Endogenous Inhibitor (S) of Platelet Aggregation

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Abstract

Human blood plasma contains an endogenous inhibitor (s) of platelet aggregation (EIPA). The EIPA activity was found to be associated with a globulin-rich and albumin-rich protein fractions of blood plasma. Data presented in this paper suggests that ELPA may regulate platelet aggregation associated with excessive production of arachidonic acid metabolites (JPMA 35 41, 1985).

Introduction

In recent years, role of arachidonic acid (AA) in platelet aggregation and thrombosis has received niuch attention. One of the main reasons for this interest has been the demonstration that AA metabolism by way of prostaglandin (PG) endoperoxide synthase pathway in platelets leads to the generation of prostaglandin endoperoxides (PGG_2, PGH_2) and thromboxane A_2 (TXA_2). Both of these products are strongly pro-aggregatory of platelets[1,2] TXA_2 has been thought to contribute to stroke, myocardial infarction and angina pectoris. [3,4]The balance of pro-aggregatory TXA2 production by platelets and anti-aggregatory prostacyclin (PGI_2) by the blood vessel wall may be an important factor in controlling thrombus formation.[5] In addition, human plasma of serum has recently been shown to contain endogenous inhibitors of prostaglandin synthesis and arachidonate lipoxygenase. [6,9]
Aspirin, an inhibitor of prostaglandin synthesis is known to inhibit platelet aggregation and its use as an antithrombotic drug has been investigated10-12 Therefore, the effects of PG synthesis inhibitors on synthesis of TXA_2 and PGI_2 is of considerable significance. The question arises, how the production of PG endoperoxides and thromboxanes is regulated during circulation. To answer this question, we investigated the effect of plasma on AA-induced platelet aggregation. The results of this study are indicative that human plasma and serum contain one or more circulating endogenous inhibitors of platelet aggregation named EIPA.

Material and Methods

Sodium arachidonate purchased from Sigma (grade 1, 99% pure) was prepared by dissolving 10 mg of arachidonic acid in 50 ul ethanol and diluting with 0.2% sodium carbonate to 2.5 ml of solution. 5 u1 of this solution was added tQ 0.7 ml of diluted washed platelet suspension to give a final -concentration of 95 p M. All other drugs and chemicals used were obtained from various commercial suppliers and were of the highest purity grade available.

Platelet Preparation

Blood was drawn from volunteers into plastic syringes. The volunteers has not taken aspirin-like drug for at least 7 days. Blood was gently mixed with heparin in plastic sterile tubes to give a final heparin concentration of 10 units! ml. The blood was then centrifuged at 200 xg for 15 min, giving a supernatant platelet-rich plasma (PRP). An aliquot of PRP was retained for testing, and the remainder centrifuged at 600 xg for 15 minutes and the resulting platelet pellet was resuspended in 0.15M NaC1 (saline) containing prostacyclin (PGI_2) 10 ng/ml to inhibit platelet stickiness during processing. This

suspension was centrifuged at 600 xg for 15 minutes and the platelet pellet was resuspended in half the original plasma volume of 0.l5M Tris-HC1 buffer, pH 7.4, containing PGI_2 10 ng/ml. After standing at room temperature for I hour to allow the PGI_2 to decay, the suspension was adjusted to about 2 X 10^8 platelets/ml before use (Table I).

Table I

Preparation of Washed Platelets.

Fresh Whole Blood (Heparinized)	
Centrifuged at 200_g for 15 min	
Platelet Rich Plasma	Red cells etc Discarded
Centrifuged at 600_g for 15 min	
Platelet Pellet (PP)	Platelet-Poor Plasma (Removed and kept on ice)
PP resuspended in 0.85% Saline containing 10NG/ML PGI_2	
Centrifuged at 600_g for 15 min	
	Supernatant Discarded
PP Resuspended in 0. IM Tris Buffer, pH 7.4, containing 10NG/ML PGI_2	
Platelet Suspension left at room temperature for at least I hour before use, for PGI_2 to decay.	

Platelet Aggregation

Aggregation was monitored with a LumiAggregometer (Model 400, Chronology; American Scientific Products, Chicago, U.S.A) using 0.5 ml aliquots of PRP or washed platelet suspension at 37 C, the volume was made upto 0.7 ml with saline and the mixture was pre-incubated with test material at 37°C

for 5 min before challenge with sodium arachidonate. The resulting aggregation was recorded by Lumi-aggregometer and expressed as percentage inhibition, compared with control, at 4 mi after challenge.

