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HUMAN LIPOPROTEIN (A)- INDUCED REDUCTION OF PLATELET AGGREGATION IS NOT MEDIATED BY APOLIPOPROTEIN (A)'S LYSINE-BINDING REGIONS.

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Materials and Methods
4. Statistical analyses
5. Results and discussion
6. References

1. ABSTRACT

Intact lipoprotein (a) (Lp (a)) is known to decrease collagen - stimulated platelet aggregation *in vitro*, though the nature of the interaction between this lipoprotein and stimulated platelets is unknown. Lysine binding regions of Lp (a) facilitate binding between it and at least one cell type. Epsilon aminocaproic acid (EACA) renders Lp (a)'s lysine binding regions incapable of cellular interaction using those regions. Washed human platelets were presented vehicle alone or with varying concentrations of Lp (a) either not exposed or previously exposed to increasing levels of EACA. In all experiments, EACA failed to affect the impact of Lp(a) on platelet aggregation. It is concluded that the lysine binding regions of Lp (a) do not mediate the intact Lp(a)-induced reduction of collagen- stimulated platelet aggregation *in vitro*.

2. INTRODUCTION

The nature of the interaction between Lp(a) mediating its dose- related reduction of *in vitro* collagen-stimulated human platelet aggregation is not clear (1). The lysine binding regions of Lp (a) mediate some of that lipoprotein's binding with other cells (2,3). As apolipoprotein (a) has lysine binding regions and apolipoprotein (a) has been shown to mediate Lp(a)'s binding to human platelets (4) it was hypothesized that such lysine binding regions would mediate at least in part, the binding of Lp(a) to human platelets(5). Further it has been shown that recombinant apolipoprotein (a) exposed to EACA reduces the apo (a) platelet interaction thus reducing the pro-aggregating impact of purified apo(a) on stimulated human platelets(5). Thus, it was the purpose of this work to determine if intact Lp (a)'s lysine binding regions play any role in the Lp (a)-mediated reduction of human platelet aggregation *in vitro*.

3. MATERIALS AND METHODS

Seven subjects participated in all experiments. The proposed study was fully explained to each subject and informed written consent was obtained. The study's protocol was approved by the Human Ethics Committee of the University of Texas, Southwestern Medical Centre at Dallas.

Lp(a) was isolated from five high level Lp(a) donors by passing human plasma over a lectin column (Sigma, St. Louis, MO) (L. Seman, personal communication). The eluate was ultracentrifuged in a Beckman benchtop ultracentrifuge (TLA 100.3 rotor, 85,000 rpm, 16 h) using a KBr final density of 1.215 g/ml to isolate Lp(a). The purity of the isolated Lp(a) was determined via rocket electrophoresis and a 2-16 % native polyacrylamide gradient gel(6-8).

Blood was drawn from the antecubital vein of each subject in 15-ml vacutainers (red top) containing 2.0 ml of acid citrate dextrose (9). The washed platelets were obtained by centrifuging whole blood at 111 g for 15 min at 30 ^{o} C to yield platelet rich plasma (PRP). The PRP was centrifuged at 2000 g for 15 minutes at 37 ^{o} C. The derived

Table 1. Collagen (2 microgram/ml)-stimulated aggregation in washed human platelets exposed to different levels of human lipoprotein (a) (Lp (a)) or Lp (a) exposed to varying epsilon aminocaproic acid (EACA) concentrations

Lp (a) concentration (mg/dl)	0 mM EACA	1 mM EACA	5 mM EACA	25 mM EACA	50 mM EACA	100 mM EACA
0	91.0±8.1	-	-	-	-	-
1	81.1±8.2	85.8±9.2	89.9±10.2	82.3±7.7	86.8±9.2	82.6±9.2
5	66.3±6.2[a]	65.0±7.3[a]	65.8±5.2[a]	61.9±5.9[a]	67.2±6.6[a]	65.0±6.2[a]
25	49.6±5.7[a]	52.4±4.4[a]	50.1±5.4[a]	50.0±6.4[a]	54.5±4.5[a]	47.2±5.8[a]
50	42.3±4.6[a]	43.1±3.5[a]	42.6±3.8[a]	42.4±5.5[a]	43.4±5.8[a]	38.7±4.1[a]
100	33.5±2.1[a]	32.5±2.2[a]	34.9±2.9[a]	31.9±3.4[a]	29.1±2.5[a]	32.7±2.8[a]

Values are the means ± SEM of platelets from 7 subjects including duplicate measures for each subject. Values with a superscript letter in a column are significantly different ($p < 0.05$) from their corresponding value in the 0 mg/dl Lp (a) column. Values with no superscript or identical superscript are identical statistically for a given row. The final platelet concentration was 2 x 10^8 /ml.

