GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Effect of Diquertin on Platelet Content of Cyclic Nucleotides

A. A. Kubatiev, Z. T. Yadigarova, I. A. Rud'ko, V. A. Bykov, and N. A. Tyukavkina

Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, *Vol. 128, No. 9, pp. 267-269, September, 1999*
Original article submitted January 14, 1999

The effects of diquertin on the content of cyclic nucleotides in human platelets was studied. Diquertin in a concentration of 5 mM increased the content of cAMP and cGMP in native and thrombin-activated platelets probably due to inhibition of phosphodiesterase. Increasing the concentration of diquertin above 5 mM did not potentiate this effect. The antiaggregation effect of diquertin was probably associated with the increase in platelet levels of cyclic nucleotides.

Key Words: *flavonoids; diquertin; platelet; cyclic nucleotides*

Flavonoids are natural plant compounds [8]. Diquertin or dihydroquercetin (DQC, 3,3',4',5,7-pentahydroxyflavanone) is a new preparation (Russia) obtained from shredded larch wood of *Larix dahurica T.* and *Larix sibirica L.* [1]. Flavonoids inhibit enzymes involved in cell activation (protein kinase C, tyrosine kinase, and phospholipase A_2). Some flavonoids are strong inhibitors of cyclic nucleotide phosphodiesterase [2,7]. The most potent phosphodiesterase inhibitors display an antiaggregation effect on human platelets *in vitro*.

Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are second messengers suppressing platelet activation. Cyclic nucleotides inhibit phospholipase C [5], reduce platelet content of cytoplasmic Ca^{2+} [4], and activate Ca^{2+}-ATPase [6]. cAMP reduces platelet content of cytoplasmic Ca^{2+} enhancing its intracellular deposition and efflux from cells [4,6]. cGMP inhibits agonist-induced Ca^{2+} mobilization from intracellular stores and Ca^{2+} entry, attenuates phospholipase C activation, and modulates protein phosphorylation [3].

Department of General Pathology and Pathophysiology, Russian Academy of Postgraduate Education, Moscow

Taking into account the role of cyclic nucleotides in platelet activation, we studied the effect of DQC on the content of cAMP and cGMP in human platelets.

MATERIALS AND METHODS

Venous blood from 20 fasting donors (12 women and 8 men) was collected into tubes with 3.8% sodium citrate (9:1 ratio). Platelets were isolated by centrifugation at 190*g*.

DQC dissolved in DMSO to final concentrations of 1, 5, and 10 mM was added to the platelet suspension and incubated at 37°C and constant agitation for 2 min. Absolute ethanol and HCl were added to the suspension, mixed, and stored at -20°C. To analyse the effect of DQC on cAMP and cGMP contents in activated platelets, the suspension of native platelets was incubated with various concentrations of DQC at 37°C and constant agitation for 2 min, thrombin (0.5 U/ml) was added, and platelet aggregation was recorded for 5 min. The reaction was stopped with absolute ethanol and HCl. The samples were mixed and stored at -20°C.

Platelet contents of cAMP and cGMP were estimated in supernatants after ethanol extraction using enzyme immunoassay test systems for quantitative analysis of cAMP and cGMP (Bioimmunogen, Russia).

0007-4888/99/0009-0890$22.00

TABLE 1. Effects of DQC on Platelet Contents of cAMP and cGMP (pmol/ml)

DQC, mM	cAMP		cGMP	
	basal	induced by thrombin (0.5 U/ml)	basal	induced by thrombin (0.5 U/ml)
Control	59.2±2.2	40.6±1.5	34.9±2.6	22.1±2.6
1	62.6±7.7	47.4±2.3	36.9±2.2	24.8±2.5
5	73.4±2.0*	62.2±2.2*	52.2±1.7**	47.3±2.2*
10	80.7±3.0**	77.6±2.3*	56.9±1.9**	51.6±1.9*

Note. *$p<0.05$ and **$p<0.01$ compared with the control.

The results were analyzed by Student's t test.

