PKC inhibitors RO 31-8220 and Gö 6983 enhance epinephrine-induced platelet aggregation in catecholamine hypo-responsive platelets by enhancing Akt phosphorylation

Sun Young Kim¹, Sewoon Kim², Jeong Mi Kim¹, Eek-hoon Jho², Seonyang Park³, Doyeun Oh⁴ & Hye Sook Yun-Choi¹,*
¹Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 151-742, ²Department of Life Science, University of Seoul, Seoul 131-743, ³College of Medicine, Seoul National University, Seoul 110-744, ⁴College of Medicine, CHA University, Seongnam 463-712, Korea

Impaired responsiveness of platelets to epinephrine (epi) and other catecholamines (CA) has been reported in approximately 20% of the healthy Korean and Japanese populations. In the present study, platelet aggregation induced by epi was potentiated by RO 31-8220 (RO) or Gö 6983 (Gö). Phosphorylated Akt (p-Akt) was very low in epi-stimulated PRP from CA-hypo-responders (CA-HY), whereas it was detected in those from CA-good responders (CA-GR). RO and Gö increased p-Akt, one of the major downstream effectors of phosphoinositol-3 kinase (PI3K), in epi-stimulated PRP from both groups. Wortmannin, a PI3K inhibitor, attenuated the RO or Gö-induced potentiation of p-Akt in epi-stimulated PRP, suggesting positive effects for RO and Gö on PI3K. TXA2 formation was increased by the addition of either RO or Gö in epi-stimulated platelets. The present data also suggest that impaired Akt phosphorylation may be responsible for epinephrine hypo-responsiveness of platelets. [BMB reports 2011; 44(2): 140-145]

INTRODUCTION

Circulating platelets are exposed to diverse tissue-releasing substances (1) and play a pivotal role in haemostasis and thrombosis (2). When platelets are activated by agonists such as adenosine diphosphate (ADP), U46619 (9, 11-dieoxy-11α, 9α-methanoepoxyprostaglandin Fα; thromboxane A2 mimetic), collagen, or thrombin, they aggregate and release their granule contents. Secreted granule contents such as ATP, ADP, serotonin, and calcium ion from dense granules as well as TXA2 formation are essential for the induction of secondary platelet aggregation (3, 4). The majority of platelet agonists are also known to activate phosphoinositol-3 kinase (PI3K) in platelets, and inhibitors of PI3K block fibrinogen binding and platelet aggregation. The lack of phosphorylated Akt, one of the major downstream effectors of the PI3K pathway, was reported to cause impaired platelet aggregation (3). Epinephrine (epinephrine) induces heterogeneous responses on human platelets. Impaired responsiveness of platelets to epinephrine and other catecholamines (CA) has been reported in approximately 20% of samples from healthy normal Korean and Japanese individuals (6, 7). The degrees of aggregation in response to other aggregation-inducing agents (AA, ADP, and U46619) are also significantly lower in platelets that are hypo-responsive to CA (8, 9). Elevated plasma concentrations of NO and cGMP have been observed in CA-hypo-responders (CA-HY) compared to CA-good responders (CA-GR) (10).

In this study, use of a widely used PKC inhibitor, RO 31-8220 (RO) increased epinephrine-induced platelet aggregation in platelet-rich plasma (PRP) from CA-GR. In addition, RO induced platelet aggregation in epinephrine-stimulated PRP from CA-HY, who are normally characterized by impaired platelet aggregation. The present study was undertaken to understand the effect of RO on epinephrine-stimulated human platelet aggregation and especially to identify the mechanism of their upregulatory effect on epinephrine-induced PRP from CA-HY.

RESULTS

RO 31-8220 and Gö 6983 potentiate platelet aggregation

The effects of five different commercially available PKC inhibitors were investigated on epinephrine-induced platelet aggregation in PRP obtained from both CA-GR and CA-HY. As shown in Fig. 1A and 1B, two PKC inhibitors, RO 31-8220 (RO), a non-selective inhibitor, and Gö 6983 (Gö), a PKC α, β, δ, γ, and ζ inhibitor, potentiated 0.1 uM epinephrine-induced platelet aggregation in PRP from CA-GR in a dose-dependent manner; RO and Gö alone did not induce platelet aggregation. Moreover, both RO and Gö induced platelet aggregation in 1 uM epinephrine-treated PRP from CA-HY. Normally, epi-
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Fig. 1. Representative tracings of the effects of RO 31-8220, Gö 6983, rottlerin, and Gö 6976 on epinephrine-induced human platelet aggregation. (A) PRP of CA-GR was stimulated by 0.1 μM epinephrine with or without RO 31-8220 (0.1, 1, and 10 μM) or Gö 6983 (0.1, 1, and 10 μM). (B) PRP of CA-HY was stimulated by 1 μM epinephrine with or without RO 31-8220 (0.1, 1, and 10 μM) or Gö 6983 (0.1, 1, and 10 μM). (C) PRP of CA-GR was stimulated by 1 μM epinephrine with or without rottlerin (1, 10, or 50 μM) and Gö 6976 (1, 10, or 50 μM). The tracings are representative figures of a minimum of three tests each of PRP from three individuals.

RO 31-8220 and Gö 6983 augment phosphorylation of Akt
The level of secreted ATP was measured to determine the effects of RO and Gö on granule secretion during platelet aggregation. ATP was not detected in either 0.1 μM epinephrine-stimulated PRP from CA-GR or 1 μM epinephrine-stimulated PRP from CA-HY, whereas negligible platelet aggregation was observed (Fig. 4A and B). Secretion of ATP was increased by RO and Gö in 0.1 μM epinephrine-stimulated PRP from both groups, as shown in Fig. 3A, B, and C.

RO 31-8220 and Gö 6983 potentiate TXA2 formation
TXA2 generation is essential for the induction of secondary platelet aggregation. The effects of RO or Gö on TXA2 production were observed during platelet aggregation. The formation of TXA2 was determined by measuring TXB2, the stable metabolite of TXA2. As shown in Fig. 4C, TXA2 production was increased by the addition of 1 μM epinephrine to PRP from CA-GR, whereas TXA2 formation was negligibly increased compared to control platelets in 0.1 μM epinephrine-stimulated PRP from CA-GR, and TXA2 formation from both CA-GR and 1 μM epinephrine-stimulated PRP from CA-HY. Upon addition of RO or Gö, TXA2 formation was enhanced in 0.1 μM epinephrine-activated PRP from both CA-GR (141.32 ± 0.3550 or 146.32 ± 0.0135 vs 46.321 ±