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Effects of Piperlongumine Isolated from *Piper longum* L. on Platelet Aggregation and VSMC Proliferation

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1. Introduction

Platelets play an important role not only in normal haemostasis but also in thrombosis at damaged blood vessels since thrombus formation occurs through the activation and aggregation of platelets [1]. Abnormal vascular smooth muscle cell (VSMC) proliferation and growth factors such as the platelet-derived growth factor (PDGF) play an important role in the development and progression of proliferative cardiovascular diseases, including hypertension, and atherosclerosis [2]. Therefore, inhibition of abnormal platelet aggregation and cell proliferation represents a promising approach for the prevention or treating of cardiovascular diseases such as thrombosis and atherosclerosis.

Plants constitute a rich source of bioactive chemicals such as phenolics, terpenoids and alkaloids [3]. Plant extracts may be an alternative to currently used medicinal source because they constitute a rich source of bioactive chemicals [4-6]. The survey of phytochemical investigations of *Piper* species has revealed a wide variety of chemical constituents produced by them, important classes among others being alkaloids/amides, lignans, neolignans and terpenes. The long pepper, *Piper longum* L., has been used in the Indian Ayurvedic system of medicine [7] and reveals numerous biological activities [8-11].

In the present study, we examined the effects of piperlongumine derived from *P. longum* on platelet aggregation and human aortic VSMCs proliferation, and investigated the inhibitory mechanism.

2. Methods

2.1. Effect on platelet aggregation

We examined the antiplatelet activity of piperlongumine derived from *P. longum*, and investigated the inhibitory mechanism related with AA pathway. The effects of piperlongumine on *in vitro* antiplatelet aggregation activity, *ex vivo* antiplatelet aggregation activity, *in vivo* antithrombotic activity, AA liberation, TXB_2, PGD_2 and 12-HETE generations, and TXA_2 synthase activity were investigated.

2.2. Effect on aortic VSMC proliferation

In the present study, we examined the antiproliferative effects of piperlongumine on human aortic VSMCs stimulated by PDGF-BB. The effects of ppiperlongumine on PDGF-BB-induced cell proliferatin, DNA synthesis, PDGF-Rβtyrosine-phosphorylation and its downstream intracellular signal pathway, such as ERK1/2, PI3'K/Akt and PLC-γ1, in human aortic VSMCs were investigated.

3. Results and Discussion

Piperine, pipernonaline, piperoctadecalidine and piperlongumine, four alkaloids derived from *P. longum*, inhibited *in vitro* rabbit platelet aggregation induced by collagen, AA and PAF, except for that induced by thrombin. Piperlongumine was the most potent in *in vitro* antiplatelet aggregation effect. Therefore, piperlongumine was further investigated the effects on *ex vivo* platelet aggregation and *in vivo* pulmonary thrombosis. The results of the present study, indicate that piperlongumine inhibited platelet aggregation in rats *ex vivo* and prevented death due to pulmonary thrombosis in mice *in vivo*. Accordingly, the present investigation was carried out to study the effects of piperine, a major constituent of *P. longum*, and piperlongumine, a potent antiplatelet and antithrombotic constituent of *P. longum*, on AA metabolism in order to elucidate a possible mechanism through AA pathway. To achieve our object, we investigated the effects of piperine and piperlongumin liberation of AA, generations of TXB_2, PGD_2 and 12-HETE, and TXA_2 synthase-activity. Our results suggest that antiplatelet effect of piperine may, at least partly, due to suppression of AA liberation and inhibition of TXA_2 synthase activity in combination with enhance the 12-HETE generation. However, piperlongumine weakly affected that AA pathway. Thus, other possible antiplatelet mechanisms are also currently under investigation.

Piperlongumine significantly inhibited the 50 ng/mL PDGF-BB-induced increase in DNA synthesis and cell number of human aoritc VSMCs. Whereas, Piperlongumine did not show any

cytotoxicity in human VSMCs in the experimental condition. These inhibitory effects were associated with inhibition of the PDGFβ-receptor phosphorylation, as well as downstream signal transduction pathway such as phosphorylation ERK1/2, PI3'K/Akt and PLC-γ1. These results suggest that piperlongumine has the anti-proliferative activity and the effect may be mediated by inhibition of the PDGF-BB-induced PDGFβ-receptor tyrosine-phosphorylation and its downstream intracellular signal pathway in human aortic VSMCs.

In conclusion, our results suggest that piperlongumine isolated from *P. longum* L. may be useful as a lead compound and new agents for the prevention or treating of cardiovascular diseases such as thrombosis and atherosclerosis.

Table 1. IC_{50} of the alkaloids isolated from *Piper longum* L. fruits on rabbit platelet aggregation.

Compounds	Collagen	AA	PAF
Piperine	213 ± 10.5	158 ± 12.2	134 ± 9.5
Pipernonaline	127 ± 8.6	166 ± 9.8	132 ± 19.6
Piperoctadecalidine	190 ± 12.4	258 ± 16.9	191 ± 20.4
Piperlongumine	16.3 ± 2.3	59.9 ± 6.5	53.1 ± 4.9
Aspirin	>200	35.6 ± 3.2	>200

Washed rabbit platelets were preincubated with samples, DMSO (0.5% control), aspirin at 37°C for 3 min in the presence of 1 mM $CaCl_2$, then platelet aggregation was induced by addition of collagen (2 μg/mL), AA (100 μM) and PAF (10 nM).

