Binding of Dipyridamole to Human Platelets and to α_1 Acid Glycoprotein and its Significance for the Inhibition of Adenosine Uptake

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ABSTRACT The interactions of dipyridamole with α_1 acid glycoprotein of plasma and with human platelets are related to inhibition of adenosine uptake by platelets. Binding studies by equilibrium gel filtration suggested that 1 mol of dipyridamole binds per mol of α_1 acid glycoprotein with a dissociation constant of 1.6 μM. Platelets contain two populations of binding sites, one with high and another with lower affinity for the drug. The binding of dipyridamole to the high-affinity sites follows a Michaelis-Menten binding pattern with a dissociation constant of 0.04 μM. Approximately 2×10^4 dipyridamole molecules are bound at the high-affinity sites of each platelet. The lower affinity sites bind the drug with a dissociation constant of 4 μM. In the presence of α_1 acid glycoprotein of plasma, the binding of dipyridamole to human platelets is inhibited. Correspondingly, the dipyridamole inhibition of adenosine uptake by platelets is reduced 1,000-fold by purified α_1 acid glycoprotein. The binding of dipyridamole to human platelets was found to be essential for its inhibition of adenosine uptake by platelets. Dipyridamole decreases the incorporation of [^{14}C]adenosine radioactivity in platelet nucleotides and reduces the [^{14}C]-ATP to [^{14}C]ADP ratio. Purified α_1 acid glycoprotein reverses these effects of dipyridamole on adenosine metabolism of platelets in a concentration-dependent manner. An equilibrium of dipyridamole binding to α_1 acid glycoprotein and to platelets is proposed.

INTRODUCTION

A number of clinical and experimental studies have suggested that dipyridamole, a vasoactive substance, can function as an antiplatelet and antithrombotic agent (1–4). In addition to these in vivo observations, inhibition of ADP-induced platelet aggregation and release reaction in vitro by dipyridamole has been observed (5, 6). The drug was previously shown to inhibit platelet phosphodiesterase (7–9) and the uptake of glucose (6), and adenosine (10–12) by platelets. Dipyridamole also potentiated the inhibitory effect of adenosine on ADP-induced platelet aggregation (7, 8, 13), and it was proposed that this is due to accumulation of adenosine which activates the platelet adenylate cyclase (14). These observations suggested a relationship between adenosine uptake by platelets and inhibition of ADP-induced platelet aggregation. Further studies on the mechanism of adenosine uptake by platelets and its inhibition by dipyridamole may provide a better understanding of platelet aggregation.

We previously reported that the degree of inhibition of ADP-induced platelet aggregation by dipyridamole was much greater with washed platelet suspension than with platelet-rich plasma (PRP)[1] (15). This difference in potency was attributed to α_1 acid glycoprotein (α_1 acid GP) of the plasma which interferes with the antiplatelet activity of dipyridamole (15).

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[1] *Abbreviations used in this paper*: α_1 acid GP, α_1 acid glycoprotein; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RA 233, dipyridamole derivative 2,6(diethanol amino)-4 piperidino(5-4d)pyrimidine.

Thus, the α_1 acid GP has been implicated in the modification of dipyridamole function as an inhibitor of adenosine uptake by platelets. In a preliminary communication we reported the inhibition of adenosine uptake and aggregation when dipyridamole is bound to platelets (16). We showed that binding of dipyridamole to washed platelets at 37°C reaches a maximum within 1 min of incubation and is not affected by temperature (4°–50°C) and pH (6–8) of the incubation mixture (16). Results concerning the metabolism of [^{14}C]adenosine in platelets in relation to the binding of dipyridamole to platelets and to α_1 acid GP are reported.

METHODS

Materials. Nonradioactive and [^{14}C]dipyridamole and dipyridamole derivative, RA 233 (specific radioactivity 7.3 μCi/mg) were gifts from Pharma Research Canada Ltd., Quebec, Canada. [U-^{14}C]adenosine (557 mCi/mmol), [U-^{14}C]-adenine (225 mCi/mmol), and [8-^{14}C]adenosine (59 mCi/mmol) were from Amersham/Searle, Arlington Heights, Ill. ^{125}I-Albumin (0.93 μCi/mg protein) was from E. R. Squibb, & Sons, Princeton, N. J. Sephadex G-25 and Sepharose 2B were obtained from Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J. Analysis of adenosine by paper electrophoresis (17) and dipyridamole by thin-layer chromatography followed by UV spectroscopy (18) showed about 97% purity.

