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Antiplatelet property of *Curcuma longa* L. rhizome-derived *ar*-turmerone

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Abstract

The antiplatelet activities of *Curcuma longa* L. rhizome-derived materials were measured using a platelet aggregometer and compared with those of aspirin as antiplatelet agent. The active constituent from the rhizome of *Curcuma longa* L. was isolated and characterized as *ar*-turmerone by various spectral analyses. At 50% inhibitory concentration (IC_{50}) value, *ar*-turmerone was effective in inhibiting platelet aggregation induced by collagen (IC_{50}, 14.4 μM) and arachidonic acid (IC_{50}, 43.6 μM). However, *ar*-turmerone had no effect on platelet activating factor or thrombin induced platelet aggregation. In comparison, *ar*-turmerone was significantly more potent platelet inhibitor than aspirin against platelet aggregation induced by collagen. These results suggested that *ar*-turmerone could be useful as a lead compound for inhibiting platelet aggregation induced by collagen and arachidonic acid.

Keywords: Antiplatelet Agents; *ar*-Turmerone; Collagen; *Curcuma longa* L.

1. Introduction

Platelet aggregation is a complex phenomenon that probably involves several intracellular biochemical pathways. Platelets activated by a number of physiological agonists, such as arachidonic acid, collagen, platelet activating factor, or thrombin, undergo a complex cascade of events that result in shape change, secretion, formation of arachidonic acid metabolites, and aggregation (Siess, 1997). Since platelets readily aggregate in response to a variety of endogenous substances and secrete various substances that cause further aggregation, they can initiate thrombus formation and precipitate thromboembolism, leading to ischemic diseases. In addition, the interactions between platelets and blood vessel walls are important in the development of thrombosis and cardiovascular diseases (Dinerman and Mehta, 1990; Hirsh, 1987; Ross, 1978). When blood vessels are damaged, platelet aggregation occurs rapidly to form haemostatic plugs or arterial thrombi at the sites of vessel injury or in regions where blood flow is disturbed. These thrombi are the source of thromboembolic complications of atherosclerosis, heart attacks, stroke, and peripheral vascular disease (Packham, 1994). Therefore, the inhibition of platelet function represents a promising approach for the prevention of thrombosis.

Plant extracts may be an alternative to currently used antiplatelet agents, because they constitute a rich source of bioactive chemicals (Cho et al., 2004; Lim et al., 2004). Since many of them are largely free from adverse effects and have excellent pharmacological actions, they could lead to the development of new classes of possibly safer and antiplatelet agents (Cho et al., 2004; Lim et al., 2004; Tsai et al., 2000). Additionally, some flavonoids and polyphenols are found to have effective inhibitory

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activities of platelet aggregation induced by collagen (Lin and Lu, 1996; Tsai et al., 2000). Therefore, much effort has been focused on the plant materials for potentially useful products as commercial antiplatelet agents or lead compounds. In East Asia, the rhizome of *Curcuma longa* L., belonging to the family Zingiberaceae, has long been considered to have natural medicinal properties such as, an analgesic in the treatment of menstrual disorders, rheumatism, and traumatic diseases because it contains a number of monoterpenoids, sesquiterpenoids, and curcuminoids (Tang and Eisenbrand, 1992). Furthermore, it has been noted that the materials of *C. longa* L. have fungicidal (Kim et al., 2003), insecticidal (Chander et al., 1991, 1992), repellent (Jilani and Saxena, 1990; Su et al., 1982), and antifeeding activities (Jilani and Saxena, 1990) against some stored-product insects (Chowdhury et al., 2000). The insect repellent and antifeeding constituents in *C. longa* L. are turmerones (Lee et al., 2001; Su et al., 1982) and curcuminoids (Chowdhury et al., 2000), respectively. However, relatively little work has been done on the inhibitory activities of platelet aggregation by the rhizome of *C. longa* L. in spite of the antiplatelet activities of *Curcuma aeruginosa* Roxb, *Curcuma domestica* Valeton, *Curcuma mangga* Valeton, and *Curcuma xanthorrhiza* Roxb (Jantan et al., 2004; Jantan et al., 2005). Active compound isolated from *C. longa* L. rhizome may be a good source for lead compound for antiplatelet agents.

