. A voucher specimen (KRIB0026906, FBM074-016) was deposited in the herbarium of the Korea Research Institute of Bioscience and Biotechnology (KRIBB). Information for all tested plant extracts is available in the website (www.ibmrc.re.kr).

Extraction, isolation and identification

The dried TDB (6.0 kg) was extracted with 100% MeOH (10.0 l) at room temperature overnight three times and evaporated using a rotary evaporator at temperature below 45 °C to obtain a crude extract (793.7 g). Next, the extract was successively partitioned using *n*-hexane, chloroform, ethyl acetate, and *n*-butanol to obtain five fractions. The fractions were analyzed by ultra-performance liquid chromatography-photodiode array-quadrupole-time of flight-mass