Purification of α_1 acid GP. The α_1 acid GP was isolated from Cohn fraction VI of pooled normal human plasma, and the homogeneity of the purified protein was established (19–21).

Binding of dipyridamole to α_1 acid GP. A Sephadex G-25 column (0.9 × 15 cm) was equilibrated with 6.5 μM [^{14}C]dipyridamole in 0.02 M Tris buffer, pH 7.2, at 27°C. Purified α_1 acid GP (19 nmol) was applied to the column after mixing with [^{14}C]dipyridamole (0–33 nmol) in a final volume of 0.5 ml and then 1.0-ml fractions were collected and analyzed for radioactivity. The areas under peaks of each elution profile were determined by integration of the area by making use of approximate equations as derived from a nonlinear regression analysis made by IBM 360 computer program (IBM Corp., White Plains, N. Y.). The molar ratio of α_1 acid GP and dipyridamole in the complex was estimated according to Hummel and Dreyer (22).

Isolation of platelets. Washed human platelets and PRP were prepared according to Mustard et al (23). The platelet suspension was kept at 37°C and used within 2–3 h after preparation.

Binding of [^{14}C]dipyridamole and [^{14}C]RA 233 to human platelets. 0.8-ml Aliquots of washed human platelet suspension or PRP (10^9 cells/ml) were incubated with specified concentrations of [^{14}C]dipyridamole or [^{14}C]RA 233 in a final volume of 1.0 ml for 5 min at 37°C and centrifuged at 12,000 *g* for 2 min at room temperature with an Eppendorf centrifuge (Brinkmann Instruments Inc., Westbury, N.Y.). Subsequently, the radioactivity of platelet pellet and supernate of each sample was determined. The recovery of total radioactivity was about 90%. The effect of purified α_1 acid GP was tested with platelet suspension incubated with α_1 acid GP of specified amount for 10 min at 37°C before the addition of varying concentrations of [^{14}C]dipyridamole. When heat-inactivated (80°C) platelets were used, the radioactivity in the platelet pellet was < 1–2%.

Sepharose 2B gel filtration of washed human platelets preincubated with dipyridamole. Washed human platelets (3.5 ml, 10^9 cells/ml) were incubated with [^{14}C]dipyridamole (1 μM) in a final vol of 4 ml for 5 min at 37°C and passed through a Sepharose 2B column (1 × 25 cm) equilibrated with calcium-free buffer (24). 1.0-ml fractions were collected and analyzed for platelets (with Coulter counter, Coulter Electronics Inc., Hialeah, Fla.) and radioactivity. In another set of experiments, 3.5 ml (2 × 10^9 cells/ml) of washed human platelet suspension was incubated with dipyridamole (10 μM) in a final vol of 4.0 ml at 37°C and subjected to gel filtration. The platelet concentration of the pooled fractions containing >10^9 cells/ml was adjusted to 10^9 cells/ml with equilibrating buffer and used for the determinations of [8-^{14}C]adenosine uptake.

Uptake of [^{14}C]adenosine by human platelets. Washed human platelet suspension or PRP of 0.8 ml (10^9 cells/ml) was incubated with [8-^{14}C]adenosine (0.1 μM) at 37°C for 5 min in a final volume of 1.0 ml. The percent of total radioactivity in the platelet pellet was estimated as described for the binding of [^{14}C]dipyridamole to platelets. Similar uptake measurements were made using platelets incubated with varying concentrations of dipyridamole for 5 min or α_1 acid GP for 10 min at 37°C. In another set of experiments, platelets were incubated with specified amounts of purified α_1 acid GP for 10 min before the addition of dipyridamole.

Incorporation of [^{14}C]adenosine into platelet adenine nucleotides. Washed human platelets or PRP (0.7 ml, 10^9 cells/ml) were incubated for 5 min at 37°C with uniformly labeled adenosine (0.1 μM) in a total volume of 1.0 ml. Aliquots of samples were mixed with equal volumes of 90% ethanol containing 7.7 mM EDTA (pH 7.4) and analyzed for adenosine metabolites by high voltage paper electrophoresis (17). The radioactivities of adenosine metabolites were estimated using washed platelet suspension and its supernate, or PRP and platelet-poor plasma (PPP). The radioactivity of adenosine metabolites in platelet pellet was estimated after subtracting the values of PPP and supernate respectively from PRP or washed platelet suspension. The estimated adenylate energy charge values of platelets incubated with [^{14}C]adenosine and [^{14}C]adenine were 0.80 and 0.90, respectively.