RESULTS

In a concentration of 1 mM, DQC had practically no effect on platelet content of cAMP. Platelet cAMP level increased after incubation of platelets with 5 and 10 mM DQC and did not differ from the control after thrombin-induced platelet aggregation in the presence of 1 mM DQC. However, 5 mM DQC elevated the content of cAMP in the suspension of thrombin-activated platelets. After incubation with 5 mM DQC, cAMP content was the same in thrombin-activated and native platelets. Similar results were obtained after incubation of platelets with 10 mM DQC (Table 1).

DQC in a concentration of 1 mM had no significant effect on the content of cGMP in native and thrombin-activated platelets. In the presence of 5 mM DQC, the level of cGMP in native and thrombin-activated platelets significantly increased compared with the control. Increasing the concentration of DQC to 10 mM did not potentiate the rise in cGMP in native and thrombin-activated platelets in comparison with 5 mM DQC. However, cGMP content in native and thrombin-activated platelets in the presence of 10 mM DQC exceeded the control levels (Table 1).

These results indicate that DQC in a concentration of 5 mM elevated cAMP and cGMP contents in native and thrombin-activated platelets.

Platelet content of cyclic nucleotides depends on the ratio between activities of anabolic and catabolic enzymes. Degradation of cyclic nucleotides is catalyzed by phosphodiesterases. Platelets contain various phosphodiesterases: cGMP-activated cAMP phosphodiesterase activating hydrolysis of cAMP and cGMP, cGMP-inhibited cAMP phosphodiesterase with a low dissociation constant, and cGMP-specific phosphodiesterase [9]. Various substances increasing platelet level of cAMP via activation of adenylate cyclase (prostaglandin E_1, prostacyclin, and forskolin) stimulate cAMP phosphodiesterase in platelets and trigger the feedback mechanism regulating platelet content of cAMP. However, nitrovasodilators contributing to the formation of cGMP via activation of soluble guanylate cyclase lead to a dose-dependent increase in the content of cAMP in platelets, because cGMP decreases the intensity of cAMP catabolism suppressing cGMP-inhibited cAMP phosphodiesterase.

Myricetin and quercetin reduce the content of cAMP under the effects of prostacyclin [7]. These flavonoids are believed to increase the concentration of cAMP via inhibition of phosphodiesterase activity. The increase in cAMP and cGMP levels in native and thrombin-activated platelets is probably due to DQC-induced phosphodiesterase inhibition. The increase in the concentration of DQC above 5 mM was not accompanied by further increase in platelet levels of cyclic nucleotides.

Thus, the antiaggregation effect of DQC probably results from the increase in platelet content of cyclic nucleotides.

REFERENCES

1. N. A. Tyukavkina, V. A. Khutoryanskii, B. N. Bazhenov, *et al., Russian Inventor's Certificate No. 2088256, Preparation for Complex Therapy of Diseases (Diquertin) and Method for Its Obtaining* [in Russian], *Byull. Izobret.*, No. 24 (1997).
2. A. Beretz and J. P. Cazenave, *Planta Med.*, **57**, No. 7, S68-S72 (1991).
3. R. Deana, M. Ruzzene, M. G. Doni, *et al., Biochim. Biophys. Acta*, **1014**, 203-206 (1989).
4. M. G. Doni, L. Cavallini, and A. Alexandre, *Biochem. J.*, **303**, 599-605 (1994).
5. M. G. Doni, R. Deana, S. Bertoncello, *et al., Biochem Biophys. Res. Commun.*, **156**, 1316-1323 (1988).
6. J. S. Johansson, L. E. Nied, and D. H. Haynes, *Biochim. Biophys. Acta*, **1105**, 19-28 (1992).
7. R. Landolfi, R. L. Mower, and M. Steiner, *Biochem. Pharmacol.*, **33**, No. 9, 1525-1530 (1984).
8. E. Middleton, *Adv. Exp. Med. Biol.*, **439**, 175-182 (1998).
9. S. Sheth and R. Colman, *Semin. Hematol.*, **31**, 110-119 (1995).