Effects of Platelet-modifying Drugs on Arterial Thromboembolism in Baboons

Aspirin Potentiates the Antithrombotic Actions of Dipyridamole and Sulfinpyrazone by Mechanism(s) Independent of Platelet Cyclooxygenase Inhibition

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Abstract

To resolve questions of drug actions, efficacy, and interactions for platelet-modifying agents used clinically, we have compared the relative capacities and mechanisms of aspirin, dipyridamole, sulfinpyrazone, and dazoxiben to prevent arterial thromboembolism in a baboon model. In 136 studies the agents were given twice daily by oral administration both singly and in combination. The antithrombotic efficacy of a given therapy was determined by its capacity to interrupt steady-state platelet utilization induced by thrombogenic arteriovenous cannulae.

When given alone, dipyridamole and sulfinpyrazone reduced the rate at which platelets were utilized by thrombus formation in a dose-dependent manner with essentially complete interruption by dipyridamole at 10 mg/kg per d. In contrast, neither aspirin (2–100 mg/kg per d) nor dazoxiben (20–100 mg/kg per d) decreased cannula platelet consumption detectably despite the striking reduction in the capacity of platelets to produce thromboxane B_2. However, aspirin, but not dazoxiben, potentiated the antithrombotic effects of dipyridamole and sulfinpyrazone in a dose-dependent fashion without changing the pharmacokinetics for any of the agents. Complete potentiation required aspirin at 20 mg/kg per d to be given with each dose of dipyridamole. Because dazoxiben's blockade of platelet thromboxane A_2 production was not associated with antithrombotic potentiation, and because complete potentiation by aspirin required a dose that fully inhibited vascular production of prostaglandin I_2 (PGI_2), we conclude that aspirin's potentiating effect on dipyridamole is independent of PGI_2 production or inhibition of thromboxane A_2 formation. In addition, because frequent repeated and synchronous dosing of aspirin was necessary, aspirin's potentiating effects appear to be produced by mechanism(s) unrelated to its potent, irreversible inhibition of platelet cyclooxygenase.

Introduction

The indications for the use of platelet-modifying drugs in the management of patients with arterial vascular disease are unclear despite extensive basic, experimental animal and clinical studies. In general, the negative clinical trials have been relatively uninformative, because of uncertainties with respect to the role of platelets in the outcome events, the mechanisms of drug actions, and the effective doses for the drugs in the trials. In the absence of established mechanisms of drug action, we reason that defining dose regimens requires objective assays with thrombotic endpoints rather than the use of in vitro biochemical measurements of less certain interpretation.

To assess the relative antithrombotic efficacy of various clinically available drug regimens, we have used a model of steady-state arterial thromboembolism in baboons that primarily involves increased platelet destruction in the thrombotic process (1). In this model, thrombogenic tubular polyurethane is inserted as an extension segment between chronic femoral cannulae to form an arteriovenous shunt. The overall rate of thrombus formation in vivo is quantified by measuring the consumption of circulating ^{51}Cr-platelets induced by the thrombogenic segment. Cannula platelet consumption correlates directly with the surface area for the polyurethane segment and remains in the steady state for months. Despite increased rates of platelet consumption, the destruction of circulating fibrinogen is not measurably increased. Moreover, the rate of platelet consumption is independent of normal variations in cannula blood flow rate and platelet count, and is unaffected by heparin anticoagulation or ancrod defibrinogenation. ^{111}In-platelet imaging of thrombogenic tubular segments of polyurethane demonstrates luminal accumulation and subsequent embolization of irregular platelet thromboemboli that are also directly demonstrable by laser light-scattering techniques (2), or by trapping in blood filters or perfused kidneys (1).

Methods

Animal studies. Normal male baboons (*Papio anubis*) weighing 8–14 kg were used. All animals were dewormed and observed to be disease-free for at least 6 wk before study. Base-line circulating platelet concentrations in the study animals averaged 382,000±129,000 platelets/μl (±1 SD), hematocrits were 35±3%, and leukocyte counts averaged 8,300±4,100/μl. All animals had a chronic arteriovenous (A-V)[1] shunt surgically implanted between the femoral artery and vein that consisted of two 25-cm lengths of Silastic tubing, 3.0 mm i.d. (Dow Corning Corp., Midland, MI), connected to 13–15-gauge Teflon vessel tips (Lifemed, Vernitron Corp., Compton, CA). In addition, the two Silastic lengths were fixed with Dacron sewing cuffs (E. I. DuPont deNemours and Company, Inc., Wilmington, DE) at skin exit sites and connected

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1. *Abbreviations used in this paper:* A-V, arteriovenous; HPLC, high pressure liquid chromatography; PGI_2, prostacyclin; TxA_2, thromboxane A_2; TxB_2, thromboxane B_2.

with a 1-cm length of blunt-edge Teflon (2.8 mm i.d.). The cannulae were sterilized by autoclaving before surgical placement. Segments of test tubing were interposed between the segments of the permanent Silastic A-V cannula with 1-cm-long and 2.8-mm i.d., blunt-edged Teflon connectors. The permanent Teflon-Silastic shunt system did not detectably shorten platelet survival or produce measurable platelet thromboemboli (1).