The radioactivity determinations were made using Intertechnique scintillation spectrometer, model SL 40 (Intertechnique, Dover, N. J.). Scintillation fluid (15 ml/sample) consisted of 0.55% PPO and 0.01% POPOP in toluene, Triton X-100 (1:1, vol/vol). The counting efficiency was 70%.

Calculations. The binding constants (K_a) for the interaction of dipyridamole with platelets were estimated using the equation (25, 26):

$$Cb/Cf = K_a(np\text{-}Cb).$$

Where Cb is the molar concentration of [^{14}C]dipyridamole bound to platelets at equilibrium; Cf, that of unbound drug; K_a the apparent association constant of the binding; n, the number of binding sites per platelet and p is the platelet concentration. A plot of Cb/Cf as a function of Cb (molar concentration) resulted in a line with a negative slope of K_a, an intercept on the ordinate of Knp, and an intercept on the abscissa of np.

From the estimated values of free dipyridamole in samples containing a constant amount of α_1 acid GP and varying [^{14}C]dipyridamole concentrations, the apparent K_a of dipyridamole with α_1 acid GP was calculated according to Koche-Weser and Sellers (27). The fraction of binding sites of platelet or that of α_1 acid GP, occupied by the drug at a

particular concentration of dipyridamole was estimated using the equation described by Paton (28).

$$Pe = \frac{X}{X + K}.$$

Where Pe is the fraction of binding sites occupied: X is the concentration of dipyridamole and K is equilibrium constant or dissociation constant (K_d).

RESULTS

Binding of dipyridamole to α_1 acid GP. Dipyridamole was shown to form a complex with α_1 acid GP (15). The molar ratio of both components in the complex was established from the elution profile of [^{14}C]dipyridamole-α_1 acid GP mixture applied on Sephadex G-25 (Fig. 1). In this figure the area of the peak above the base line corresponds to the amount of [^{14}C]dipyridamole bound to α_1 acid GP, whereas the area of the trough that follows represents the amount of [^{14}C]dipyridamole removed by α_1 acid GP from the buffer that equilibrates the gel. The integrated areas are therefore, essentially, equal. As the concentration of dipyridamole that was applied to the column increased from 0 to 66 μM, the area of the trough progressively decreased and finally resulted in a positive peak (not illustrated).

Samples with excess dipyridamole in relation to α_1 acid GP gave an elution profile with two positive peaks, whereas samples containing dipyridamole and α_1 acid GP in equimolar ratio in the complex gave an elution profile without a peak or trough. The molar ratio of the components in the complex estimated according to Hummel and Dreyer (22) suggests that 1 mol of dipyridamole binds to 1 mol of α_1 acid GP to form a complex with a K_d of 1.6 μM.

Binding of dipyridamole to human platelets. The concentration dependence of dipyridamole binding to washed platelets and its modification by α_1 acid GP or PPP is shown in Fig. 2. The binding of [^{14}C]dipyridamole to platelets was inhibited by 0.25 mg of purified α_1 acid GP or 0.2 ml of PPP (plasma contains 0.7–0.9 mg α_1 acid GP/ml). As shown in Fig. 2, the [^{14}C]dipyridamole binding pattern to platelets in the presence of 0.2 ml plasma was similar to that observed with washed platelets preincubated with 0.25 mg of α_1 acid GP. These results indicate that purified α_1 acid GP or plasma can significantly inhibit the binding of [^{14}C]dipyridamole to human platelets.

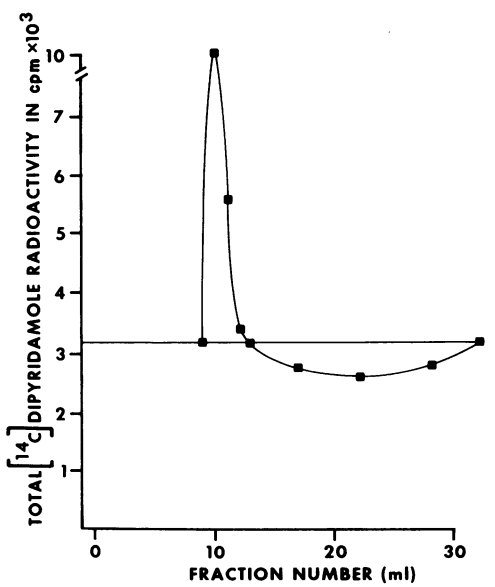

FIGURE 1 The elution profile of [^{14}C]dipyridamole and α_1 acid GP on Sephadex G-25 column equilibrated with 6.5 μM [^{14}C]dipyridamole in buffer. The sample applied on the column contained 6 μM of [^{14}C]dipyridamole and 38 μM of α_1 acid GP. The horizontal line represents the relative [^{14}C]dipyridamole radioactivity of the equilibrating solution. The total [^{14}C]dipyridamole radioactivity of each fraction was plotted against the fraction number.

