

Activity of Endogenous Inhibitor(s) of Platelet Aggregation in Plasma of Normal Males, Females and Pregnant Women

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Abstract

The EIPA activity in human plasmas obtained from adult males, females, pregnant females and foetal umbilical cord was determined. EIPA activity was highest in the following order of potency; adult females > adult males > pregnant females > foetal umbilical cord plasma. The data suggest the EIPA may vary according to sex or pregnancy in females. activity, is highest in females than in males and (JPMA 35: 217, 1985).

Introduction

Endogenous inhibitor (s) of platelet aggregation (EIPA) exist in human blood plasma and serum¹. A preliminary analysis of human plasma Cohn fractions revealed that most of EIPA activity is associated with albumin-rich and α -globulin rich protein fractions. Furthermore, it has been observed that EIPA is non-dialyzable and labile to heating for 10 min at 100°C or to incubation with pronase at 37°C, indicating that EIPA is proteinaceous in character.

The *in vivo* role of EIPA remains to be defined though it has been postulated² that EIPA may regulate platelet aggregation triggered by arachidonic acid (AA) metabolites. Since albumin (a part of EIPA) is known to bind fatty acids^{2,3} we suggested that EIPA may inhibit platelet aggregation in the blood stream that might otherwise be triggered by small amounts of free AA.

However, it is not known if EIPA activity differs according to sex. We extended this study to elucidate possible sex differences in EIPA activity and to investigate possible changes that may be associated with pregnancy.

Material and Methods

Blood samples were collected and divided into four groups from human sources comprising of: a) twelve men (age range 20-40 years), b) thirteen unmarried women (age range 22-37 years), c) eleven pregnant women (gestational ages 20-36 weeks of mixed parity who had normal pregnancies), d) six umbilical venous cord samples from babies born at term. In groups a-d, blood was obtained from the antecubital vein by venepuncture and subjects had not taken any non-steroidal anti-inflammatory drugs in the preceding week.

All blood samples were taken into tubes containing heparin (10 units/ml of blood). Blood was centrifuged at 1,000 xg for 15 mins at 4°C, plasma was separated and stored at 20°C until analyzed. Samples displaying any degree of haemolysis were discarded. Albumin concentration was determined as described in the Technical Bulletin No. 630 (Sigma, USA) with human plasma albumin as a reference. The inhibitory effect of various plasmas on arachidonate-induced platelet aggregation (EIPA activity) was measured as follows:

Platelet Aggregation

Platelet aggregation was monitored with a Lumi-Aggregometer (Model 400, chronolog; American Scientific Products, Chicago, U.S.A) using 0.5ml aliquots of PRP or washed platelet suspension 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 min after challenge.

Platelet Preparation

Blood was drawn from volunteers into plastic syringes. The volunteers had 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 resulting platelet pellet was resuspended in 0.15M NaCl (saline) containing prostacyclin (PGI₂) 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 0.15M TrisHCl buffer, pH 7.4, containing PGI₂ to decay; the suspension was adjusted to about 2×10^8 platelets/ml before use (Table 1).

Table**Inhibition of Arachidonate – Induced Platelet Aggregation (EIPA) by Various Human Plasmas and their Albumin Concentration.**

Subject	Albumin (mg/ml)	EIPA activity (IC 50% v/v)
A. Male	53.2±0.5 (8)	0.03±0.03 (12) P<0.05 vs B
B. Female	51.0±0.9 (13)	0.02±0.01 (13) P<0.05 vs C
C. Pregnant female	42.4±0.8 (11)	0.44±0.06 (11) P<0.05 vs A
D. Foetal/ umbilical cord	36.5±3.1 (4)	0.57±0.03 (6) P<0.001vs A

Number of subjects in brackets. IC 50 is the concentration (% v/v) required to inhibit arachidonate – induced platelet aggregation by 50%.

Paired Students t–test was used to calculate P values.

Values for significance between different human plasmas were based on paired students t-test.

Results and Discussion

The EIPA activity of human plasmas obtained from adult males, females, pregnant females and foetal umbilical cord was tested. The EIPA activity of various plasma samples was concentration dependent.

The IC_{50} values (plasma concentration required to inhibit AA induced platelet aggregation by 50%) of all four groups and plasma albumin contents are given in Table. These results are also presented graphically in Fig.

