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Effects of Green Tea Catechins on Human Platelet Aggregation and Experimental Thrombosis

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Introduction

Platelet aggregation is a complex phenomenon which probably involves several intracellular biochemical pathways. There is increasing evidence that internal Ca^{2+} stores play a central role in the response of platelets to activating agents. Platelet aggregation requires agonist-induced conformational change of platelet membrane GPIIb/IIIa, and the subsequent binding of fibrinogen to this activated GPIIb/IIIa complex. The interaction of fibrinogen with the GPIIb/IIIa receptor appears to be mediated by an Arg-Gly-Asp (RGD) sequence within the fibrinogen molecule. At approximately 50000 copies per platelet, GPIIb/IIIa is the most abundant glycoprotein on the platelet surface. The GPIIb/IIIa complex is calcium dependent, in that it dissociates into GPIIb and GPIIIa subunits at Ca^{2+} concentrations below about 1 μM. In addition, phosphoinositide breakdown is important for agonist-induced platelet activation. This breakdown generates two active products, diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3), and the latter triggers intracellular Ca^{2+} mobilization. Another important mediator is thromboxane (TX) A_2, which is the major cyclooxygenase product derived from arachidonic acid (AA). TXA_2 is a potent platelet aggregator, vasoconstrictor, and bronchoconstrictor.

Green tea is the dried young leaves of *Camellia sinensis*, also known as *Thea sinensis L.*, an infusion of which is widely consumed as a beverage. Green tea constituents, especially green tea catechins (GTC), exhibit a range of pharmacological effects including anticarcinogenic activity and prevention of cardiovascular diseases. In the present study, we investigated the antithrombotic effects and mechanism of GTC and EGCG, a major compound of GTC. The effects of GTC on the platelet aggregation *in vitro*, murine pulmonary thrombosis *in vivo*, rat platelet aggregation *ex vivo*, coagulation parameters, the binding of FITC-conjugated fibrinogen to platelet GPIIb/IIIa, thromboxane A_2 formation and intracellular free calcium levels in platelets were tested. The effect of

GTC on the second messenger, IP3 level was also examined.

Methods

1. Platelet aggregation assay was performed as Born and Cross described (1963).
2. Ex vivo platelet aggregation assay was performed as previously described (Yuk et al., 2000).
3. Calcium measurement (Kang et al., 2003).
4. IP_3 and TXB_2 formations were assayed by EIA kit.
5. GPIIb/IIIa binding assay were performed as previously described (Zhang et al., 2001).
6. In vivo antithrombotic assay were performed as DiMinno and Silver (1983) described.
7. Bleeding time measurement (Hornstra et al., 198)

Results and discussion

The results of antithrombotic activity show that GTC and EGCG prevent death due to pulmonary thrombosis induced by platelet aggregation in a dose-dependent manner. GTC and EGCG also prolonged the mouse tail bleeding time compared to control. Ex vivo study in rats indicate that GTC and EGCG show the inhibitory effects on platelet aggregation when administered orally. However, they did not change the coagulation parameters such as PT, APTT and TT. These results show that the antithrombotic activities of GTC and EGCG may be mediated by the inhibition of platelet aggregaand they may not directly act on the release of thromboplastin and/or thrombin formation.

In the in vitro study, GTC and EGCG inhibited human platelet aggregations induced by AA, ADP, collagen, epinephrine, and calcium iono, A23187, in a concentration-dependent manner. GTC significantly inhibited fibrinogen binding to human platelet surface GPIIb/IIIa complex, but failed to inhibit binding to purified GPIIb/IIIa complex. These results indicate that the antiplatelet activity of GTC may be due to inhibition of an intracellular pathway preceding GPIIb/IIIa complex exposure.Numerous reports suggest that Ca^{2+} is involved in major cellular processes such as vascular disorders and platelet activation, and a number of signaling mechanisms can cause an increase in cytosolic Ca^{2+} concentrations in platelets. A rise in cytosolic Ca^{2+} levels stimulates enzymes involved in platelet activation which are not fully functional at the low Ca^{2+} concentrations present in resting platelets. In the present study, GTC significantly inhibited platelet aggregation induced by collagen and thrombin, both of which increase cytosolic Ca^{2+} levels. GTC also inhibited platelet aggregation mediated by the calcium ionophore A23187, indicating that the observed effects of GTC may be mediated by impairment of Ca^{2+} influx. We also investigated the effects of GTC on intracellular calcium levels in platelets, thapsigargin-induced platelet aggregation and extracellular Ca^{2+}-mediated platelet aggregation. Pretreatment of human platelets with GTC for 8 min markedly inhibited the rise in

$[Ca^{2+}]i$ normally induced by thrombin. Thapsigargin is a potent inhibitor of the Ca^{2+} ion pump proteins located in the membranes of the sarcoplasmic and endoplasmic reticula of skeletal and cardiac muscle and brain tissue. Thapsigargin increases the $[Ca^{2+}]i$ by inhibition of Ca^{2+}-ATPase. GTC strongly inhibited thapsigargin-induced human platelet aggregation. GTC also inhibited extracellular calcium-mediated platelet aggregation induced by collagen. These results show that the inhibition of increases in $[Ca^{2+}]i$ by GTC may be due to inhibition of both calcium release from intracellular stores and calcium influx from outside the cell. GTC inhibited not only the release of intracellular Ca^{2+} but also the influx of extracellular Ca^{2+}. Hara et al. reported the hypotensive effect of tea catechins in rats, and we have previously reported the effects of green tea catechins on vascular smooth muscle tension and $^{45}Ca^{2+}$ uptake. These previous results support our current proposition that the antiplatelet activity of GTC may be due to blocking of calcium mobilization.

Moreover, IP_3, which is produced by phospholipase C, is involved in the release of calcium from the DTS following platelet aggregation, and the phosphoinositide breakdown induced by thrombin was significantly inhibited by GTC.

TXA_2, which is the major cyclooxygenase product derived from arachidonic acid (AA). is a potent platelet aggregator, vasoconstrictor and bronchoconstrictor. GTC also inhibited AA and collagen-induced TXA_2 formation, in a concentration-dependent manner.

Taken together, these observations suggest GTC and EGCG have the antithrombotic activities and the modes of antithrombotic action may be due to the anti-platelet activities rather than anticoagulation activi And the antiplatelet activity of GTC may be mediated by inhibition of TXA_2 and IP_3 formations as well as cytoplasmic calcium increase, which leads to the inhibition of fibrinogen-GPIIb/IIIa bindings.

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