A 50-cm segment of tubular polyurethane (4.0 mm i.d., Biomer, Ethicon, Inc., Sommerville, NJ) was inserted as the thrombogenic extension piece into the permanent A-V shunt. This thrombogenic segment induced platelet thrombus formation on the luminal surface that was measured as cannula platelet consumption (Table I).

Drugs mixed with applesauce were administered orally to baboons bearing the thrombogenic polyurethane cannulae using varying doses and regimens (see Results) beginning 2 d before platelet survival studies were initiated, and throughout the 5-d period required to perform this determination. Dipyridamole was a gift from Boehringer-Ingelheim (Elmsford, NY), sulfinpyrazone was a gift from CIBA-Geigy Corp., Pharmaceuticals Division (Summit, NJ), and dazoxiben was a gift from Pfizer, Inc. (Groton, CT).

Laboratory studies. Platelet counts were measured during each study with an electronic particle counter on peripheral blood collected in 2 mg/ml EDTA (1). The platelet count of 27 normal baboons was 432,000±123,000/μl.

Platelet survival was determined by measuring the disappearance of radioactivity from blood sampled six to eight times after injection of autologous ^{51}Cr-labeled platelets (1). The initial sample was drawn 1 h after the infusion of labeled platelets. All blood and plasma samples were counted for radioactivity using a gamma spectrometer (Nuclear Chicago, Chicago, IL). Platelet survival time was analyzed by computer fitting to gamma functions (1, 3) and was 5.4±0.4 d (±1 SD) in 27 normal male baboons. The proportion of labeled platelets remaining within the systemic circulation after infusion (i.e., "recovery") was calculated from the platelet activity per milliliter, extrapolated to zero time, multiplied by the estimated blood volume (70 ml/kg of body wt), and divided by the platelet ^{51}Cr activity injected. Recovery values averaged 85±7% in the control animals. In view of the steady-state requirement for the system of analysis we excluded four studies in which the cannula became occluded during the period of observation.

A mathematical model was used to separate the rate of senescent platelet removal from the rate of platelet utilization induced directly or indirectly by the thrombogenic cannula. Thereby, the rate of cannula-associated platelet consumption was calculated (1). This analysis is based on the Mills–Dornhorst model of platelet disappearance (4) and is expressed mathematically by:

$$\tau = \frac{1 - e^{-kT}}{k}, \quad (1)$$

where T is the normal platelet survival time in untreated control animals, and τ is the experimental platelet survival time. The parameter k, appearing in both the numerator and denominator of Eq. 1, is a rate constant equivalent to the fraction of platelets destroyed daily by random, extrinsic processes. The rate of platelet destruction by the cannula material (platelets/square centimeter per day) was calculated by multiplying the factor k times estimated blood volume and platelet count, and dividing by recovery and the area of exposed polyurethane surface (62.8 cm^2). Based upon the control studies, T was assigned a value of 5.4 d in all calculations. Since approximately half of all control animals had a platelet survival time < 5.4 d, analysis of the control data according to Eq. 1 predicted a finite value of platelet destruction which averaged 0.8±1.2 × 10^8 platelets/square centimeter per day (±1 SD) due to the variability in normal platelet lifespan. This base-line value was considered to be the lower limit of sensitivity for the method.

Standardized template bleeding times were performed on the shaved volar surface of the forearm as described previously (5).

The production of thromboxane A_2, measured as the stable metab-

Table I. Studies of Platelet-modifying Agents in Baboons

Drug dosage						
Dipyridamole	Sulfinpyrazone	Aspirin	Dazoxiben	Animals	Cannula platelet consumption	Significance vs. controls
mg/kg per d	*mg/kg per d*	*mg/kg per d*	*mg/kg per d*	*n*	*plats/cm² per d × 10⁻⁸*	*P*
Untreated				15	19.3±0.9	—
2.5				6	13.8±2.4	=0.02
10				6	2.5±0.8	<0.001
	20			5	14.8±2.9	=0.06
		2		5	17.0±1.6	>0.2
		20		5	20.1±3.2	>0.5
		100		3	20.2±4.8	>0.5
			20	5	19.5±2.3	>0.5
			50	5	16.8±3.0	>0.2
			100	5	18.4±2.8	>0.5
2.5		10		5	11.4±1.9	<0.001
2.5		15		4	8.2±2.8	<0.001
2.5		20		6	2.7±0.9	<0.001
2.5			20	5	17.4±2.0	>0.3
2.5			100	3	16.1±2.7	>0.1
	20	10		6	9.7±2.6	<0.001
	20	20		6	6.8±2.6	<0.001
	20	40		6	6.0±1.8	<0.001
2.5	10			4	13.0±1.1	<0.005
2.5	20			11	10.0±0.8	<0.001
2.5	40			5	8.6±2.6	<0.001
2.5	20	10		6	11.1±2.4	<0.001

olite thromboxane B_2 (TxB_2) in whole blood was determined using a radioimmunoassay (New England Nuclear, Boston, MA) for samples collected by a method adapted from Lewy et al. (6). 2 ml of blood drawn by plastic syringe without anticoagulant was immediately transferred to a 12 × 100-mm glass tube containing 10 U of thrombin and mixed rapidly before clotting. The resultant blood clot was incubated at 37°C for 30 min. Serum was separated by centrifugation at 1,000 *g* for 10 min and stored frozen at −20°C until the time of assay. Samples for serum TxB_2 measurements were obtained preceding initiation of drug therapy, and 1 h after the morning dose on the second day of oral drug administration.