Results and Discussion

Sodium arachidonate has been shown to produce aggregation of human platelets suspended in plasma.[13] In this study we have shown thatsodium arachidonate is 10 times more potent in inducing aggregation of human platelets suspended in buffer than in plasma (PRP). Maximal aggregation in PRP was obtained with 1.8 mM arachidonate, whereas maximal aggregation of washed platelets suspended in buffer was obtained with 0.19 mM arachidonate (Fig. 1).

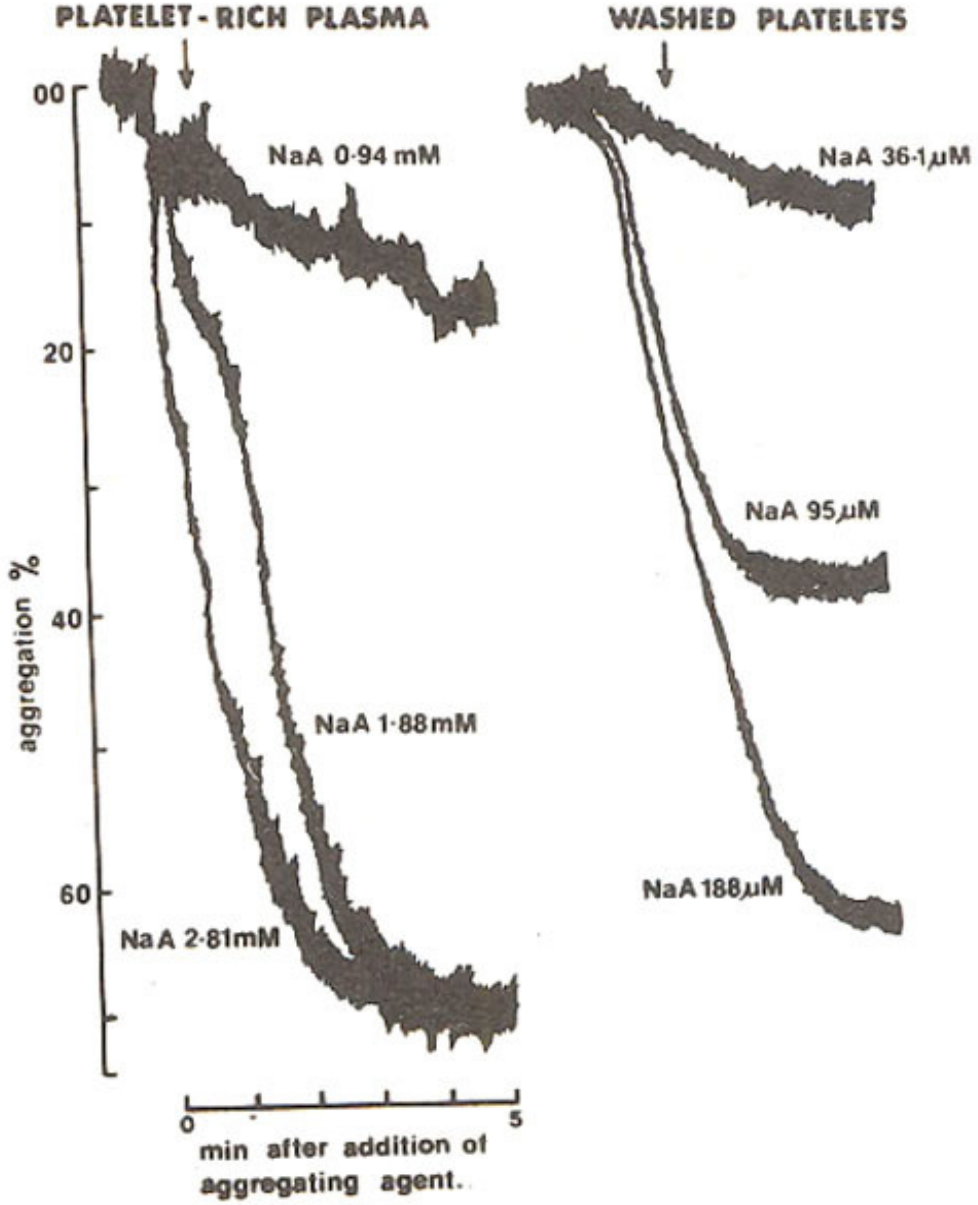

Fig. 1. Effect of sodium arachidonate (NaA) on human platelets suspended in plasma or washed platelets suspended in buffer.

