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Inhibitory effect of *Andrographis paniculata* extract and its active diterpenoids on platelet aggregation

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Abstract

Andrographis paniculata has been widely used for the prevention and treatment of common cold especially in Asia and Scandinavia. The three active diterpenoids from this plant, including aqueous plant extracts, were investigated for the inhibitory effect on platelet aggregation *in vitro*. The results indicated that andrographolide (AP_1) and 14-deoxy-11,12-didehydroandrographolide (AP_3) significantly inhibited thrombin-induced platelet aggregation in a concentration-(1–100 μM) and time-dependent manner while neoandrographolide (AP_4) had little or no activity. AP_3 exhibited higher antiplatelet activity than AP_1 with IC_{50} values ranging from 10 to 50 μM. The inhibitory mechanism of AP_1 and AP_3 on platelet aggregation was also evaluated and the results indicated that the inhibition of extracellular signal-regulated kinase1/2 (ERK1/2) pathway may contribute to antiplatelet activity of these two compounds. In addition, standardized aqueous extracts of *A. paniculata* containing different amounts of AP_3 inhibited thrombin-induced aggregation to different degrees. The extracts significantly decreased platelet aggregation in a concentration-(10–100 μg/ml) and time-dependent manner. However, the extract with high level of AP_3 (Extract B) (IC_{50} values = 50–75 μg/ml) showed less inhibitory activity against thrombin than the extract with lower level of AP_3 (Extract A) (IC_{50} values = 25–50 μg/ml). These results indicate that the standardized *A. paniculata* extract may contain other antiplatelet compounds rather than AP_1 and AP_3, which contribute to high antiplatelet activity. Therefore, the consumption of *A. paniculata* products may help to prevent or treat some cardiovascular disorders i.e. thrombosis; however, it should be used with caution by patients with bleeding disorders.

Keywords: *Andrographis paniculata*; Diterpenoids; Inhibition; Aggregation; Thrombin; ERK1/2

1. Introduction

Andrographis paniculata (Burm. f.) Nees (Acanthaceae) has been used as a traditional medicine in India, China, Thailand, and Scandinavia. The aerial parts of the plant (leaves and stems) are normally used for extraction of the active phytochemicals. Extracts of the plant and their constituents have been reported to exhibit a wide spectrum of biological activities of therapeutic importance including antibacterial, antiviral (Chang et al., 1991; Calabrese et al., 2000), anti-inflammatory (Shen et al., 2002), antimalarial (Misra et al., 1992), immuno-stimulant (Puri et al., 1993; See et al., 2002), hepatoprotective (Kapil et al., 1993), antithrombotic (Zhao and Fang, 1991), anticancer (Matsuda et al., 1994), hypoglycemic (Zhang and Tan, 2000), and hypotensive (Zhang and Tan, 1996) properties. Standardized extract of *A. paniculata* (SHA-10) containing andrographolide and deoxyandrographolide has been used to reduce the prevalence and intensity of the symptoms in uncomplicated common cold (Caceres et al., 1997; Hancke et al., 1995; Melchior et al., 2000).

The active constituents of *A. paniculata* are diterpene lactones including andrographolide (AP_1), 14-deoxy-11,12-

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didehydroandrographolide (AP_3), neoandrographolide (AP_4) (Fig. 1). Andrographolide is considered to be the most active and important constituent in this plant. However, each component possesses some different potency in pharmacological activities. For instance, AP_1 shows high activity for anti-inflammation (Shen et al., 2000) and hepatoprotection against galactosamine and paracetamol intoxication (Chander et al., 1995), AP_3 produces a potent hypotensive effect (Zhang et al., 1998), whilst AP_4 has greater activity against malaria (Misra et al., 1992). The differences in pharmacological activities of these compounds may be related to their structures. For example, recent study on structure-activity relationships of andrographolide analogues and their pharmacological activities indicated that AP_3 synthesized from AP_1 inhibited α-glucosidase stronger than AP_1 itself (Dai et al., 2006). The flexible chain between the butyrolactone moiety and the two six-membered rings may be critical to α-glucosidase inhibitory activity. Therefore, they suggested that probably AP_3 might play an important role in the *A. paniculata* plant extract exerting antidiabetic activity. In addition, the different amounts of each active principle in this plant are also very important for the therapeutic efficacy or side effects of this plant. Our previous study reported that among these active compounds, the amount of AP_3 dramatically increased during storage of crude powders and extracts (Pholphana et al., 2004). An increase of AP_3 in the old plant materials may be due to the degradation of AP_1 during the storage time to yield AP_3 as the major degraded product. Lomlim et al. (2003) indicated that the crystalline form of AP_1 was highly stable at 70 °C (75% relative humidity) over a period of 3 months while its amorphous form degraded promptly during 2-months under the same condition. The major degradation product of AP_1 was AP_3. He et al. (2003) also reported that AP_3 is one of the AP_1 metabolites isolated from rat urine, faeces, and the content of the small intestine. The presence of this possible breakdown product in the stored plant extract may increase or reduce the pharmacological activities of this extract.

