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Effect of dipyridamole and its monoglucuronide derivative on adenosine uptake by human platelets and ADP-induced platelet aggregation

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Dipyridamole, a vasoactive substance, is a potent inhibitor of adenosine uptake by human platelets [1, 2]. The drug also inhibits platelet aggregation [2, 3] and release reaction *in vitro* [2]. Several clinical and experimental studies suggested that dipyridamole alters platelet function *in vivo* [4–6]. Recently it was shown that dipyridamole is more active *in vivo* than under *in vitro* conditions [7]. A possibility therefore exists that dipyridamole is less potent than its metabolite. Previously, it was shown that dipyridamole is readily metabolized into its mono- and diglucuronide derivatives within 30 min after the intravenous administration of the drug [8]. We, therefore, compared the inhibitory potencies of dipyridamole and its monoglucuronide derivative on ADP-induced platelet aggregation of washed platelet suspension as well as on adenosine uptake by platelets. We found that the monoglucuronide derivative of dipyridamole is less active than the parent compound.

Dipyridamole (Persantin®) and its monoglucuronide derivative were gifts from Pharma Research Canada, Montreal and Dr. K. Thomae, Biberach, Germany. Stock solution of dipyridamole monoglucuronide (1 mM, pH 6.5) was made in 0.15 M NaCl. Dipyridamole (1 mM) was dissolved in 0.1 N HCl, then the solution was adjusted to pH 4.3 with 0.1 N NaOH. Further dilutions were made in 0.15 M NaCl. The pH of the platelet suspending medium after addition of these solutions was between 7.3 and 7.4. The [^{14}C]8-adenosine (59 mCi/m-mole) purchased from Amersham/Searle, (Arlington Heights), was used to prepare a stock solution of 1 μM in 0.03 M Tris buffer, pH 7.2 (Trizma-HCl, Sigma, St. Louis, Mo). Washed human platelets (10^9 cells/ml) were prepared according to Mustard *et al.* [9]. The inhibitory effect of dipyridamole and its derivative on ADP-induced platelet aggregation was studied as described before [10]. The adenosine uptake by washed platelets was determined with 1.0 ml samples containing 0.81 ml washed platelet suspension (10^9 cells/ml), 0.1 ml of the tested compound and 0.1 ml of 1 μM [^{14}C]8-adenosine stock solution. The platelets were incubated with [^{14}C]8-adenosine for 5 min at 37° and centrifuged for 2 min at 7,600 *g* and 22° (Eppendorff centrifuge, Brinkman Instruments). The radioactivity in platelet pellet and supernates was estimated after mixing with 15 ml scintillation fluid containing triton X-100, by using liquid scintillation counter. The percent of total [^{14}C]adenosine radioactivity in platelet pellet was estimated from the radioactivity measured in platelet pellet and the supernatant. It has been found that these conditions for measurements of adenosine uptake are not rate limiting. The uptake of adenosine by platelets is a slow process reaching equilibrium after 30–60 min incubation. In fact, values obtained after 5 min incubation measure the initial rate of uptake.

The effect of dipyridamole and its monoglucuronide derivative on the ADP-induced platelet aggregation of washed platelets is shown in Table 1. Under identical conditions, dipyridamole exerted greater inhibition on platelet aggregation than its glucuronide derivative. Correspondingly, the potency of dipyridamole for 50 per cent of inhibition of adenosine uptake by platelets is 200-fold higher than its monoglucuronide derivative (Fig. 1).

We have demonstrated previously that dipyridamole forms complexes with acid glycoproteins of human plasma that interfere with the inhibitory effects of this compound on ADP-induced platelet aggregation [10], and adenosine uptake (unpublished observation). Therefore, it seemed interesting to compare binding of dipyridamole and dipyridamole monoglucuronide to acid glycoproteins. This has been done as described previously [10]. In brief, 0.2 ml of 3% Cohn Fraction VI (Pentex Biochemicals, Kankakee, Ill.) used as a source of acid glycoproteins was mixed either with 0.2 ml dipyridamole (2×10^{-4} M), or with 0.2 ml dipyridamole monoglucuronide (2×10^{-4} M) and applied to Sephadex G 25 column. Protein bound and pro-

