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EPINEPHRINE POTENTIATION OF IN VIVO STIMULI REVERSES ASPIRIN INHIBITION OF PLATELET THROMBUS FORMATION IN STENOSED CANINE CORONARY ARTERIES

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ABSTRACT

In 18 anesthetized dogs with a 70% mechanically produced coronary artery stenosis, blood flow measured with an electromagnetic flowmeter showed cyclical reductions in flow due to periodic acute platelet thrombus formation. These were abolished in eight of nine dogs with 2.5 mg/kg of aspirin given intravenously and in nine of nine dogs with 5 mg/kg of aspirin. However in 14 of 18 dogs the cyclical flow reductions were temporarily renewed with the infusion of epinephrine 0.4 ug/kg/min. Human platelets inhibited with aspirin can be reactivated with physiologic amounts of epinephrine. We postulate that in patients with atherosclerotic stenotic lesions the use of aspirin to inhibit arterial thrombus formation may be less effective when they have elevated catecholamines.

INTRODUCTION

Recent evidence suggests that platelets and thrombosis in stenosed human coronary arteries play an important role in progression of coronary artery disease as well as in the pathogenesis of the acute coronary syndromes of unstable angina, myocardial infarction and sudden death (1). The primary therapeutic agent used to reduce morbidity and mortality in these syndromes has been daily aspirin. It is of interest that the majority of clinical trials with aspirin for prevention of reinfarction or cardiac death showed favorable trends but were not statistically significant (2,3). However, two recent studies in patients with unstable angina showed a highly significant reduction in myocardial infarction using 324 mg aspirin/day (4), or 1.3 gm of aspirin/day (5). There have not been however adequate studies in animals which mimic the patient problem, to allow for evaluation of therapeutic interventions.

Key Words - coronary thrombosis, aspirin, epinephrine

We previously reported a model of coronary artery stenosis with moderate to severe intimal and medial damage which produced periodic cyclical reductions in coronary blood flow due to periodic thrombus formation(6-8). This model simulates the problem in patients with ulcerative coronary artery stenosis leading to thrombus formation, as has been reported for patients with unstable angina (9). We observed previously that cyclical reductions in coronary blood flow could be abolished with high dose aspirin (20 mg/kg) but not with heparin (1 mg/kg) (6). Finally, our preliminary animal studies suggested that the intravenous administration of epinephrine appeared to reinitiate acute thrombus formation and cyclical flow reductions in spite of pretreatment with 5 mg/kg of aspirin (10).

In view of the recent concern about high dose aspirin inhibiting PGI_2 production as well as platelet thromboxane A_2 production, we elected to study 2.5 mg/kg and 5 mg/kg doses of aspirin in our model. Thus we proposed to determine if the low dose of aspirin protects against acute coronary platelet thrombus formation in stenosed dog coronary arteries and prevents renewed acute thrombus formation when the plasma epinephrine level is raised. This has potential clinical relevance since patients taking low dose aspirin to protect against an acute coronary thrombotic event raise their plasma catecholamines in a variety of ways such as smoking, (11) stress (12,13), exercise (14,15,16) and possibly some forms of hypertension (17).

METHODS

Twenty mongrel dogs (24-32 kg) of either sex were anesthetized with 3 mg/kg of morphine sulfate followed by 20 mg/kg of sodium pentobarbital. The dogs were ventilated with room air, the chest was opened and the heart exposed. The circumflex coronary artery was dissected free, and an electromagnetic flowmeter (EMF) probe was placed on it to measure coronary blood flow continuously. The artery was clamped several times with a vascular clamp to produce moderate intimal damage in the area to be stenosed. A plastic encircling cylinder 4 mm in length was placed externally around the outside of the coronary artery distal to the EMF probe producing a 70-75% stenosis as previously described (6-8). With this amount of stenosis, coronary blood flow does not decline from control unstenosed levels, but there is no reactive hyperemic response to a temporary 20 second complete occlusion. With this stenosis and the intimal damage it produces, acute platelet thrombi periodically develop in the narrowed lumen and then embolize, restoring coronary artery blood flow to control levels again, producing cyclical blood flow reductions (CFRs). In 18 of 20 dogs CFRs occurred and were observed for 1/2 hour. Then the dogs were given an IV infusion of epinephrine (0.4 ug/kg/min) for 15 minutes. The size and frequency of the cyclical reductions in flow were recorded. The dogs were then divided into two groups. Group A received 2.5 mg/kg of aspirin intravenously; this is approximately twice the equivalent of the small (80 mg) aspirin tablet. Group B received 5 mg/kg of aspirin, the rough equivalent of one standard aspirin tablet in a 70 kg adult male. The aspirin was weighed and dissolved in warm saline keeping the temperature below 50°C and used within 2 hours as aspirin is known to be unstable in solution, decomposing to salicyclic and acetic acid (18). Both groups were monitored for 30 min at which time the epinephrine infusion was repeated and monitoring was continued.