This suggests that plasma antagonizes the action of arachidonate on platelets.
Since PG endoperoxides and TXA_2 are known to induce aggregation, the differential effect of AA may indicate that plasma is probably inhibiting platelet PG endoperoxide and TXA_2 formation.

Therefore for further experiments, washed platelets suspended in buffer were used to screen the effects of plasma, serum, plasma fractions (Cohn I, II, IV-4 and V) and the commonly used plasma expanders dextran T40 and T70.

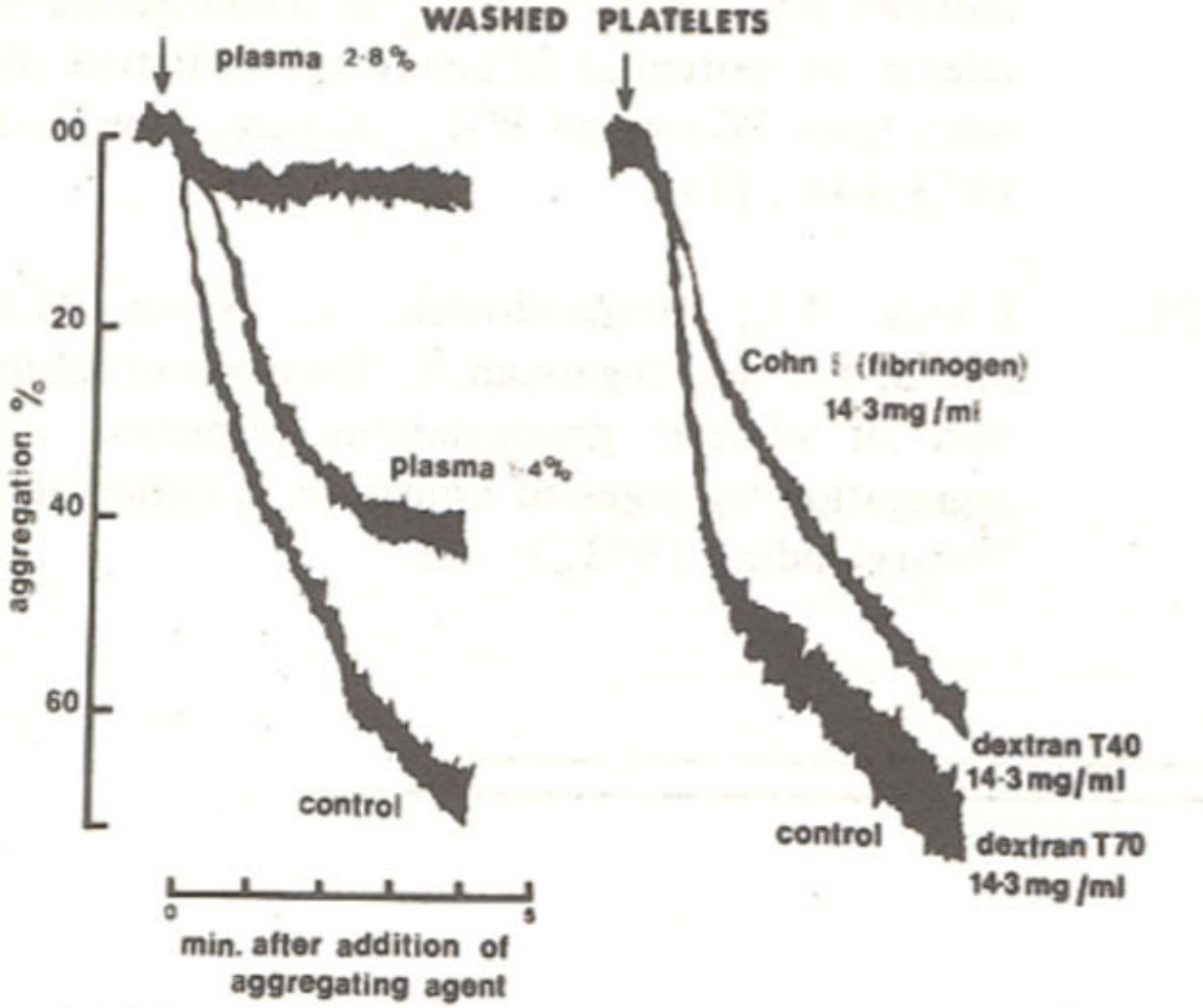

Fig. 2. Effect of human plasma, fibrinogen (Cohn I) and dextrans T40 or T70 on arachidonate induced aggregation of human washed platelets.