Table 2. Effect of piperlongumine on pulmonary thrombosis in mice.

	Dose (mg/kg)	No. of Dead or paralyzed /No. tested	Protection (%)
Control	0.5% CMC	41/49	16.33
Piperlongumine	50	25/48	47.92
Aspirin	50	18/46	39.93

Piperlongumine was orally administered 90 min before i.v. injection of epinephrine (13.2 μg/mouse) plus collagen (114 μg/mouse).

Fig. 1. Effect on arachidonic acid liberation induced by collagen.

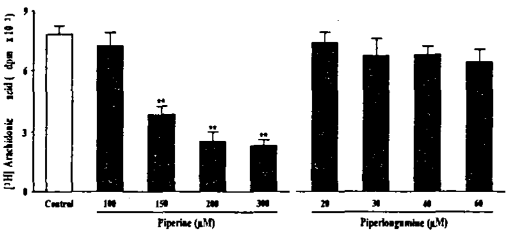


Fig. 2. Effect on TXB_2 generations.

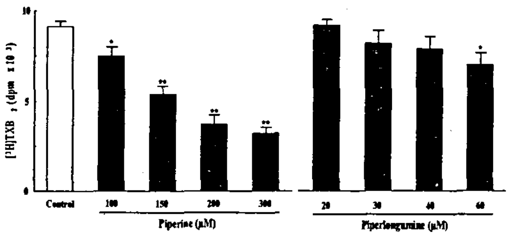


Fig. 3. Effect on PGD_2 generations.

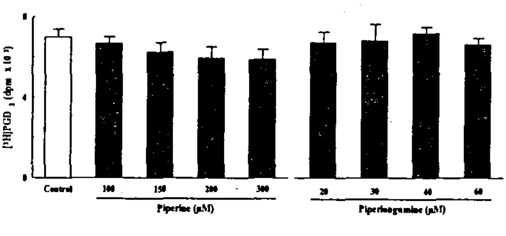


Fig. 4. Effect on 12-HETE generations.

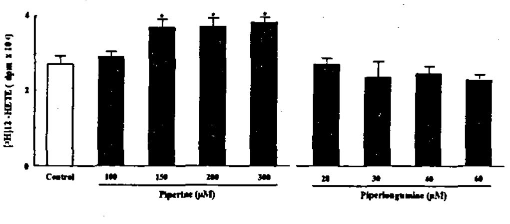


Fig. 5. Effect of piperine on TXA_2 synthase activity

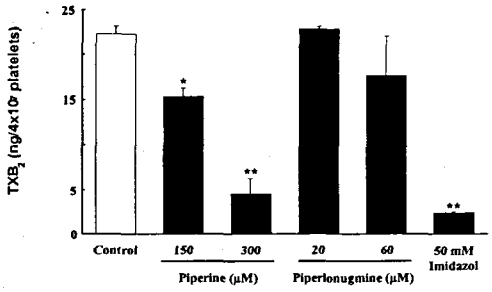


Fig. 6. Effect of piperlongumine on human vSMC proliferation

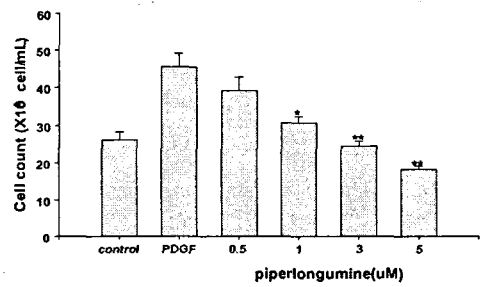
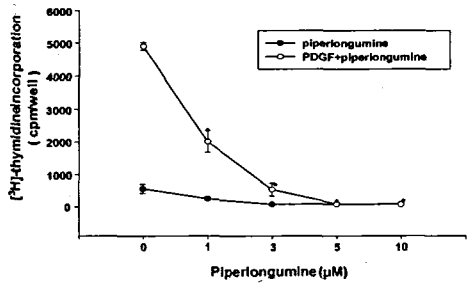
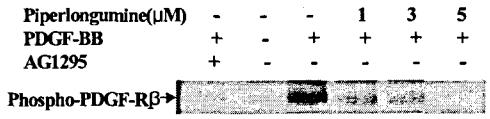
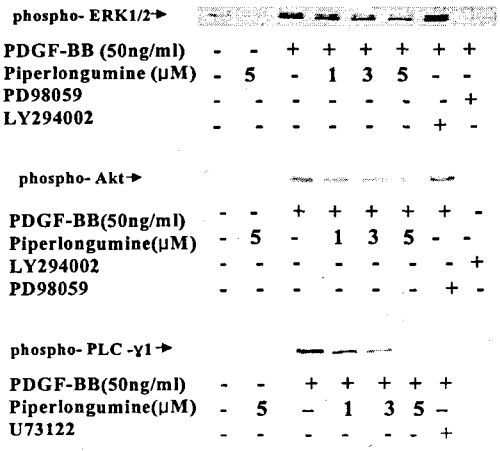


Fig. 7. Effect of piperlongumine on DNA synthesis of PDGF-BB-stimulated human vSMCs







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