RESULTS

Group A (n = 9) 2.5 mg/kg of aspirin.

All dogs in group A showed CFRs (8±3/30 min) during the initial observation period (Figure 1) (Table 1) and all showed increased frequency, and size of CFRs during the epinephrine infusion (Figure 1) (Table 1-A). Eight of 9 dogs showed abolition of CFRs after 2.5 mg/kg of aspirin (Figure 2), and the ninth dog showed decreased size and frequency of CFR's (Table 1A). In 7 of 8 dogs in which CFR's stopped with aspirin, the infusion of epinephrine 30 minutes after the aspirin caused renewal of CFR's for 13±6 minutes (Figure 2) (Table 1-A).

TABLE 1-A (n=9)

Cyclic Blood Flow Reductions (CFR's)

	Frequency (CFRs/30 min)	Average size (ml/min)	Aortic blood pressure (mm Hg)	Heart rate (beats/min)
Control n=9	8±3	39±8	112±19	122±28
	p<.05	p<.02	p<.05	NS
Peak Response to IV Epi	11±2	57±11	123±16	110±16
After 2.5 mg/kg ASA n=1	4*	15±4*	110*	124*
Peak repeat response to IV Epi n=7	5±3	29±5	p<.05 125±12	p<.05 114±15

TABLE 1-B (n=9)

Control n=9	7±2	44±7	108±11	117±14
	p<.05	p<.02	p<.05	NS
Peak response to IV Epi	12±3	67±7	129±15	108±15
After 5.0 mg/kg ASA	0	0	111±13	112±14
			p<.02	NS
Peak repeat response to IV Epi n=7	4±2	39±5	126±12	110±16

* Values shown are for the 1 dog of the 9 that showed decreased size and frequency of CFRs after aspirin, but not complete abolition.

Data were analyzed using a paired t-test and are represented as the mean±SD.

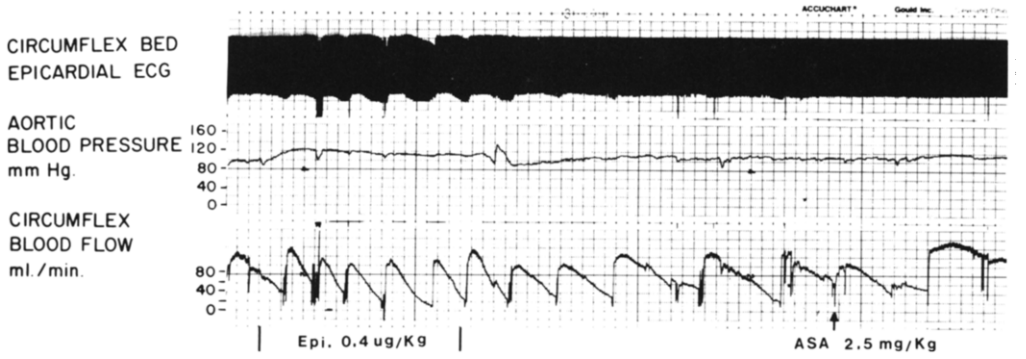

FIG 1. Control cyclical flow reductions in coronary blood flow shown on the far left show an increase in frequency and rate of flow decline when epinephrine is infused IV for 15 minutes. In the center the rate of cyclical flow reductions return to pre-epi flow rates. On the right 2.5 mg/kg of aspirin (ASA) is given IV and cyclical flow reductions due to periodic acute platelet thrombus formation are abolished.