Platelet plays a key role in the physiological hemostatic process and pathologic thrombosis. AP_1 has been demonstrated to possess antiplatelet activity and to inhibit platelet activating factor (PAF)-induced human blood platelet aggregation in a dose-dependent manner (Amroyan et al., 1999). There is still no report comparing the antiplatelet activity of these pure compounds (AP_1, AP_3, and AP_4) isolated from this plant. The antiplatelet activity of these three compounds may be developed further to use as antiplatelet aggregation drugs to treat some cardiovascular diseases. However, when this plant is used for the treatment of common cold or taken with other antiplatelet or antithrombotic agents such as aspirin, the signs of platelet dysfunction should be also concerned as it may increase the risk of bruising and bleeding. In addition, if AP_3 or AP_4 also has significant antiplatelet activity, there will be a possibility that the adverse effects of long storage of dried powders or extracts may occur when this plant preparation is used. Therefore, the present study investigates the inhibitory effect of three active diterpenoids (AP_1, AP_3, AP_4) isolated from *A. paniculata* on platelet aggregation *in vitro* and some possible mechanisms involved in this inhibitory activity. Standardized extracts, both fresh and old harvest, containing different proportions of these compounds have also been investigated.

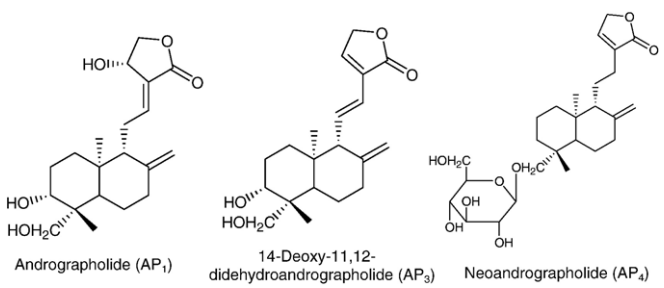

Fig. 1. Structures of andrographolide (AP_1), 14-deoxy-11,12-didehydroandrographolide (AP_3) and neoandrographolide (AP_4).

2. Materials and methods

2.1. Drugs and chemicals

Three standard diterpenoids, andrographolide (AP_1), 14-deoxy-11,12-didehydro-andrographolide (AP_3), and neoandrographolide (AP_4), were purified and identified using TLC, UV spectrum, IR and NMR by the Laboratory of Natural Products, Chulabhorn Research Institute, Thailand. Anti-phospho p-44/42 MAP Kinase (Thr202/Tyr204) antibody was purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-mouse Ig, horseradish peroxidase linked whole antibody was from Amersham Biosciences (Buckinghamshire,UK). Thrombin and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA), and pentobarbital was obtained from Vetbutal (Polfa, Poland). All other reagents were of analytical grade.

2.2. Preparation of plant extracts

A. paniculata plant was kindly identified by Dr. Wongsatit Chuakul and a voucher specimen was deposited at the Pharmaceutical Botany Mahidol Herbarium, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand (PBM 3760). Two dried *A. paniculata* raw materials were selected. One was recently harvested from Nakornpathom province (January, 2005), Extract A; and the other was another raw plant material stored at room temperature for 2 years (January, 2003), Extract B. These two plant materials were selected as they contained different level of only AP_3 while AP_1 and AP_4 were present at the same levels. Four hundred grams of aerial parts of *A. paniculata* were extracted with 4 l of hot water (70–75 °C) for 1 h. The extract was then filtered and collected. The residue was re-extracted twice with 4 l of hot water each time. The combined water extracts were lyophilized by using a freeze dry method. The %yield of extracts was between 27–30%. Five milligrams of plant extracts were dissolved in 5.0 ml of hot water (70–75 °C) and vigorously shaken. The extracts were left at room temperature until they cooled down and then filtered through a 0.45 μm nylon membrane (13 mm, Orange Scientific, Belgium) prior to