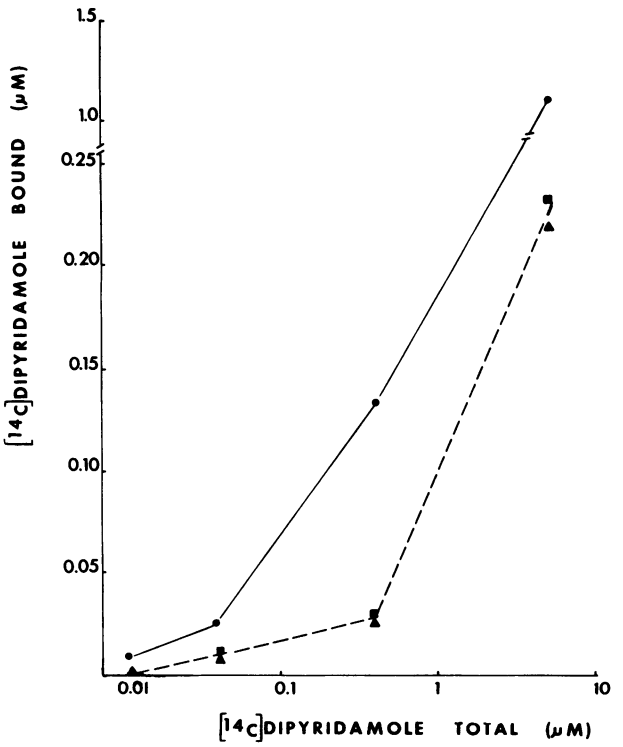

FIGURE 2 The effect of α_1 acid GP or plasma on the binding of [^{14}C]dipyridamole to washed human platelets. The concentration of dipyridamole bound to 0.5×10^9 platelets was plotted against the concentration of [^{14}C]dipyridamole added to washed platelet suspension. The total [^{14}C]-dipyridamole bound to washed human platelets (●) in the presence of 0.25 mg of α_1 acid GP (■) and with 0.2 ml plasma (▲) are shown. Each value is an average of three determinations.

It was previously shown that the amount of dipyridamole bound to washed platelets was eightfold higher as compared to platelets suspended in plasma (16). About 3.0±0.8% of total radioactivity in the platelet pellet was observed when platelet suspensions or platelets in plasma (10^9 cells/ml) were incubated with 1 μM concentration of [^{14}C]RA 233 for 5 min at 37°C. This value is close to that observed with platelets in plasma treated with [^{14}C]dipyridamole (1 μM) under identical conditions (3.5±1.7%). The radioactivity of ^{125}I-albumin was used as a marker of extracellular space in the platelets, and was 2.0±0.8% of the total pellet volume.

The total ^{14}C-radioactivity in platelet pellet increased with the addition of increasing concentrations of [^{14}C]-dipyridamole to platelet suspension (Fig. 3A). Binding of dipyridamole was clearly higher for all points of the curve. The shape of the curve suggests the existence of more than one binding system with different affinities. The data in Fig. 3A was plotted according to the equation of Rosenthal (25, 26). The relationship between the bound-to-free ratio and the total [^{14}C]-dipyridamole bound is not linear over a range of dipyridamole concentrations, indicating that not all binding sites behave identically as the saturation occurs (Fig. 3B). The biphasic curve in Fig. 3B indicates the presence of two types of binding sites for dipyridamole in human platelets. From the analysis of the intercept and slope of the first (left) part of the curve in Fig. 3B, the estimated number of dipyridamole-binding sites of high affinity per platelet and apparent K_d were 2×10^4 and 0.04 μM, respectively. The nearly horizontal part of the curve with an apparent K_d of ≅4 μM suggests the involvement of a non-saturable phenomenon. The binding ability of high-affinity sites in platelets is ≅100-fold greater than that of low-affinity sites. The data in Fig. 3B, when transformed in Lineweaver-Burke plots gave an apparent K_m of 0.06 μM for the binding of drug to high-affinity-binding sites of the platelets. The binding of dipyridamole to platelet followed saturation kinetics up to a concentration 0.1 μM. Similar plots on the binding of [^{14}C]dipyridamole to platelets in plasma (PRP) suggested that plasma significantly inhibited the binding of the drug (0.02 μM–0.17 mM) to platelets and did not follow a pattern definitive of competitive or noncompetitive type of inhibition (results not included). It was noticed that a 10-fold excess unlabeled dipyridamole reduced the total radioactivity of [^{14}C]dipyridamole (0.1 μM) in washed platelet pellet by 85%. This provides evidence that the labeled and unlabeled dipyridamole behave identically with respect to their interactions with washed platelets.