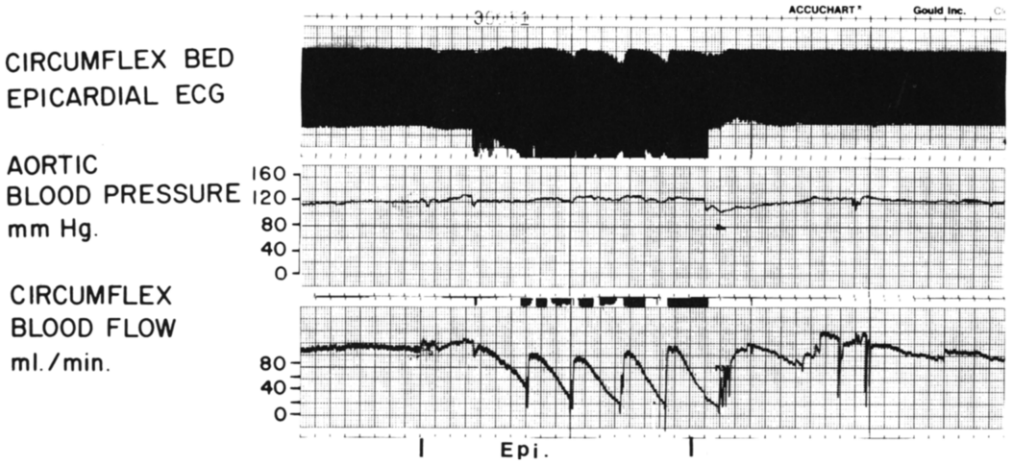

FIG 2. On the left the coronary blood flow is constant with no cyclical flow reductions or periodic acute thrombus formation due to the aspirin administration. However in the center, cyclical flow reductions return during the infusion of epinephrine (Epi for 15 min) in spite of pretreatment with aspirin.

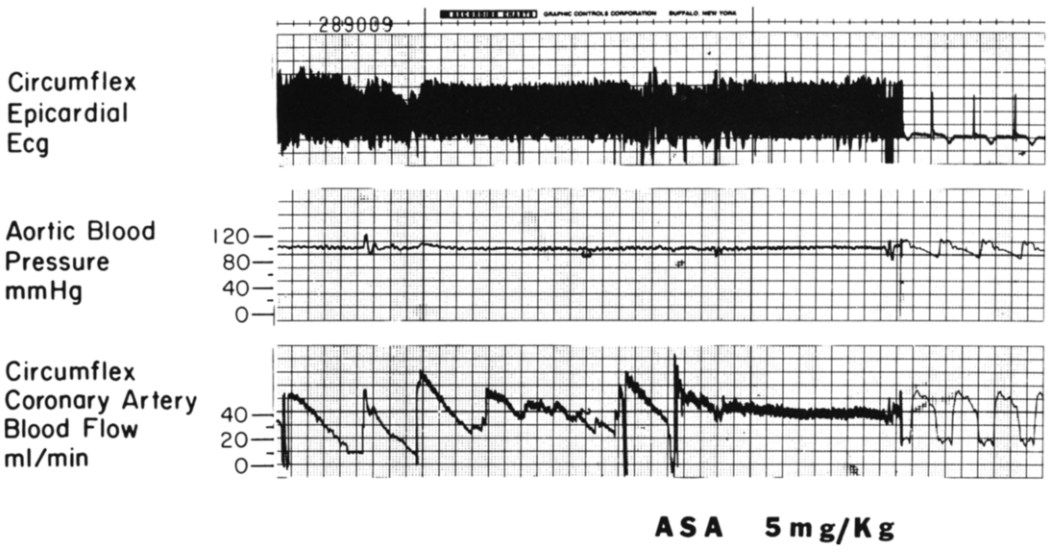

FIG. 3. Cyclical flow reductions due to acute platelet thrombus formation in the stenosed coronary artery are shown on the left and are abolished by 5 mg/kg of aspirin (ASA) given IV. Recording paper speed in 0.1 mm/sec. On the far right 4 cardiac cycles are presented at fast paper speed of 25 mm/sec, and shows normal ECG and a normal phasic coronary flow pattern for a 70% stenosis.

