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Apoptosis of human melanoma cells induced by the novel compounds propolin A and propolin B from Taiwenese propolis

Chia-Nan Chen [a], Chia-Li Wu [b], Jen-Kun Lin [a,*]

[a] *Graduate Institute of Biochemistry and Molecular Biology, College of Medicine, National Taiwan University, Section 1, Jen-Ai Road, Taipei 100, Taiwan, ROC*
[b] *Department of Chemistry, Tamkang University, Tamsui 251, Taiwan, ROC*

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Abstract

We recently demonstrated that two new prenylflavanones, propolin A and propolin B, isolated and characterized from Taiwanese propolis, induced cytotoxicity effect in human melanoma A2058 cells and shows a strong capability to scavenge free radicals. In this study, propolin A effectively induced a cytotoxic effect on five different cancer cell lines. Similar results were obtained for propolin B. DNA flow cytometric analysis and DNA fragmentation ladder indicated that propolin A and propolin B actively induced apoptosis in A2058 cells. To address the mechanism of the apoptosis effect of propolin A and propolin B, we evaluated the apoptosis-related proteins in A2058 cells. The levels of procaspase-8, Bid, procaspase-3, DFF45, and PARP were decreased in dose- and time course-dependent manners. Furthermore, also found propolin A and propolin B was capable of releasing cytochrome *c* from mitochondria to cytosol. The findings suggest that propolin A and propolin B may activate a mitochondria-mediated apoptosis pathway. On the other hand, our data show that propolin B inhibitied xanthine oxidase activity more efficiently than propolin A or CAPE. However, CAPE suppressed ROS-induced DNA strand breakage more efficiently than propolin A or propolin B. All these results indicated that propolin A and propolin B may trigger apoptosis of A2058 cells through mitochondria-dependent pathways and also shown that propolin A and propolin B were strong antioxidants.

Keywords: Propolin A; Propolin B; Taiwanese propolis; Apoptosis; Xanthine oxidase; Human melanoma cells

1. Introduction

A natural honeybee product, propolis, is a resinous material gathered by honey bees from the buds and bark of certain trees and plants, and used inside their hives [1]. It is a kind of crude medicine that has long been used as a folk remedy chiefly in Europe [2]. Propolis contains various chemical components and exhibits a broad spectrum of biological activities including antitumor [3], antioxidant [4], antibacterial [5], antiviral [6], antifungal [7], and anti-inflammatory activities [7].

Many natural products used in cancer chemotherapy, including taxol [8], adriamycin [9], VP16 [10], and camptothecin [10], have apoptosis-inducing activity. We previously identified two novel prenylflavanones from Taiwanese propolis, flavonoid compounds with hydrated geranyl side chains, namely, propolin A and propolin B (Fig. 1) [11]. Flavonoids are found naturally in fruits and vegetables and have been indicated in chemoprevention or anticancer activity [12,13]. The anticancer activities of flavonoids are due to induced apoptosis [14]. Many flavonoids have also been shown to induce apoptosis in cancer cells include quercetin

* Corresponding author. Tel.: +886 2 2356 2213; fax: +886 2 2391 8944.
E-mail address: jklin@ha.mc.ntu.edu.tw (J.-K. Lin).

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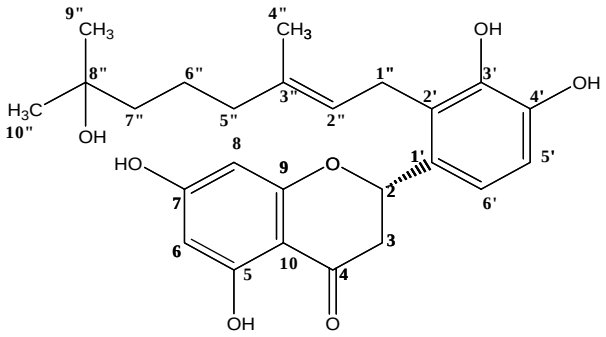
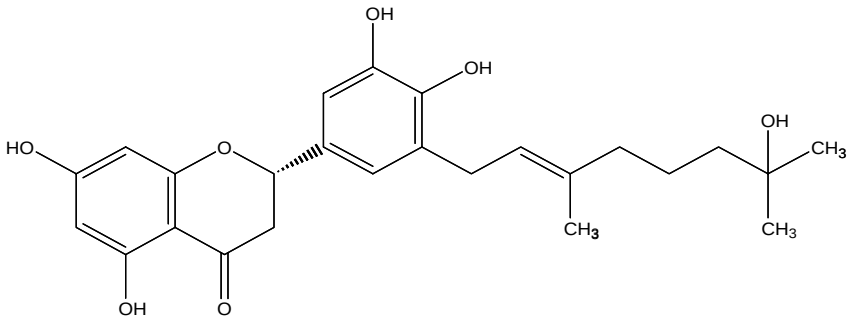


Propolin A



Propolin B

Fig. 1. Structures of propolin A and propolin B.

[15], bicalein [16], genistein [17], tangeritin [18] and sophoranone [19]. Propolin A and propolin B have hydrated geranyl side chains that differ from these flavonoids, but chemical structures similar to sophoranone that it has isoprenyl side chains.

Apoptosis, the physiological mode of cell death, is related to the regulation of development and homeostasis. Its morphological characteristics include plasma membrane blebbing, cell shrinkage, nuclear condensation, chromosomal DNA fragmentation, and formation of apoptotic bodies [20]. Many recent reports have indicated that many anticancer drugs or cancer chemopreventive agents act through the induction of apoptosis to prevent tumor promotion and progression. The process of apoptosis consists of different phases, including initiation, execution and degradation [21], and is activated by two major pathways. One is receptor-induced apoptosis that includes of the TNF-R1, Fas (CD95), and TRAIL-receptors [22,23]. In the so-called 'extrinsic' cell death pathway, a cytoplasmic death domain forms and trimerization of the receptor (TNF-R1, Fas, and TRAIL) and recruits TRADD and FADD. The other major route leading to apoptosis is the 'intrinsic' cell death pathway, activated by proapoptotic factors from the mitochondria, including cytochrome *c* and Apaf-1 [24,25].

We have demonstrated that propolin A and propolin B are cytotoxic to human melanoma A2058 cells, rat C6 glioma cells, and human leukemia cells (HL-60). In the present study, both propolin A and propolin B were found effectively induced cytotoxic effects of several cancer cell lines occur in a dose-dependent manner. Furthermore, we demonstrate that apoptosis induced by propolin A and propolin B in A2058 cells may through caspases-dependent pathways, and also that propolin A and propolin B can significantly inhibit xanthine oxidase activity, suggesting that they are strong antioxidants.

2. Materials and methods

2.1. Cell culture and cytotoxicity assay

Human melanoma cells (A2058), human breast cancer cells (MCF-7), human neuroblastoma cells (IMR-32), and rat glioma cells (C6) were cultured in Dulbecoo's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (Gibco) 1% penicillin–streptomycin. Cells were maintained at 37 °C in a humidified atmosphere at