Uptake of [^{14}C]adenosine by washed human platelets and PRP. When tested with washed platelets, 0.5 nM dipyridamole inhibited the uptake of

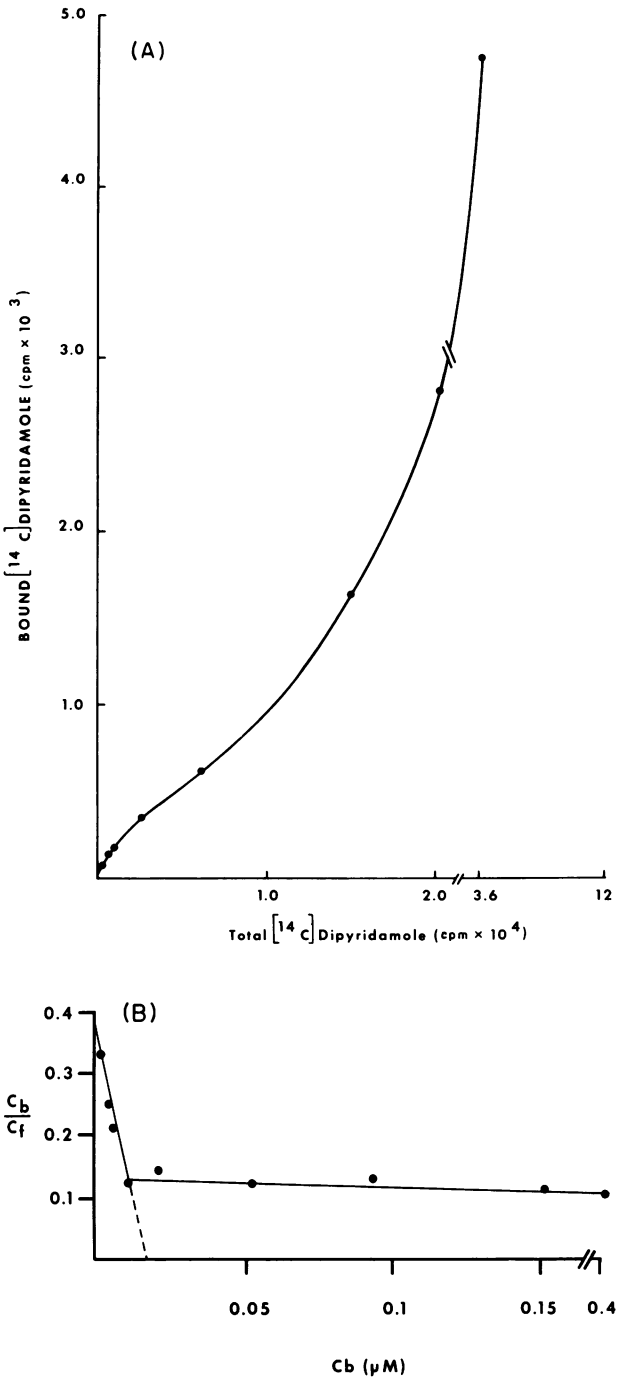

FIGURE 3 (A) Binding of [^{14}C]dipyridamole to washed human platelets (2×10^9 cells/ml) as a function of concentration of labeled dipyridamole in the 1-ml incubation mixture. Each point is an average value of four determinations (B) Rosenthal plots of [^{14}C]dipyridamole bound to washed human platelets. The *CB/Cf is the molar ratio of* [^{14}C]dipyridamole bound to washed human platelets (0.5 $\times 10^9$ cells) vs. unbound [^{14}C]dipyridamole. The *Cb* is a micromolar concentration of [^{14}C]dipyridamole bound to platelets.