Table 1. Effect of dipyridamole and dipyridamole monoglucuronide on the ADP-induced aggregation of washed human platelets*

	Light transmission increase (LTU)	
Concentration of compound†	Dipyridamole	Dipyridamole Monoglucuronide
10^{-4} M	0.2 ± 0.2	2.3 ± 0.7
10^{-5}	1.2 ± 0.5	2.6 ± 0.2
None	3.2 ± 1.1	3.2 ± 1.1

* Aliquots, 0.1 ml each of the tested compound or 0.9% NaCl were incubated with 0.7 ml platelet suspension (10^9 platelets/ml) for 1 min at 37° with constant stirring in Payton aggregometer (Payton Associates, Scarborough, Ont.). Then 0.1 ml of 0.5% human fibrinogen (Kabi, Stockholm, Sweden) and 0.1 ml ADP (10^{-4} M) were added and aggregation was revealed by changes in light transmission and expressed in arbitrary light transmission units (LTU). The data represent mean values and standard deviations from 4 experiments.

† Values refer to the final concentration of the drug in platelet suspension.

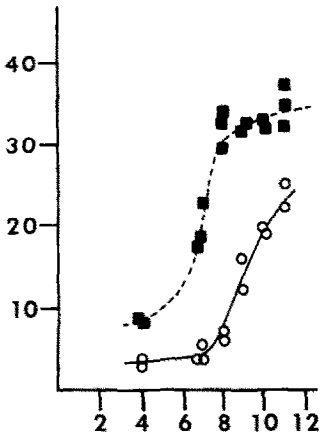

Fig. 1. The concentration dependent effect of dipyridamole (○——○), and its monoglucuronide derivative (■——■) on [^{14}C]8-adenosine uptake by platelets. Washed human platelets of 0.8 ml (10^9 cells/ml) in a total vol. of 1 ml were incubated with varying concentrations of dipyridamole or its derivative for 5 min at 37° before the addition of 0.1 μM [^{14}C]8-adenosine. The total radioactivity of platelet pellet and supernatant of each sample was determined after incubating the platelets with [^{14}C]8-adenosine for 5 min at 37°. Abscissa:-log concentration of the tested compound (M); ordinate: percent of the total ^{14}C-adenosine radioactivity. The uptake of adenosine without drugs was 30 percent. The concentration of the drug and its derivative required to produce 50 percent of inhibition on adenosine uptake by platelets are 0.1 μM and 0.5 nM, respectively.

tein-free ligands were eluted by means of 0.05 M Tris buffer, pH 7.2. The concentration of both ligands in the eluted samples was estimated by measuring absorbancy at 415 nm. It has been found that under these experimental conditions, the percentages of dipyridamole and dipyridamole monoglucuronide bound to protein were 43 and 42, respectively.

This experiment suggests that dipyridamole monoglucuronide and dipyridamole have the same affinity to plasma acid glycoproteins. Therefore, it cannot be expected that dipyridamole monoglucuronide might have stronger antiplatelet activity in the plasma than the parent compound. Accordingly, we found in 5 experiments that dipyridamole monoglucuronide (10^{-4} M) added to platelet rich plasma did not inhibit ADP-induced platelet aggregation at all while dipyridamole, at the same concentration, caused 23 ± 1.8 per cent inhibition.

These results indicate that the increased potency of dipyridamole under *in vivo* conditions is not due to its conversion to monoglucuronide derivative. The results also suggest that the hydroxyl group of dipyridamole linked to glucuronic acid is involved in the pharmacological action of the drug since it is not available when converted into a glucuronide ester. The decreased potency of the derivative can also result from the diminished binding of the glucuronide derivative to platelets since it was shown that the binding of dipyridamole to these cells is required for its pharmacological action [11].

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Specialized Center for Thrombosis Research, Department of Medicine and Physiology, Temple University Health Sciences Center, Philadelphia, PA 19140, U.S.A.

KUCHIBHOTHLA SUBBARAO
BOGUSLAW RUCINSKI
STEFAN NIEWIAROWSKI

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