Concentrations of dipyridamole in plasma were determined by using a sensitive and specific high performance liquid chromatography (HPLC) method based on paired-ion chromatography and fluorescence detection (7). This assay has been used in two recent clinical studies to characterize the pharmacokinetics of dipyridamole (8, 9). The lower limit of sensitivity for dipyridamole using this assay was 1 ng/ml. The coefficients of variation for the calibration curves for the assay of unknown samples were usually between 5 and 10%. Because dipyridamole could have theoretically produced its inhibitory effect on platelet function by preventing the removal of adenosine released from hemolyzing erythrocytes, plasma hemoglobin levels were measured using the standard cyanmethemoglobin technique in normal control animals and in cannula-bearing animals both before and after oral dipyridamole therapy. Values in 11 animals before therapy (1–12 mg/dl) were equivalent to values obtained after drug treatment (4–13 mg/dl; $P > 0.3$).

Sulfinpyrazone and its sulfide metabolite in plasma were measured by means of a sensitive and specific HPLC method using reversed-phase chromatography and ultraviolet light detection (10, 11). Sulfinpyrazone was assayed employing a triple extraction with ethylene dichloride, while the sulfide metabolite was assayed separately using a triple extraction with chlorobutane; the mobile phases differed slightly for the respective chromatographies. This assay has been used in a clinical study to determine the kinetics and metabolism of sulfinpyrazone (11). The lower limit of sensitivity for the parent drug and its metabolites was ~10 ng/ml. The coefficients of variation for the calibration curves for the assay of unknown samples were generally in the range of 5–15%.

A sensitive and specific HPLC method was developed for determining concentrations of acetylsalicylic acid and salicylic acid in plasma (Nash, P. V., and T. D. Bjornsson, manuscript submitted for publication). The blood samples were treated with physostigmine during collection to prevent hydrolysis of aspirin to salicylic acid (12). The method involved a triple extraction of 0.5 ml of acidified plasma with methyl-*tert*-butyl ether using caffeic acid as the internal standard. Extraction recovery was 90–95%. The combined organic phase was evaporated to dryness under nitrogen while the tubes were kept in an ice-water bath. The residue was redissolved in 0.2 ml of mobile phase. Chromatographic conditions involved a reversed-phase column and a mobile phase consisting of methanol:0.025 M ammonium phosphate, pH 2.5 (32:68), and a flow rate of 1 ml/min. The effluent was monitored at 234 nm using an ultraviolet detector. Retention times for the internal standard, aspirin, and salicylic acid were 7, 12, and 16 min, respectively. The lower limit of sensitivity was ~25 and 100 ng/ml for aspirin and salicylic acid, respectively; the assay was linear over a range of from 25 ng/ml to 50 μg/ml. The coefficients of variation for the calibration curves for the assay of unknown samples were usually between 5 and 10%.

The blood samples for drug analysis were collected over one dosing interval at 0, 1, 2, 4, 7, and 12 h, after the morning dose on day 4 of the study. 5–10 animals were studied for each drug or drug combination. The area under the plasma concentration vs. time curve over the dosing interval, AUC, was calculated using the trapezoidal rule. Subsequently, the average drug concentration over a dosing interval at steady state, C_{ss}, was calculated as follows (13): $C_{ss} = AUC/\theta$, where θ is the dosing interval.

All statistical analysis and curve fitting were done using the PROPHET system of the Division of Research Resources, National Institutes of Health. Statistical comparisons were made using Student's *t* test (two-tailed) for paired and unpaired data when the data were normally distributed or by the Wilcoxon Mann–Whitney rank sum test for the remaining results (14). To avoid the possibility that an individual drug treatment might achieve statistical significance by chance, dose-response relationships were established for each agent. All data in the results section are given as the mean±SE.

Results

Oral administration of dipyridamole decreased cannula platelet consumption in a dose-dependent manner with nearly complete interruption at 10 mg/kg per d (in two divided doses), whereas oral aspirin (2–100 mg/kg per d) had no measurable effect on cannula platelet consumption (Table I). These results confirm independently the previously reported experience (1).