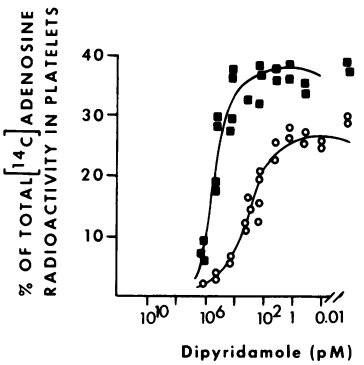

FIGURE 4 The concentration dependent dipyridamole inhibition of adenosine uptake by washed human platelets and PRP. A relation between percent of total [^{14}C]-adenosine radioactivity in washed platelets (○) or PRP (■) and concentration of dipyridamole is shown.

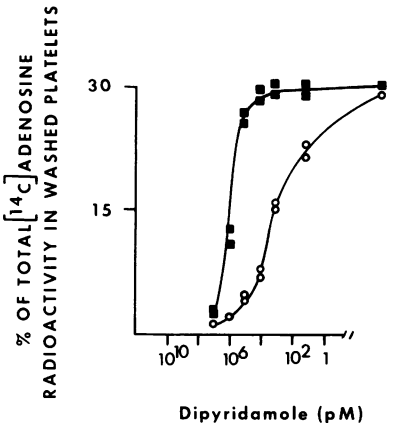

FIGURE 5 The effect of purified α_1 acid GP on dipyridamole inhibition of [^{14}C]adenosine uptake by washed human platelets. Washed human platelets (10^9 cells/ml) were incubated with purified α_1 acid GP (1 mg/ml) for 10 min at 37°C before the addition of dipyridamole and [^{14}C]adenosine to the incubating mixture. The percent of total [^{14}C]adenosine radioactivity in washed platelets (○) and in the presence of α_1 acid GP (■) was plotted against the concentration of dipyridamole.

adenosine by 50%. However, a 1,000-fold higher concentration of the drug (0.5 μM) was necessary to produce similar inhibition in PRP (Fig. 4). Addition of α_1 acid GP (1 mg/ml) to washed platelet suspension (Fig. 5) increased the I_{50} value[2] of dipyridamole from 0.5 nM to 0.5 μM as observed with PRP. Dipyridamole (0.1 μM) inhibited adenosine uptake by washed human platelets by >75%, whereas, purified α_1 acid GP at a concentration of 0.05 and 0.25 mg/ml reversed the inhibitory effect of dipyridamole by 7 and 85%, respectively. These results suggest that α_1 acid GP in plasma is responsible for the diminished inhibitory potency of dipyridamole on adenosine uptake by PRP.

Effect of dipyridamole on the incorporation of [^{14}C]adenosine radioactivity into adenine nucleotides. The major radioactive metabolites of platelets incubated with [^{14}C]adenosine were ATP, ADP, AMP, IMP, hypoxanthine, and inosine. Less than 3% of total [^{14}C]adenosine was incorporated into AMP and IMP. The distribution of [^{14}C]adenosine radioactivity among ATP, ADP, and hypoxanthine plus inosine in washed human platelets or PRP incubated with 0.1 μM dipyridamole alone and in combination with 0.05 or 0.25 mg/ml of α_1 acid GP is shown in Fig. 6. Dipyridamole inhibited [^{14}C]adenosine uptake by the washed platelets and altered the distribution of adenosine radioactivity among platelet adenine nucleotides. The percent of total [^{14}C]adenosine radioactivity incorporated into ATP, ADP, and hypoxanthine plus inosine of washed platelets were about 46, 21, and 24, respectively. Purified α_1 acid GP slightly altered the [^{14}C]adenosine-radioactivity incorporation pattern in these metabolites. Incubation of washed platelets with dipyridamole resulted in a significant decrease of total [^{14}C]adenosine radioactivity incorporated into ATP (from 46 to 13%). Consequently, there was a fourfold decrease in the [^{14}C]ATP/[^{14}C]ADP ratio, whereas, the incorporation of radioactivity into hypoxanthine plus inosine was increased from 24 to 42%. These changes produced by 0.1 μM of dipyridamole