Fig. 2 shows a tracing from a typical experiment where human plasma inhibited AA-induced platelet aggregation in a concentration related manner. Plasma Cohn fractions IV-4 (a- globulins), Cohn V (albumin) (Fig. 3) and haptoglobin inhibited aggregation in a dose-dependent manner. Cohn I (fibrinogen), Cohn II (Gamma-globulins) and dextran T40 and T70 were ineffective. Complete results are summarized in Table II.

These results demonstrate a novel finding that human blood plasma and serum potently inhibit AA-induced platelet aggregation. The inhibitory effect of plasma or serum was concentration dependent and restricted to the plasma protein fractions rich in a- globulins and albumin content. This correlated

well with our previousstudies in this laboratory that plasma inhibitory activity separated into two fractions by gel filtration chromatography on sephadex G-l00. Furthermore, we found that inhibitory activity was heat-labile and nondialyzable through a cellophane membrane indicating a large molecular size. We conclude that human blood plasma and serum contain endogenous factors that inhibit platelet aggregation (EIPA). The mechanism by which EIPA inhibits AA induced aggregation is not known at present. However, it is known that platelet aggregation can also be caused by other physiologically important substances such as ADP, collagen, epinephrine and thrombin.[14] Though these compounds induce platelet aggregation, AA could be a key factor in triggering platelet aggregation and thrombosis. The evidence in favour of this may be summarized as follow : 1) AA induces platelet aggregation both in vitro and in vivo[15,16] and the synthesis of endoperoxide and TXA_2 17;2)These same prostanoids and TXA_2 are formed during blood clotting and during the aggregation of platelets induced by AA, ADP, Collagen, epinephrine, and thrombin. [17,20] 3)Platelet aggregation induced by small amounts of ADP, epinephrine or collagen is greatly enhanced by a concentration of AA that by itself does not cause aggregation.[13] 4)Inhibitors of prostaglandin synthesis, such as aspirin inhibit platelet aggregation in response to collagen or epinephrine or AA[21,13]

Esterified AA is present in phospholipids, cholesterol esters and triglycerides of blood and other tissues. Non-esterified AA is known to be bound to plasma albumin. Albumin, a part of the plasma EIPA activity (Fig. 3, Table II),