Peak plasma dipyridamole concentrations occurred at 1–2 h after the 2.5-mg/kg per d dosing regimen and at 1–4 h after the 10-mg/kg per d regimen. The mean average steady-state plasma concentrations of dipyridamole were 26±7 and 79±31 ng/ml for these two dosage regimens, respectively (Table II), with an overall range of 10–327 ng/ml in plasma concentrations. Peak plasma sulfinpyrazone concentrations were observed at 2–4 h after the 20 mg/kg per d dosing regimen and at 1–7 h after the 100-mg/kg per d regimen. The mean average steady-state plasma concentrations of sulfinpyrazone were 1.05±0.20 and 12.52±2.56 μg/ml for these regimens, respectively (Table

Table II. Average Steady-state Plasma Drug Concentrations and Ranges in Concentrations After Oral Administration in Baboons

Drug treatment	Animals	Average steady-state concentration*
mg/kg per d	*n*	*ng/ml*
Dipyridamole (2.5)	5	26±7 (12–46)
Dipyridamole (10)	5	79±31 (21–197)
Dipyridamole (2.5) + ASA (20)	10	26±6 (5–58)
		μg/ml
Sulfinpyrazone (20)	5	1.05±0.20 (0.37–1.53)
Sulfide metabolite		7.91±1.80 (3.02–10.90)
Sulfinpyrazone (100)	5	12.52±2.56 (7.11–20.06)
Sulfide metabolite		20.12±5.83 (12.07–43.07)
Sulfinpyrazone (20) + ASA (20)	5	1.27±0.34 (0.41–2.38)
Sulfide metabolite		8.77±4.55 (1.15–26.59)
		μg/ml
Aspirin (20)	10	0.67±0.25 (0–2.81)
Salicylic acid		3.76±0.82 (0–7.99)
Aspirin (20) + dipyridamole (2.5)	10	0.35±0.04 (0.15–0.50)
Salicylic acid		2.98±0.71 (1.03–8.33)
Aspirin (20) + Sulfinpyrazone (20)	5	0.60±0.16 (0.09–1.48)
Salicylic acid		3.81±0.83 (0.34–6.80)

ASA, acetyl salicylic acid.
* Values in parenthesis represent the observed range.

Table III. Effect of Aspirin and Dazoxiben on Platelet Production of TxA_2

Drug treatment	Animals	Serum TxB_2	
		Production	Base line
mg/kg per d	*n*	*pmol/ml*	%
Base line			
(No treatment)	9	3352±436	100
Aspirin			
2	5	400±134	13.9±6.1
20	5	20±6	0.6±0.2
100	5	8±4	0.2±0.06
Dazoxiben			
20	5	672±88	23.7±5.1
100	4	4±1	0.1±0.02

II), with an overall range of 0.08–47.19 μg/ml in plasma concentrations. Steady-state plasma concentrations of the sulfide metabolite were 2–8 times higher than those of the parent drug. Peak plasma concentrations of both aspirin and its metabolite salicylic acid occurred at 1–2 h after the 20 mg/kg per d dosage regimen. The mean average steady-state plasma concentrations of aspirin and salicylic acid were 0.67±0.25 and 3.76±0.82 μg/ml, respectively (Table II).

All doses of oral aspirin inhibited the production of thromboxane A_2 (TxA_2) by platelets (Table III). Doses > 20 mg/kg per d reduced the production of TxA_2 by platelets to <1% compared with base-line controls.

The effect of aspirin on the capacity of dipyridamole to normalize cannula platelet consumption was then examined. In these studies animals received a small, constant dose of oral dipyridamole (2.5 mg/kg per d in two divided doses), sufficient to reduce cannula platelet consumption minimally, but significantly ($P = 0.02$), when used alone (Table I). Oral aspirin was combined at increasing doses with the small fixed dose of dipyridamole in a twice-daily regimen. Aspirin given together with dipyridamole produced a dose-dependent reduction in cannula platelet consumption (Fig. 1), although aspirin alone

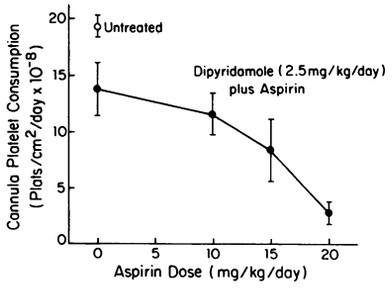

Figure 1. Potentiating effect of aspirin on dipyridamole. Baboons with consumptive cannulae received small constant doses of dipyridamole in combination with progressively increased doses of aspirin (●). The effect is near maximal at 20 mg/kg per d aspirin. (○), Untreated control value. Animals given aspirin (20 mg/kg per d) plus dipyridamole had significantly less platelet destruction than animals given dipyridamole only ($P < 0.001$).

was without effect (Table I). Interruption of platelet consumption was largely complete at the dose of 20 mg/kg per d aspirin in combination with dipyridamole 2.5 mg/kg per d. This dose of aspirin reduced the production of TxB_2 by the platelets to 0.6±0.2% of the base-line value (Table III). The average steady-state plasma concentrations of dipyridamole and aspirin following simultaneous dosing of 2.5 mg/kg per d and 20 mg/kg per d were 26±6 ng/ml and 0.35±0.04 μg/ml, respectively (Table II). These concentrations were not significantly different from values obtained when these doses were administered separately, $P = 0.970$ (dipyridamole) and $P = 0.229$ (aspirin).