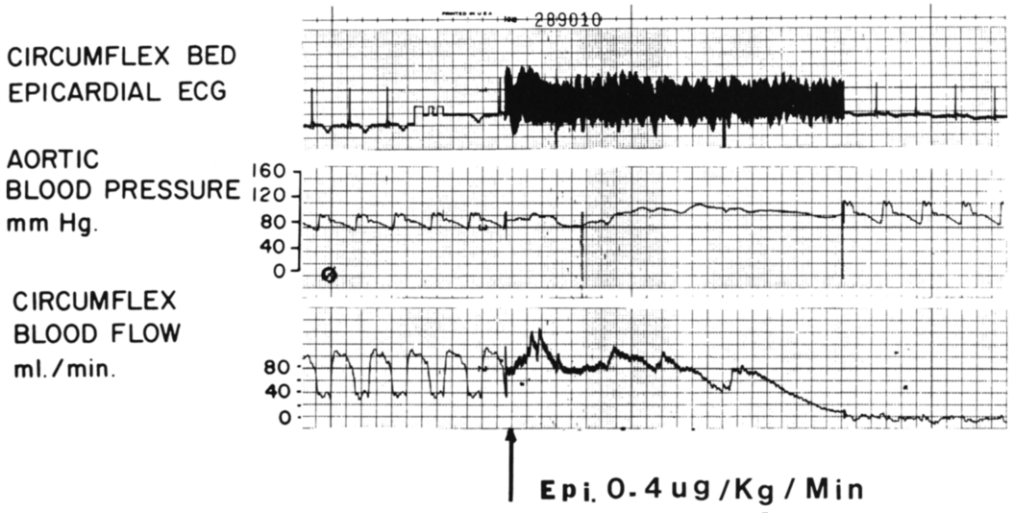

FIG. 4. Phasic coronary blood flow, arterial blood pressure and ECG are shown on the left at a fast recording paper speed of 25 mm/sec. In the center at slow paper speed the infusion of epinephrine (Epi) for 15 minutes causes a renewal of coronary blood flow decline, in spite of pretreatment with aspirin (ASA).

Group B (n=9): 5 mg/kg of aspirin.

In group B (9 dogs) all dogs had CFR's (7±2/30 min) and the size and frequency were also exacerbated by the epinephrine infusion (Table 1-B). In all 9 dogs the CFR's were abolished by 5 mg/kg of aspirin (Table 1B, Figure 3). In 7 of these 9 dogs CFR's were renewed by the IV epinephrine infusion (Table 1B, Figure 4), and one of these dogs died from complete coronary occlusion and ventricular fibrillation (Figure 5). The frequency and the size of CFRs for Group A dogs before and after aspirin and after the epinephrine challenge are shown in Table 1-A. The same data for group B dogs are shown in Table 1-B.

Arterial blood pressure increased and heart rate was reflexly slowed by the IV infusion of epinephrine in both groups of dogs (Table 1-A and 1-B).

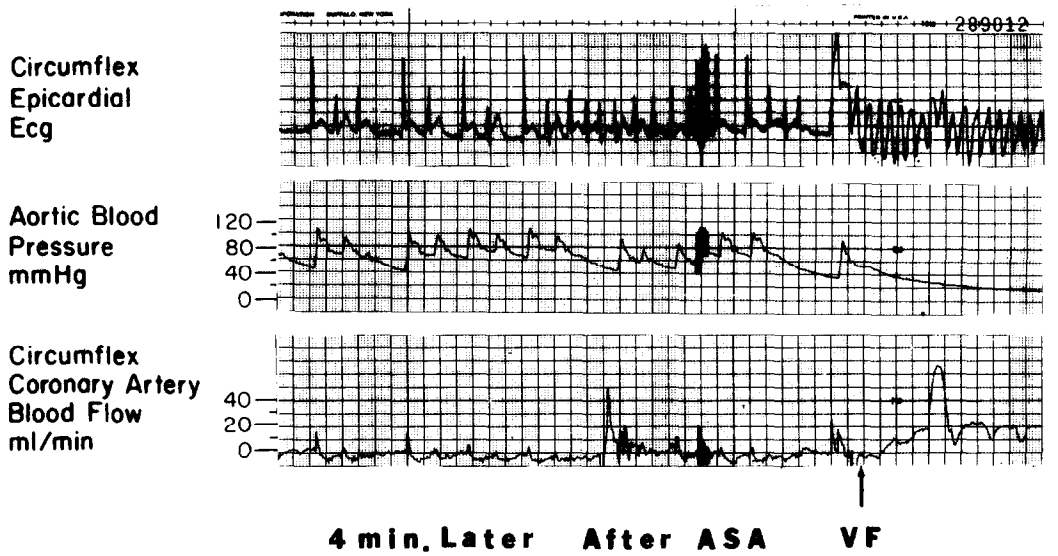

FIG. 5. Coronary blood flow has declined to zero due to a complete thrombotic coronary occlusion, and the dog died from ventricular fibrillation (VF) in spite of the pretreatment with 5 mg/kg of aspirin (ASA).