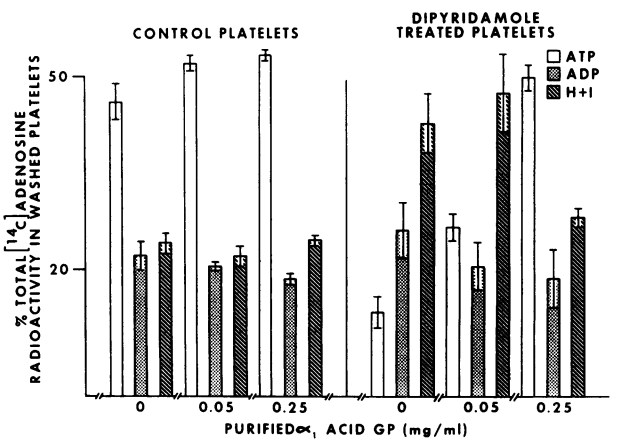

FIGURE 6 The effect of dipyridamole and α_1 acid GP on the appearance of [^{14}C]adenosine radioactivity among ATP, ADP, and hypoxanthine plus inosine (H + I) of washed human platelets (10^9 cells/ml). Control platelets or platelets treated with 0.1 μM dipyridamole were incubated with purified α_1 acid GP (0.05 or 0.25 mg/ml) and the percent of total [^{14}C]adenosine radioactivity among ATP, ADP, and hypoxanthine plus inosine of platelet pellet was determined. The variance bars indicate the standard deviations out of results of four determinations.

[2] I_{50}, concentration of dipyridamole required to inhibit adenosine uptake by platlets by 50%.

could be reversed by the addition of purified α_1 acid GP to washed platelet suspension. Accordingly, 0.1 μM of dipyridamole failed to alter the distribution of [^{14}C]adenosine-radioactivity among adenine nucleotides of platelets suspended in plasma.

The relationship between inhibition of adenosine uptake and binding of dipyridamole to platelets. When washed human platelets (10^9 cells/ml) were incubated with [^{14}C]dipyridamole (1 μM) and subjected to gel filtration on Sepharose 2B column, it was found that the early fractions contained platelets without [^{14}C]dipyridamole-radioactivity, whereas the latter fractions showed the radioactivity and did not contain platelets (results not included). It is therefore possible to separate [^{14}C]dipyridamole bound to platelets by gel filtration. Recovery of about 95% of total radioactivity was achieved by this method.

The effect of removal of dipyridamole from washed platelets on the inhibition of adenosine uptake by platelets is shown in Table I. The percent of total [^{14}C]adenosine-radioactivity in platelet pellet was estimated using platelet suspension obtained before and after Sepharose 2B gel filtration and compared with similar samples obtained after incubating the platelets with dipyridamole. The gel filtration of platelets alone resulted in a 20% loss in the ability of washed platelets to take up [^{14}C]adenosine. Separation of dipyridamole from washed platelets by gel filtration completely abolished the inhibitory potency of dipyridamole on adenosine uptake by washed human platelets. The binding of dipyridamole to platelets is therefore essential for its inhibition on adenosine uptake by platelets. It was observed that adenosine (10 mM) did not inhibit the binding of dipyridamole (1 μM) to washed platelets.

TABLE I
Effect of Removal of Dipyridamole from Washed Human Platelets on [^{14}C]Adenosine Uptake

	Percent of total [^{14}C]adenosine radioactivity in platelet pellet gel filtration	
	Before	After
Washed platelets (control)	20.5±3.4	16.5±2.1
Washed platelets incubated with 10 μM dipyridamole	2.2±0.9	16.0±2.0

Washed human platelets (2×10^9 cells/ml) were incubated with 10 μM dipyridamole for 5 min at 37°C and subjected to Sepharose 2B gel filtration. The percent of total [^{14}C]adenosine radioactivity in platelet pellet was estimated in platelets before and after gel filtration after adjusting the platelet counts in the pooled fractions to 10^9 cells/ml. Mean±SD; $n = 10$. For details refer to Methods.