Dazoxiben, a thromboxane synthetase inhibitor, was studied to determine if cannula platelet consumption was decreased when the formation of thromboxane A_2 was specifically blocked. A dose of 100 mg/kg per d prolonged the bleeding time in four baboons significantly over base-line values (14.2±2.4 min vs. 3.3±0.8; $P < 0.01$), and decreased the production of TxA_2 by platelets to <1% of control values (Table III). Oral dazoxiben at 20 and 100 mg/kg per d (given in two divided doses) failed to affect cannula platelet consumption when given alone (Table I). Moreover, despite the blockade of platelet production of thromboxane A_2, dazoxiben (20 or 100 mg/kg per d) in association with a small dose of dipyridamole (2.5 mg/kg per d) did not change cannula platelet consumption beyond that produced by dipyridamole alone (Table I).

To evaluate the possibility that the potentiating effect of aspirin on dipyridamole might be produced by aspirin's conversion in vivo to salicylic acid, salicylic acid was administered orally in amounts equivalent to the dose of aspirin shown to be effective in combination with the small fixed dose of dipyridamole. Cannula platelet consumption was neither decreased by salicylic acid alone nor did salicylic acid in combination with dipyridamole have a potentiating effect (Table IV).

The potentiating effect of aspirin on dipyridamole was then evaluated with respect to the timing and frequency of the oral doses (Table V). In one set of studies aspirin was given 2 h after the administration of dipyridamole; in another set of studies aspirin was administered only with the first daily dose of dipyridamole but not with the second dose of dipyridamole. As shown in Table V, a significant proportion of aspirin's potentiating effect on dipyridamole was lost when aspirin was given 2 h after dipyridamole or when it was given only with the first daily dose of dipyridamole. Thus, complete potentiation of dipyridamole by aspirin depended upon giving the aspirin

Table IV. Effects of Combination Dipyridamole-Salicylic Acid on Cannula Platelet Consumption in Baboons

Drug dosage			Cannula platelet consumption	
Dipyridamole	Salicylic acid	Animals	Rate	Significance (vs. controls)
mg/kg per d	*mg/kg per d*	*n*	*plats/cm² per day × 10⁻⁸*	*P*
—	—	15	19.3±0.9	—
—	20	4	18.0±2.5	>0.5
2.5	—	6	13.8±2.4*	<0.02
2.5	20	6	13.5±1.9*	<0.01

* Difference between values not significant at $P > 0.5$.

Table V. Effects of Varying Dipyridamole (1.25 mg/kg Twice Daily) Plus Aspirin Regimens on Cannula Platelet Consumption in Baboons

Study group	Aspirin regimen	Animals	Cannula platelet consumption	
			Rate	Significance (vs. group 1)
		n	*plats/cm² per day × 10⁻⁸*	*P*
1	10 mg/kg twice daily with dipyridamole	6	2.7±0.9	—
2	10 mg/kg twice daily but 2 h after dipyridamole	7	8.6±2.0	<0.05
3	10 mg/kg with first dipyridamole only	7	12.0±2.4	<0.01

in amounts of 20 mg/kg per d simultaneously and with each dose of dipyridamole.

To examine the effects of combining other drugs with dipyridamole, sulfinpyrazone, another agent with documented antithrombotic efficacy in animals and man, was assessed. Increasing doses of sulfinpyrazone (10, 20, and 40 mg/kg per d) were given together with the fixed small dose of dipyridamole (Table I). In contrast to aspirin, sulfinpyrazone did not potentiate the capacity of dipyridamole to interrupt platelet consumption. Although a modest reduction in platelet consumption was observed with increasing sulfinpyrazone, the results were not different from those obtained in animals given dipyridamole only ($P > 0.15$).

The effect of aspirin on sulfinpyrazone's antithrombotic capacity was also assessed. Sulfinpyrazone was given orally twice daily at small constant doses (20 mg/kg per d) that produced minimal ($P = 0.06$) effects on cannula platelet consumption when used alone; oral aspirin was administered simultaneously at increasing doses in combination with sulfinpyrazone (Fig. 2, Table I). Aspirin produced a significant potentiation of sulfinpyrazone's capacity to reduce cannula platelet consumption, and the potentiating effect was maximal when the dose of aspirin reached 20 mg/kg per d. Interestingly, no additional enhancement was noted at higher doses of aspirin despite the fact that the interruption of cannula platelet consumption was incomplete. The average steady-state plasma concentrations of sulfinpyrazone and aspirin after concurrent dosing (20 mg/kg per d) of both drugs were 1.27±0.34 and 0.60±0.16 μg/ml, respectively (Table II). These blood levels were not significantly different from values obtained when these drugs were given separately at the same doses, $P = 0.595$ (sulfinpyrazone) and $P = 0.875$ (aspirin).

