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Short communication

Piperlongumine, a constituent of *Piper longum* L., inhibits rabbit platelet aggregation as a thromboxane A_2 receptor antagonist

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Abstract

Piper longum L. has been used as a crude drug for the treatment of the disorder of peripherally poor blood circulation in Asia. In the present study, we examined the effect of piperlongumine, a constituent of *P. longum* L., on rabbit platelet aggregation. Piperlongumine concentration-dependently inhibited platelet aggregation induced by thromboxane A_2 receptor agonist U46619, but it only slightly inhibited thrombin-induced one. Piperlongumine also inhibited U46619-induced phosphatidylinositol hydrolysis and the binding of [^{3}H]SQ29548 to thromboxane A_2 receptor with a similar concentration-dependency to the aggregation. It is assumed that piperlongumine inhibits platelet aggregation as a thromboxane A_2 receptor antagonist.

Keywords: Platelet aggregation; Piperlongumine; Thromboxane A_2 receptor

1. Introduction

Blood vessel wall injury triggers sudden platelet activation and platelet plug formation, followed by the occurrence of blood coagulation and the formation of fibrin-containing thrombi that occludes the site of injury. These events limit vital blood loss at a site of injured tissue. However, platelet plug and thrombi may often block narrow and diseased vessels, leading to ischemia and/or destruction in vital organs (Aronow, 2004; McNicol and Israels, 2003). In addition, platelet aggregation and subsequent thrombus formation occur in coronary and cerebral arteries, causing myocardial infarction and stroke, respectively (Stoyioglou and Jaff, 2004). The platelet aggregation is regulated by a lot of physiological agonists, such as collagen, platelet-activating factor, adenosine 5′-diphosphate (ADP) and thromboxane A_2 (TXA_2) (McNicol and Israels, 2003).

When TXA_2, a metabolite of arachidonic acid, is released from activated platelets, it binds TXA_2 receptors (prostanoid TP receptor) and causes platelet shape change and aggregation (Ohkubo et al., 1996), as a positive feedback mediator. Prostanoid TP receptor interacts with heterotrimeric G proteins, $G_{q/11}$, $G_{i/o}$ and $G_{12/13}$ (Djellas et al., 1999; Offermanns et al., 1994; Shenker et al., 1991; Ushikubi et al., 1994). It is known that the stimulation of prostanoid TP receptor results in platelet shape change mainly through $G_{12/13}$ pathway (Klages et al., 1999), and subsequent aggregation through $G_{q/11}$ pathway (Offermanns et al., 1997).

TXA_2-induced platelet aggregation is important for thrombus formation in vascular beds. At present, non-steroidal anti-inflammatory drugs (NSAIDs) and a TXA_2 synthase inhibitor are clinically used for reducing TXA_2-mediated platelet aggregation by decreasing TXA_2 synthesis in platelets, such as aspirin, indomethacin and ozagrel (Catella-Lawson et al., 2001; Kawano et al., 2001). Therefore, it is thought that the regulation of prostanoid TP receptor-mediated platelet aggregation is clinically important for thrombosis formation.

Piper longum L. has been used as a crude drug to improve intestinal disorder, asthma and peripherally poor circulation in Asia. In particular, the improving activity of the peripherally

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poor blood circulation is well known. On the other hand, it has been shown that platelet function affects the peripherally poor blood circulation through the platelet aggregation and thrombosis formation (Nagatome et al., 2005). *P. longum* L. contains various constituents, such as piperine, piperidine, pipernonaline and piperlongumine (Yang et al., 2002). Although piperine is a constituent of both *P. longum* L. and *Piper nigrum* L., piperlongumine is that of *P. longum* L., but not *P. nigrum* L.. In addition, the pharmacological effect of piperlongumine on platelet function remains to be solved. In the present study, we examined the effect of piperlongumine on rabbit platelet aggregation, and show that piperlongumine inhibits platelet aggregation as a prostanoid TP receptor antagonist.

2. Materials and methods

2.1. Materials

Piperlongumine (Fig. 1A) was purchased from Sigma-Aldrich (St Louis, MO, U.S.A). 9,11-Dideoxy-9α,11α-epoxymethanoprostaglandin $F_2\alpha$ (U46619) and [1*S*-[1α,2α (*Z*),3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazine]methyl]-7-oxabicyclo [2.2.1]hept-2-yl]-5-heptenoic acid (SQ29548) were obtained from Cayman Chemical Company (Ann Arbor, MI, U.S.A). Thrombin was from Wako Pure Chemicals (Osaka, Japan). [^{3}H] Inositol and [^{3}H]SQ29548 were from Perkin Elmer Life Science (Boston, MA, U.S.A). All other chemicals used were of reagent grade or the highest quality available.