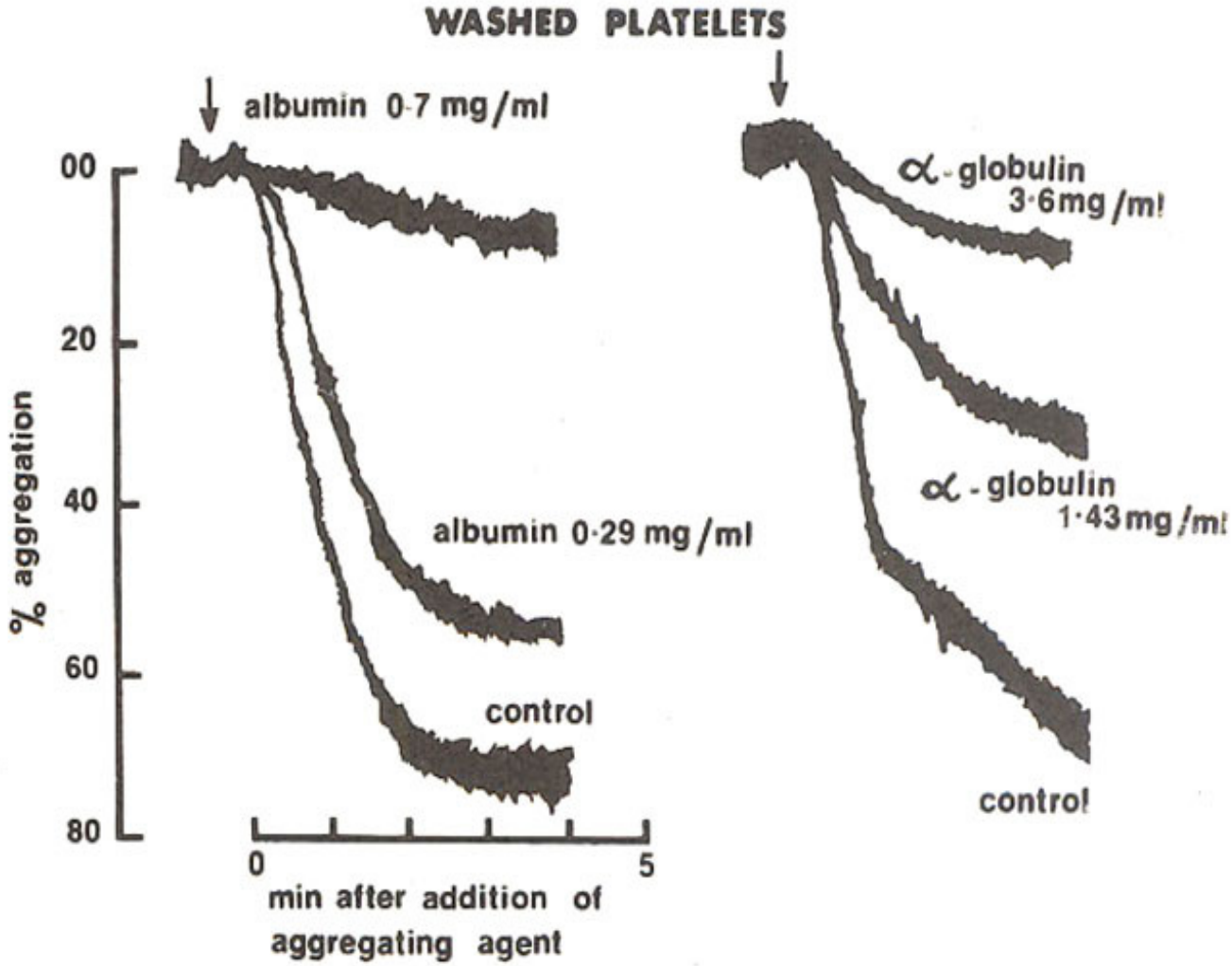

Fig. 3. Inhibition of arachidonate induced aggregation of human washed platelets by plasma albumin (Cohn V) or α - globulins (Cohn IV - 4).

Table II

Inhibition of Arachidonate – Induced Aggregation of Human Blood Platelets.

Test Material (Human)	Concentration (% v/v)		Mean % inhibition ± SEM
Plasma	0.07		19.4 ± 3.1
	0.3		36.1 ± 3.7
	1.4		68.8 ± 3.5
	2.8		88.8 ± 6.4
Serum	0.07		12.3 ± 1.9
	0.3		30.4 ± 4.1
	1.4		65.4 ± 3.9
	2.8		79.7 ± 9.8
Umbilical Cord Plasma	0.3		15.9 ± 2.8
	1.4		55.5 ± 7.5
	2.8		65.6 ± 5.9
Fibrinogen (Cohn I)	14.3	mg/ml	11.9 ± 11.5
Gamma-globulin (Cohn II)	14.3	mg/ml	14.2 ± 4.6
α - Globulin (Cohn IV-4)	1.13	mg/ml	53.2 ± 2.5
	2.81	mg/ml	84.9 ± 2.1
Albumin (Cohn V)	0.29	mg/ml	23.5 ± 6.8
	0.71	mg/ml	63.4 ± 8.5
	1.42	mg/ml	91.7 ± 3.6
Haptoglobin	3.6	mg/ml	30.8 ± 10.5
	7.2	mg/ml	55.9 ± 6.7
Dextran T40	14.3	mg/ml	8.5 ± 4.9
Dextran T70	14.3	mg/ml	14.6 ± 2.8

Results are means ± SEM of 3-7 determinations. Aggregation in test suspension was compared with that in control, 4 min after challenge with a 95 μM arachidonate.

Haptoglobin was purified according to Saeed et al (1980). Purified Cohn fractions were obtained from Sigma and Miles U.S.A.

may therefore be an important factor in controlling haemostasis, Because aggregation of platelets tends to be self-recruiting and the amounts o prostacyclin in the circulating plasma are too small to inhibit aggregation, this proposed function of albumin would be expected to have a survival value. Since anti-aggregatory effect of albumin has a ceiling (Fig.3) aggregation due to massive liberation of AA. as

might occur (in thrombosis) with damage to the vessel wall, would be unimpaired.
The present findings also indicate a potential advantage of FIPA over dextran as a plasma expander. We propose that after trauma such as surgery when tissue injury presumably releases AA from disrupted cell membranes, intravenous infusions of FIPA might help to prevent post operative thrombosis. Infuced dextran will increase the plasma volume temporarily but it will also dilute the plasma proteins and may leave the platelets more prone to aggre gation. The isolation of IIPA and elucidation of the mechanisms by which EIPA acts is currently under study in our laboratory.

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