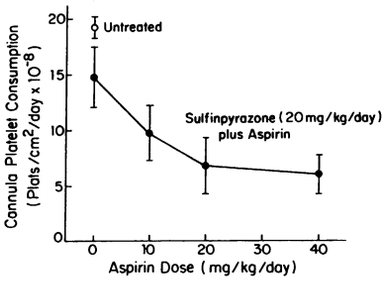

Figure 2. Effect of aspirin in combination with sulfinpyrazone. (●), When aspirin is given at increasing doses in combination with a minimally effective dose of sulfinpyrazone (20 mg/kg per d), a modest enhancement is observed in the inhibition of cannula platelet consumption in baboons. (○), Untreated control value. Animals given aspirin (40 mg/kg per d) plus sulfinpyrazone had significantly less platelet destruction than animals given sulfinpyrazone only ($P < 0.05$).

The effects of combining aspirin, sulfinpyrazone, and dipyridamole were then studied (Table I). There was no further enhancement produced by the triple combination over the effect produced by aspirin together with either dipyridamole or sulfinpyrazone. Moreover, no additive effect of either sulfinpyrazone or dipyridamole was evident in the full combination beyond that produced by aspirin's potentiation.

Discussion

A number of studies that used different platelet-active drugs in laboratory tests, various experimental thrombosis models, and clinical trials (15–19) have produced conflicting data. This result may in part be explained by: (*a*) the in vivo relevance of in vitro testing has generally not been established for the methods employed, (*b*) the experimental animal models have not been sufficiently quantitative, or have included multiple uncontrolled variables, (*c*) the role of platelets in the pathophysiology of the study outcome event has been uncertain, and (*d*) the mechanism of drug action for the clinically available agents has not usually been defined. In this context, platelet survival measurements have been more predictive than many other laboratory testing procedures in that they directly reflect increased platelet destruction due to thrombus formation (20), and have correlated with antithrombotic efficacy and dosage regimen in a number of experimental and clinical settings (1, 15–18, 21–35).

The capacity of dipyridamole to decrease cannula platelet consumption in a dose-dependent fashion (Table I) correlates well with its ability to normalize platelet survival in patients with artificial heart valves (21, 22), A-V cannulae (20), coronary artery disease (28, 29), and renal microvascular disease (36), and its efficacy in preventing thromboembolism in patients with prosthetic heart valves (23). Moreover, the dose and resulting blood levels required in the present baboon model to achieve complete interruption of cannula platelet consumption, i.e., 10 mg/kg per d, are in reasonable accord with the dose reported to normalize platelet survival (21, 24, 26) and prevent thromboembolism in patients with artificial heart valves, i.e., 5–7 mg/kg per d (23).

Similarly, the dose-dependent inhibition by sulfinpyrazone of cannula platelet consumption in baboons (1, and Table I) correlates with its capacity to normalize platelet survival in patients with artificial heart valves (30), valvular heart disease (31), transient ischemia attacks (32), and coronary atherosclerotic disease (33), and with its ability to reduce thrombotic events in patients with A-V cannula (25), valvular heart disease (31), postmyocardial infarction (34), and saphenous vein coronary artery bypass (35). However, the dose of sulfinpyrazone required in baboons to block completely cannula thrombus

formation is about one order of magnitude greater than that shown to have detectable effects on platelet survival time and reduction in thrombotic occlusion in humans (37). There are at least three possible explanations for this discrepancy. First, the pharmacokinetics of sulfinpyrazone may be different in the baboon compared with humans. For instance, the plasma levels of sulfinpyrazone in baboons documented in the present study (Table II), averaging between 1 and 10 μg/ml after twice daily doses of 20–100 mg/kg per d, are of the same order of magnitude as the plasma levels achieved in humans using <0.1 of the dose, i.e. a single dose of 200 mg (37). Second, the present dosing regimen involved twice-daily administration rather than the usual four times daily regimen used in patients. Third, antithrombotic efficacy in humans using smaller doses than those required in the baboons has been observed in uremic patients treated by chronic hemodialysis via A-V cannulae (25). Since patients undergoing chronic hemodialysis are known to have significant platelet dysfunction (38), and plasma drug binding of acidic drugs such as sulfinpyrazone is decreased in renal failure (39), the administration of a dose that would normally be incompletely effective, could be antithrombotic in dialysis patients, whereas patients without associated hemostatic abnormalities may be more resistant to that dose of sulfinpyrazone.