DISCUSSION

Epinephrine is known to increase platelet activity and aggregation of human platelets (19), to be synergistic with other platelet stimuli such as ADP and collagen in man (19) and to be synergistic with these other stimuli in some species such as the dog (20). We and others have shown that with this model there is a significant amount of intimal damage exposing collagen which is a strong stimulus for platelet aggregation and acute thrombus formation (8,21). In addition, we have observed by light microscopy that there are significant numbers of damaged red cells trapped in the thrombus in the stenosed lumen (8). It seems reasonable to assume that some red cells are injured by the turbulent blood flow existing in a severe coronary artery stenosis or caught by the strands of fibrin in the forming clot thus producing the damaged red cells seen in the thrombus. The damaged red cells release ADP, another potent stimulus for platelet aggregation, and acute thrombus formation (22,23).

Epinephrine is known to enhance the proaggregatory stimulation of platelet activity by ADP or collagen (19). Aspirin blocks the platelet production of thromboxane A_2, and thereby inhibits platelet aggregation and acute platelet thrombus formation. However, the epinephrine can apparently overcome the inhibitory effects of aspirin temporarily.

Rao and his colleagues have shown that human platelets inhibited with aspirin could be reactivated with physiologic amounts of epinephrine in an in vitro study (24). Furthermore, they have demonstrated that epinephrine reverses the inhibitory influence of aspirin on platelet-vessel wall interaction (25).

Inhibition of CFRs by aspirin is probably due to the blocking of the production of endoperoxides PGG_2 and PGH_2, and of thromboxane A_2. Thus epinephrine probably does not reverse the inhibition by aspirin but rather potentiates the effects of other agonists such as ADP from damaged red cells, thrombin, and the exposed collagen due to intimal damage (19,20).

We have previously studied 10 patients with angiographically discrete lesions in peripheral arteries resembling those produced in our model, who had frequent periodic episodes of claudication at rest due to peripheral vascular disease. Blood flow velocity was measured continuously for 2 hours with a CW Doppler flow meter placed on the skin over the area of stenosis with the patient lying quietly. In 6 of 10 patients at least one decline in blood flow velocity similar to a "CFR" in our animal model occurred followed by a rapid return to control velocity levels (24). All 10 patients were given 2 aspirin tablets and were restudied 1 hour later. None of the patients who had demonstrated a flow velocity decline before aspirin had any when studied after aspirin. However, in this study, no method was used to raise the patient's plasma epinephrine levels, to see if a decline in blood flow velocity might be provoked.

A large number of clinical trials have been done comparing aspirin to placebo in patients with coronary artery disease (27). Indeed the first suggestion that daily aspirin might reduce the risk of coronary death was reported in 1953 before we knew that aspirin affected platelets (28). However, most of these studies demonstrated a favorable trend but not a statistically significant benefit for any cardiac event (27). A recent VA cooperative study did show a highly statistically significant reduction in death and infarction in outpatients with unstable angina taking 324 mg of aspirin daily, when compared to a matched group of patients taking only placebo (4).

We report here that low dose aspirin inhibits acute thrombus formation in our model but that epinephrine infusions can renew acute thrombus formation. We have preliminary data to show that low dose ethanol (blood alcohol level .13 gm/dl) can inhibit acute thrombus formation and CFR's in our model but the CFR's are renewed with an epinephrine infusion (29). However, if the aspirin and ethanol are given together they act synergistically to prevent renewed CFRs by epinephrine infusion (29). The possibility that this synergism may be important clinically has not been studied.

The plasma levels achieved with this dose of epinephrine averaged 800-1400 pg/ml (unpublished observations). This is within the range observed for some clinical situations such as during acute myocardial infarction (30). Somewhat lower levels are observed in patients with congestive heart failure (31) or with stress (32) or during smoking (11). This concentration of epinephrine is within the range which can potentiate the effects of other agonists. We have also shown that CFRs occur in rabbit and monkey carotid arteries instrumented as described above (33,34,35). These CFRs can be abolished with 5 mg/kg of aspirin but they are renewed with the same epinephrine dose of 0.4 ug/kg/min for 15 minutes.

Two factors suggest that the present study may be clinically relevant. First, the dogs are anesthetized and barbiturate anesthesia moderately inhibits platelet function in a dose dependent fashion (36). Secondly, human platelets are more sensitive to catecholamines than dog platelets (37). Thus the epinephrine enhancement of thrombus formation may be even more significant in man than it was in the present animal study.

In summary, our model of coronary artery stenosis and thrombosis shows that low dose aspirin can abolish acute thrombus formation, but this phenomenon can be renewed with physiologic amounts of IV epinephrine. This may have some clinical relevance since patients with known coronary artery disease and unstable angina have variably elevated catecholamines by a variety of mechanisms. We feel that any experimental agent studied and considered for patient use should protect against acute platelet thrombus formation as well as elevated epinephrine levels.

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