Table 2. Proof of elimination of Lp(a) lysine site binding capability

^{125}I-ligand	Buffer Moleculesx 10^4 /cell	EACA Molecules x 10^4 /cell	Percent inhibition
Lp (a)	3.2±0.3	1.5±0.2[a]	48.1±3.9

Lp(a) at 100 mg/dl incubated without or with 100 mM EACA in the presence of U937 cells (2 X 10^8/ml) in PSIII buffer. The means (± SEM) are those of triplicate determinations. Lp(a)/EACA incubation was for 1 min at room temperature followed by dialysis and then Lp(a) incubation with cells. The superscript a denotes a significant ($p < 0.05$) difference.

platelet pellet was suspended in platelet suspension I (PS I) and further washed once in each of PS II and PS III based on the method of Mustard *et al* (10). PS I, II, and III contained Tyrodes buffer [NaCl (137 mM), KCl (2.7 mM), $NaHCO_3$ (12 mM), NaH_2PO_4 (0.4 mM), MgCl (1 mM), $CaCl_2$ (2 mM), glucose (5.5 mM) at pH 7.35, and osmolarity 200-300 mosmol, containing 0.35 % bovine serum albumin, HEPES (2.5 mM)] and apyrase (PS I and II, 30 *m*g/ml, PS III (3 *m*g/ml). PS I also contained heparin (50 units/ml). Final platelet concentrations in PS III were adjusted to 2 x 10^8/ml with the aid of a Coulter Counter (Model ZM, Coulter Electronics Ltd., Miami, FL). Lp (a) with or without EACA exposure (1 minute at room temperature) was dialyzed overnight at 4°C to remove EACA. Platelets in PS III were then incubated with 0, 1, 5, 25, 50, and 100 mg/dl Lp (a) or with vehicle (0.15 M phosphate buffered saline (PBS) pH 7.4). A portion of the platelet suspension (0.5 ml) was placed in a siliconized cuvette and then incubated for 1 minute at 37°C with Lp (a) (exposed or not exposed to EACA) or vehicle. The aggregation response was made using an 800 B Payton aggregometer (Payton Instruments, Buffalo, NY) with stirring at 900 rpm at 37 °C. The degree of aggregation was measured by taking the distance in cm from the baseline to the vertical midpoint of the aggregation plateau at 5 minutes post-agonist (equine type I collagen, Hormon Chemie, Munich, Germany) introduction to the platelet. This distance was expressed as a percentage of the distance between baseline (Lp(a)(with or with out EACA exposure)/platelet mix (0 % change in light transmittance)and a Tyrodes buffer blank (100 % change in light transmittance). Proof that the lysine binding regions on Lp(a) were blocked was established using the technique of Scanu *et al.* (3) employing U 937 cells.

4. STATISTICAL ANALYSES

A two-way analysis of variance (ANOVA) was used to determine if there was a statistically significant effect of Lp (a) concentrations (with and without EACA) on platelet aggregation. In all cases, it was the mean (of duplicate measures) levels of the seven platelet donors that were used in the statistical analyses. The means for each subject were then used to generate subject group aggregation means in Table 1. A t-test was applied to the data in Table 2 showing that the assay was working.

5. RESULTS AND DISCUSSION

The data is contained in Table 1. Increasing concentrations of EACA up to 100 mM resulted in no change in Lp (a)-induced reduction of levels relative to the absence of EACA. The statistical analysis revealed no statistically significant interaction between EACA and Lp (a). Proof that the assay was working is contained in Table 2.

The similar pattern of platelet aggregation response to increasing concentrations of Lp (a) in the presence or absence of EACA suggests that the lysine binding regions of Lp (a) are not mediating the platelet aggregation response to intact Lp (a)(Table 1). Proof that the assay was working is included in Table 2. Thus the significance of these findings it is possibly another epitope or epitopes mediating the intact Lp (a)-platelet interaction that induce the drop in platelet aggregation(1). These results stand in contrast to other results suggesting that apo (a) would mediate an increase in Lp(a)-induced platelet aggregation(5).

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