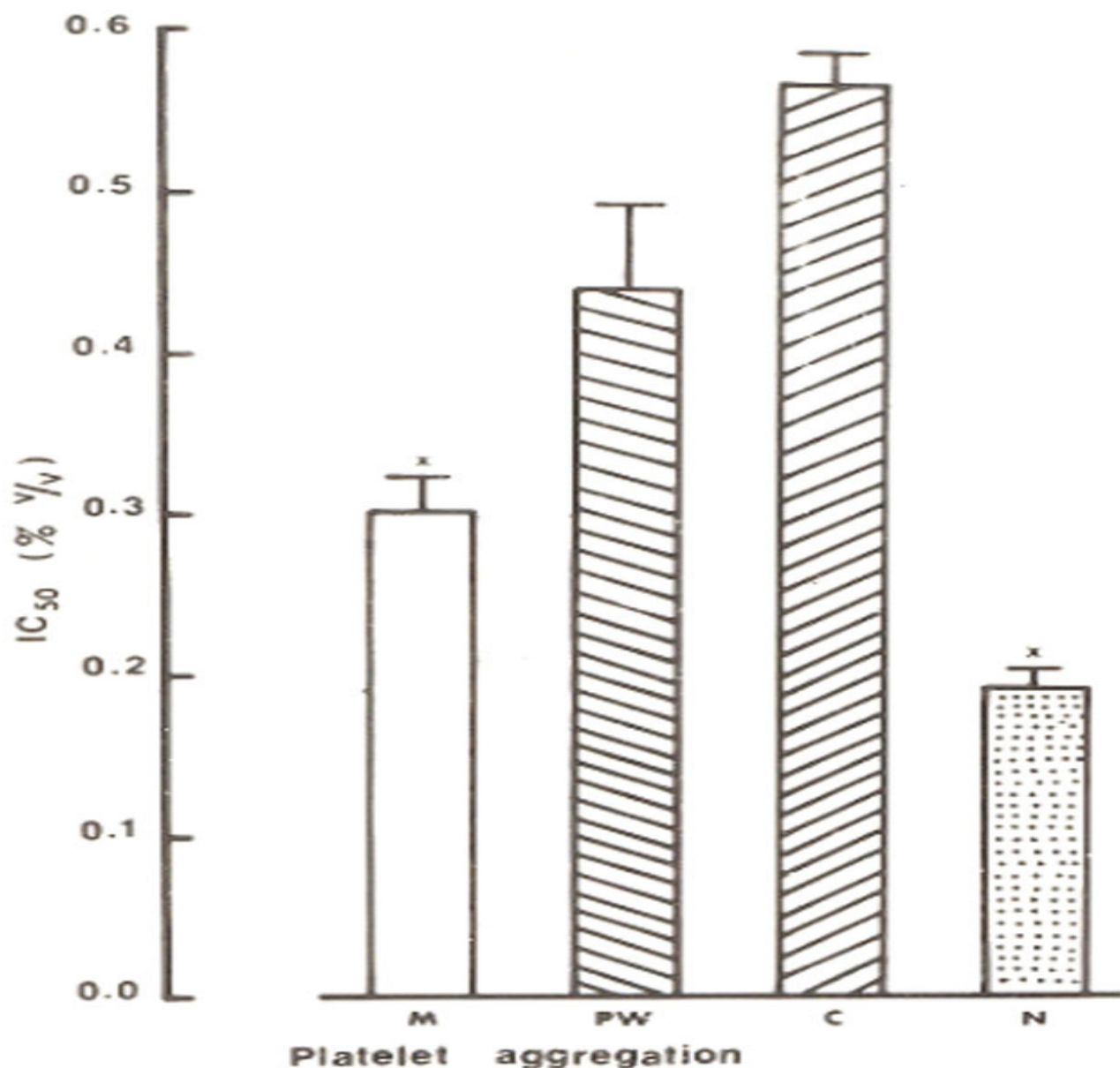


Fig. Comparative inhibitory effects of various human plasmas on AA-induced platelet aggregation. IC_{50} is the concentration (% v/v) which inhibits AA-induced aggregation by 50%.

M; adult man (n=12), PW; pregnant woman (n=11), C; human umbilical cordblood plasma (n=6), N; non-pregnant woman (n=13).

$P < 0.05$ compared with pregnant female plasma.

We have shown that EIPA activity in plasma, derived from adult females and males, was significantly

($P < 0.05$) higher than in plasma from pregnant females and foetal umbilical cord. These results demonstrate significant differences between the EIPA activities according to sex. and physiological state. The greater potency for inhibition of AA-induced platelet aggregation displayed by plasma from non-pregnant women when compared with men or pregnant women provides a source for considerable speculation. Does the male/female difference reflect the greater likelihood of men to suffer a thrombotic episode? Though the question cannot be answered at this time, however it is of interest to note that Doll et al.⁴ have recently shown that among non-smokers, men are six times more likely than women to suffer a thrombotic episode. Such a causal relationship is unlikely but we would like to speculate that EIPA may contribute to the development or outcome of these events.

The relatively poor ability of umbilical cord plasma to inhibit AA-induced platelet aggregation when compared with adult plasma is suggestive that the endogenous inhibitor (s) of platelet aggregation (EIPA) do not cross the placenta from mother to the foetus to any significant extent. The significance of the low EIPA : activity in cord plasma is uncertain. This finding however, is consistent with the high level of plasma AA metabolites such as prostaglandin (PG) E and F that exist in healthy preterm infants as reported by Mitchell et al.⁵ Furthermore, these two PG's do not induce aggregation of human platelets.⁶

In conclusion, an appreciation of the significance of these interesting preliminary results awaits the outcome of ongoing work on the identity and role of plasma EIPA.

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