The lack of aspirin's efficacy to interrupt cannula platelet consumption in the present model (Table I) is in agreement with the inability of aspirin to prolong platelet survival time in patients with artificial heart valves (21, 24, 30), or atherosclerotic coronary artery disease (28, 40), and the ineffectiveness of aspirin to reduce mortality in the secondary prevention of myocardial infarction (41). However, aspirin has clearly been shown to be clinically effective in reducing thromboembolic complications of artificial heart valves (42), thrombotic occlusion of A-V cannulae in uremic patients (43), stroke and death in patients with transient ischemia attack (44), myocardial infarction in men with unstable angina (45), and possibly in reducing the occlusion of saphenous vein coronary artery bypass grafts (46). It may be important in this context to note that an additional hemostatic abnormality was associated with the beneficial effect of aspirin in the studies of artificial heart valves (oral anticoagulation) and A-V cannulae (platelet dysfunction in uremics treated with chronic hemodialysis), and that the pathogenesis of transient ischemic attacks and unstable angina may involve vasospasm rather than thrombosis. The reported antithrombotic effect of aspirin in patients undergoing saphenous vein coronary artery bypass is provisional, in that the study involved a relatively small number of patients with an unusually high frequency of graft occlusion in the control group. Thus, aspirin may exert limited antithrombotic effects unless it is used in association with another antithrombotic drug or perturbation of the hemostatic mechanism, or in a setting where blockade of the vasoconstrictor TxA_2 is relevant. Our results in the baboon are consistent with this formulation.

Whereas aspirin alone fails to modify platelet consumption in the present studies, aspirin clearly potentiates the efficacy of low doses of dipyridamole; i.e., 2.5 mg/kg dipyridamole has little although significant capacity to interrupt platelet consumption alone, but is greatly potentiated by the addition of aspirin at 20 mg/kg per d (Fig. 1; Table I). Moreover, the full potentiation of that dose depends upon administering aspirin simultaneously with every dose of dipyridamole (Table V). These results in the baboon are in accord with the capacity of the combination aspirin and dipyridamole to prolong platelet survival in patients with Dacron vascular grafts (26, 27) and coronary artery atherosclerosis (28). In these patient studies the combination shown to be effective was approximately 3 mg/kg per d of dipyridamole and 15 mg/kg per d aspirin, doses that are similar to the 2.5 and 20 mg/kg per d, respectively, used in the present baboon studies. The data regarding normalization of platelet survival time also correlate with the capacity of this same combination regimen to reduce saphenous vein bypass graft occlusion rates in patients undergoing coronary artery bypass surgery when the therapy is initiated before surgery (47, 48) and to reduce "coronary incidence" (sudden death plus both fatal and nonfatal myocardial infarction) in patients for 2 yr after acute myocardial infarction (49).

The mechanism whereby aspirin potentiates the antithrombotic activity of dipyridamole is not evident from our studies. The possibility that the effect might be mediated through aspirin's conversion to salicylic acid is excluded by the data reported in Table IV. Also, the pharmacokinetic results (Table II) indicate that aspirin does not produce significant alterations in the plasma levels of dipyridamole, or vice versa. This lack of pharmacokinetic interaction between aspirin and dipyridamole has recently been reported for humans (18).

The possibility that inhibition of the cyclooxygenase–TxA_2 pathway might be involved in the potentiation of dipyridamole appears to be excluded by the results with dazoxiben (Table I) and studies evaluating the effects of variations in dose and timing of administering the drug combination (Table V). The thromboxane synthetase inhibitor dazoxiben (50) is similar to aspirin in that it fails to inhibit cannula platelet consumption when used alone in doses sufficient to prolong the bleeding time and to decrease platelet TxA_2 production to <1% of base-line control values (Tables I and III). However, in contrast to aspirin, dazoxiben has no potentiating effect on dipyridamole (Table I). Thus, the specific blockade of TxA_2 formation does not by itself produce detectable antithrombotic activity or potentiation of dipyridamole's antithrombotic activity in this model. These results are at variance with a set of studies carried out in rabbits (51).

The possibility that the endoperoxides prostaglandin G_2 (PGG_2) and prostaglandin H_2 (PGH_2) might mediate platelet participation in thrombotic processes independent of TxA_2 also appears to be excluded by aspirin's dose- and time-dependent potentiation of dipyridamole (Tables I and V); i.e., aspirin's full effect requires simultaneous and repeated dosing at 20 mg/kg per d, a dose that completely and irreversibly blocks platelet cyclooxygenase-dependent formation of the endoperoxides, TxA_2 and prostaglandin I_2 (PGI_2) (Table III; 52). Other potential mechanisms of aspirin's antithrombotic effect that are not excluded by our studies include the possibilities that aspirin could reduce the amount of platelet consumption in forming thrombus by enhancing the rate of disaggregation of reversibly attached platelets, increasing some platelet-active product of the lipoxygenase pathway (53), or by acetylation of other platelet or plasma proteins (52, 54). Evidence that aspirin produces a reversible platelet hemostatic defect is also provided by the observation that platelets taken from normal donors who had ingested aspirin exhibited only transient impairment in platelet plug forming capability when infused into severely thrombocytopenic recipients (55).