2. Methods

2.1. Chemicals

Borneol, 1,8-cineole, and sabinene were purchased from Fluka Chemical Corp. (Milwaukee, WI, USA). Turmerone was kindly provided by Dr. Byeoung-Soo Park, Seoul National University, South Korea. Collagen, arachidonic acid, and thrombin were obtained from Chrono-Log Co. (Havertown, PA, USA). Platelet activating factor (PAF) was obtained from Sigma (St. Louis, MO, USA). Other chemicals were of analytical grade.

2.2. Extraction and isolation

Dried rhizome (3.1 kg) from *C. longa* L. was purchased from Boeun medicinal herb shop at Kyungdong Market (Seoul). It was finely powdered, extracted twice with methanol (5 l) at room temperature for two days and filtered. The combined filtrate was concentrated under vacuum at 35 °C to yield about 10.1% (based on the weight of the dried rhizome). The extract (313 g) was sequentially partitioned into hexane (115.7 g), chloroform (81.4 g), ethyl acetate (51.7 g), butanol (17.2 g), and water (47.0 g) for subsequent bioassay. The organic solvent portions were concentrated to dryness by rotary evaporation at 35 °C, while the water portion was freeze-dried. Active component of the hexane fraction was isolated as described by Lee et al. (2001).

Because of its good antiplatelet activity against collagen-induced platelet aggregation, the hexane fraction (10 g) was chromatographed on a silica gel column (Merck 70–230 mesh, 500 g, 6 i.d. × 67 cm), and successively eluted with chloroform/methanol (between 50:1 and 30:1). Column fractions were collected and analyzed by TLC (silica gel G). Fractions with a similar TLC pattern were pooled. The insecticidal fraction (1.6 g) was successively rechromatographed on a silica gel column, using with hexane/ethyl acetate (30:1). For further separation of the biologically active constituent, a preparatory HPLC (Spectra System P2000, Thermo Separation Products) was used. The column was μPorasil (19 i.d. × 300 mm, Waters) using hexane/ethyl acetate (200:1) at a flow rate of 4 ml min^{-1} and detected at 243 nm. Finally, a potent bioactive compound (105 mg) was isolated. Structural determination of the active isolate was made by spectroscopic analysis. ^{1}H and ^{13}C NMR spectra were recorded in deuterochloroform with a Bruker AM-500 spectrometer at 400 and 100 MHz, respectively. Chemical shifts were reported as δ values downfield from an internal standard of Me_4 Si. Mass spectra were obtained on a JEOL GSX 400 spectrometer.

2.3. Preparation of washed rabbit platelets

Platelet rich plasma was obtained from healthy male white rabbit blood, anticoagulated with a one-tenth volume of 1% EDTA by centrifugation at 230*g* for 10 min at room temperature. Platelets were sedimented by centrifugation of platelet-rich plasma (PRP) at 800*g* for 15 min and then washed twice with Hepes buffer (137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 5.6 mM glucose and 3.8 mM Hepes, pH 6.5) containing 0.35% bovine serum albumin and 0.4 mM EDTA. The washed platelets were resuspended in Hepes buffer (pH 7.4). The platelets number was counted by Coulter Counter (Coulter Electronics, Hialeah, FL, USA) and adjusted to a concentration of 3×10^8 platelets ml^{-1}.

2.4. Aggregation of washed rabbit platelets

Platelet aggregation was measured using an aggregometer (470-vs, Chrono-log Co. PA, USA) as previously described (Rho et al., 1996). Briefly, washed platelets (3×10^8 platelets ml^{-1}) were incubated at 37 °C in the aggregometer with various concentrations of samples for 3 min in the presence of 1 mM $CaCl_2$, then platelet aggregation was induced by addition of collagen (2 μg ml^{-1}), arachidonic acid (100 μM), thrombin (0.1 unit ml^{-1}), or PAF (10 nM). The resulting aggregation, measured as the change in light transmission, was recorded for 10 min. Each inhibition rate was obtained