DISCUSSION

By our data, it can be concluded that the binding of dipyridamole to human platelets is essential for its inhibition of adenosine uptake by platelets. We find at least two populations of dipyridamole binding sites in platelets, one characterized by high affinity ($K_d = 0.04$ μM) and another by low affinity ($K_d = 4$ μM) for the drug. The high-affinity binding sites of platelets are saturable with Michaelis-Menten kinetics and $\cong 2 \times 10^4$ dipyridamole molecules bind per cell. The nonsaturating, low-affinity binding pattern of the drug to platelets suggests the presence of either a large number of low-affinity binding sites in platelets or an adsorption phenomenon. The difference in the amount of dipyridamole bound to high- and low-affinity binding sites in platelets is expected to be much greater at a low dipyridamole to platelet ratio, where the drug predominantly binds to high-affinity binding sites of the platelets. From the K_d values, it can be suggested that at low concentrations of dipyridamole (below 0.1 μM), the low-affinity binding system makes a negligible contribution, whereas at high concentrations of dipyridamole (above 1 μM) both systems contribute to the binding of dipyridamole to platelets. We found the dipyridamole at a very low concentration (500 pM), exerted about 50% inhibition of adenosine uptake by washed platelets (Fig. 4). This supports the notion that the binding of dipyridamole to high-affinity sites of platelets are probably responsible for the inhibition of adenosine uptake by platelets. However, dipyridamole binding sites of the platelets appear to be distinct from adenosine-binding sites since high concentrations of adenosine did not inhibit the binding of dipyridamole to platelets. The incubation of platelets with 0.1 μM dipyridamole resulted in the inhibition of [^{14}C]adenosine incorporation into platelet ATP (Fig. 6). Because the low-affinity sites of platelets had a binding constant $K_d = 4$ μM, the data presented in Fig. 6, measured with 0.1 μM dipyridamole would show minimal contribution from the low-affinity binding sites (only 2% are saturated at 0.1 μM) despite the existence of larger numbers of low-affinity sites than high-affinity binding sites in platelets. By contrast, at the same concentration of the drug (0.1 μM), the high-affinity binding sites are: 70% saturated. Therefore we concluded that high-affinity binding sites are essentially involved in the inhibition of [^{14}C]adenosine incorporation into platelet ATP. The decreased [^{14}C]ATP content of platelets treated with dipyridamole may be either due to potentiation of platelet adenylate cyclase by the accumulated adenosine (14) in the presence of dipyridamole or due to activation of platelet ATPase. However, dipyridamole (1 mM) has been shown to inhibit (Na^+, K^+) dependent ATPase activity of erythrocytes by 15% (29).

Since ATP supplies energy required for platelet aggregation (30), it is possible that the decrease of [^{14}C]ATP content in platelets by dipyridamole contributes to the antiplatelet activity of the drug.

Sixma et al. (31), demonstrated the presence of two types (low- and high-K_m system) of adenosine transport mechanisms in washed human platelets. The increased level of [^{14}C]hypoxanthine and [^{14}C]inosine in dipyridamole-treated platelets can be explained on the basis of inhibition of low K_m adenosine transport mechanism. It was previously shown that dipyridamole also inhibits the adenosine uptake by erythrocytes (32, 33). Since dipyridamole binds to erythrocytes (34), it is possible that the mechanism of inhibition of adenosine uptake by erythrocytes is also related to the binding of dipyridamole to erythrocytes membranes.

The effect of low concentration of dipyridamole on [^{14}C]adenosine uptake and its incorporation into platelet nucleotides could be reversed by α_1 acid GP of plasma (Fig. 6). Dipyridamole binds to α_1 acid GP of plasma forming a complex (15, 35). In agreement with equilibrium dialysis method (35), the molar ratio of dipyridamole to α_1 acid GP as determined by Hummel and Dreyer's method (22) was 1:1 with an apparent K_d of 1.6 μM, which is about 40-fold higher than K_d for high-affinity binding sites of the platelets. These results therefore suggest that α_1 acid GP can inhibit the binding of dipyridamole to high-affinity binding sites of the platelets, thereby blocking the inhibitory effect of dipyridamole on adenosine uptake by platelets.

Using a spectrofluorometric method (36), we estimated the level of circulating dipyridamole in the plasma of 12 human volunteers who ingested several dosages of dipyridamole (0.48–3.6 mg/kg body wt). We also measured the percent inhibition of [^{14}C]adenosine uptake by their platelets. The concentration of dipyridamole required to exert 50% inhibition of [^{14}C]-adenosine uptake by platelets suspended in plasma (PRP) under in vitro and in vivo conditions were found to be of same order of magnitude (0.2–0.5 μM). This indicates that the mode of action of dipyridamole on the adenosine uptake by platelets in vivo probably involves the binding of the drug to high-affinity sites of the platelets.

Since dipyridamole binds to platelets and to α_1 acid GP of plasma, we suggest that dipyridamole exists in a dynamic state of equilibrium between α_1 acid GP and platelets. The effect of dipyridamole on adenosine uptake by platelets may therefore depend on the level of circulating drug and on the level of α_1 acid GP. The level of α_1 acid GP in plasma was shown to vary under different conditions of inflammation and tissue injury (37, 38). It follows that the pharmacological effectiveness of dipyridamole as inhibitor of adenosine uptake by platelets can be evaluated on the basis of relative concentrations of circulating dipyridamole and α_1 acid GP in plasma.

The data suggest the presence of binding sites for dipyridamole in platelets that are involved in the adenosine transport across cell surface. Further studies on the isolation and characterization of these sites from platelets may help to explain the adenosine transport mechanism in cells.

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