2.2. Preparation of washed platelets

Fresh blood was obtained from male rabbits (Japanese white rabbits weighing about 2.5–3.5 kg), collected into plastic tubes containing acid citrate dextrose solution (1/6 volume of blood), composed of citric acid (65 mM), trisodium citrate (85 mM), and dextrose (2%). Then, the blood was centrifuged at 250 ×*g* for 12 min to obtain platelet-rich plasma. The platelet-rich plasma was centrifuged at 90 ×*g* to remove contaminated erythrocytes and leukocytes, and then centrifuged at 450 ×*g* for 15 min at room temperature (20–25 °C). The pellet was washed twice with Tyrode/HEPES solution (NaCl 138.3 mM, KCl 2.68 mM, $MgCl_2$ 1.0 mM, $NaHCO_3$ 4.0 mM, HEPES 10 mM, glucose 0.1% and bovine serum albumin 0.35% at pH 6.35). The resultant pellet was resuspended in the second Tyrode/HEPES solution (pH 7.35) with a final density of $3–5\times10^8$ platelets/ml (Ohkubo et al., 1996). All experimental procedures were performed in accordance with the guidelines of the Animal Experimentation Committee of Tohoku University Graduate School of Pharmaceutical Sciences.

2.3. Determination of platelet aggregation

Platelet aggregation was determined by a standard turbidometric method using an aggregometer (PAM-6C, Merbanix, Tokyo, Japan) (Ohkubo et al., 1996). The levels of light transmission were calibrated as 0% for a platelet suspension and 100% for the Tyrode/HEPES solution (pH 7.35). Platelet suspension (3×10^8 platelets/ml, 0.3 ml) in a cuvette was preincubated for 3 min and then with 1 mM $CaCl_2$ for 3 min at 37 °C under continuous stirring at 1000 rpm. After the further preincubation with piperlongumine for 5 min, platelet aggregation was monitored after the addition of U46619.

2.4. Measurement of inositol phosphates

Washed platelets in albumin-free Tyrode/HEPES solution (pH 7.35) were labeled with 25 μCi/ml [^{3}H]inositol at 37 °C for 1 h. Then, platelets were washed with albumin-containing Tyrode/HEPES solution (pH 7.35), and resuspended at 3×10^8 platelets/ml. After the preincubation for 10 min, platelets were treated with indicated concentrations of piperlongumine in the presence of LiCl (10 mM) for 5 min. Then, platelets were stimulated with U46619 for 15 min. The reactions were terminated by addition of equal volume of ice-cold 10% trichloroacetic acid (TCA). The TCA

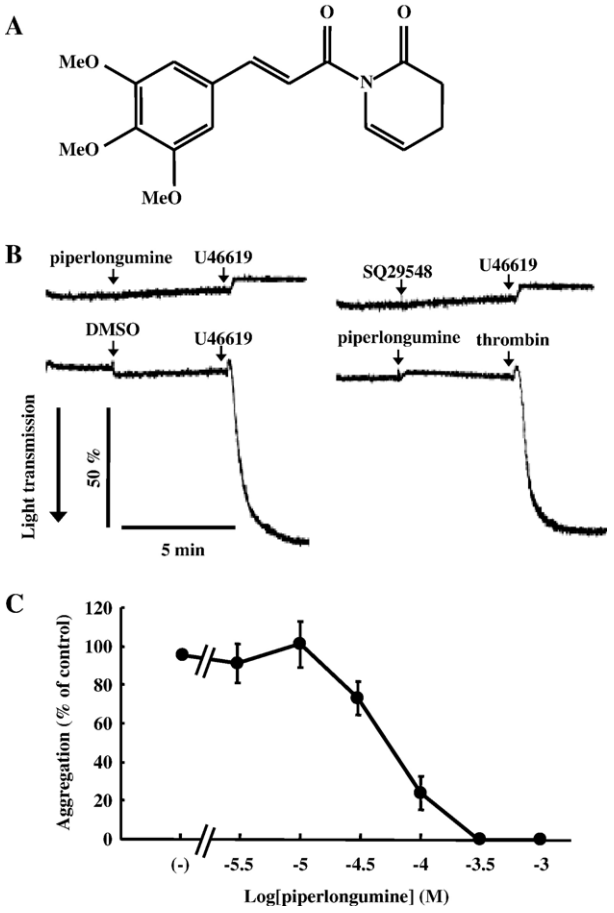

Fig. 1. Effect of piperlongumine on U46619-induced platelet aggregation. (A) Chemical structure of piperlongumine. (B) Characteristics of piperlongumine in platelet aggregation. Piperlongumine (100 μM), SQ29548 (3 μM) or DMSO (final concentration of 1%) was preincubated for 5 min before the addition of U46619 (3 μM) or thrombin (0.05 U/ml) in the presence of 1 mM $CaCl_2$, as shown with an arrow. (C) Concentration-dependent inhibition of U46619-induced platelet aggregation by piperlongumine. Piperlongumine (3–1000 μM) or DMSO (control) was preincubated for 5 min before addition of U46619 (3 μM) in the presence of 1 mM $CaCl_2$. The aggregation in the presence of piperlongumine was expressed as % of that in the absence of the compound. Results were means±S.E.M. for four individual experiments.