Our results with the combination of aspirin and dipyridamole also have relevance to an understanding of the antithrom-

botic mechanism of dipyridamole. It has been proposed that dipyridamole acts to elevate platelet cyclic AMP (cAMP) by both blocking platelet phosphodiesterase-dependent breakdown and increasing cAMP formation by PGI_2-mediated stimulation of platelet adenylatecyclase (56). However, the capacity of aspirin to potentiate the antithrombotic effects of dipyridamole despite the use of repeated and relatively high doses of aspirin sufficient to block cyclooxygenase-dependent production of PGI_2 (Table III), appears to exclude a role for PGI_2 in the antithrombotic mechanism of dipyridamole. It seems more likely that an increase in blood adenosine levels may be involved, because dipyridamole is a potent inhibitor of vascular and erythrocyte adenosine uptake (57, 58) and produces an elevation in plasma adenosine levels (59). The higher plasma concentrations of adenosine could mediate the inhibition of platelet reactivity by stimulating platelet adenylate cyclase activity (60). For example, in vitro 10 μM dipyridamole markedly inhibits the uptake of adenosine by endothelial cells and produces potent antiaggregating activity that is not blocked by aspirin, i.e., in the absence of prostacyclin (57). The vasodilatory effects of dipyridamole also appear to be related to the elevation of plasma adenosine levels.

The results in these studies showing efficacy for sulfinpyrazone but not for aspirin or dazoxiben (Table I) when used singly indicate that the mechanism of sulfinpyrazone's antithrombotic action is unrelated to its effects on arachidonic acid metabolism (61). This argument also applies to the sulfide metabolite of sulfinpyrazone, which has been reported to be ~10 times more potent as an antiplatelet agent as the parent drug (62), and achieves considerably higher plasma levels in baboons than have been observed in man (Table II). The plasma sulfide levels averaged ~8 and 20 μg/ml after an oral dose of 20 and 100 mg/kg per d in the present study, whereas peak sulfide levels in normal human subjects after 200 mg of sulfinpyrazone averaged 2.6 μg/ml (11). In addition, the inhibitory effects reported for sulfinpyrazone with respect to competitive blockade of cyclooxygenase activity are probably irrelevant at the blood concentrations achieved by clinical therapy (37). Thus, although there are data to suggest that sulfinpyrazone may also mediate its therapeutic effect through actions on the endothelium (63), the antithrombotic mechanism for this drug remains to be clarified.

Aspirin also potentiates the antithrombotic effect of sulfinpyrazone with a maximal effect at 20 mg/kg per d (Table I), the same dose found to be optimal when aspirin is combined with dipyridamole. These results are consistent with the trend observed in the Canadian Stroke Study in which sulfinpyrazone and aspirin together may have been more effective than aspirin alone in reducing stroke and death (44, 64). The potentiation of sulfinpyrazone's antithrombotic effect by aspirin also indicates that aspirin and sulfinpyrazone have different antithrombotic mechanisms that are independent of their reported inhibitory effects on cyclooxygenase (52, 61). No formal clinical trials involving known thrombosis-related outcome events have definitively tested this postulated enhanced antithrombotic efficacy of an aspirin-sulfinpyrazone combination.

In considering our experimental results, it is important to bear in mind that the present model primarily assesses platelet–surface and platelet–platelet interactions in the formation of thrombus initiated by an artificial surface under arterial flow conditions at a site remote from normal or damaged vascular tissues. Thus, the system is presumably not influenced by effects of the immediately adjacent vessel wall, e.g., local removal of prothrombotic factors such as ADP and thrombin, or the local release of antithrombotic substances such as PGI_2 and tissue plasminogen activator. Thus, the results of the present studies have their most obvious clinical relevance to patients with prosthetic heart valves, A-V cannulae, and prosthetic vascular grafts. However, in view of the concordance between drug effects on platelet consumption in the present model, and patients with atherosclerotic vascular disease, together with the demonstrated clinical usefulness in reducing thrombotic occlusion of saphenous vein bypass grafts (47, 48), the results may also be relevant to treatment strategies in the management of patients with vascular disease.

The results reported in this paper may have a number of implications for the design of future controlled clinical trials assessing antiplatelet agents. First, in view of present inadequate understanding of the mechanisms whereby these drugs act, the selection of dose and regimen to be used in future trials should be based on dose-response studies using thrombotic endpoints rather than biochemical measurements without proven significance. Second, the recommendation to test low-dose, infrequent-administration regimens of aspirin (15, 19) may not be valid for some situations because that proposal is based primarily on the results of aspirin's inhibition of platelet and vascular wall cyclooxygenase (19), a process not necessarily related to aspirin's antithrombotic effects. However, the recent report that low dose aspirin improves patency of saphenous vein coronary artery bypass grafts (46) may support the low dose approach if this result is validated. Third, an effective combination of aspirin and dipyridamole requires multiple, simultaneous oral administration of relatively large amounts of aspirin with each dose of dipyridamole, such as the regimen used in the saphenous vein coronary artery bypass study, i.e., 75 mg of dipyridamole together with 330 mg of aspirin given as a combination three times daily (47, 48). Fourth, a definitive controlled clinical trial comparing the antithrombotic efficacy of aspirin vs. aspirin plus dipyridamole should be carried out using that dose regimen. Finally, consideration might be given to carrying out a controlled clinical trial designed to compare aspirin with the combination of aspirin plus sulfinpyrazone.

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