The Effects of Oligolysines and Polylysines on Human Platelet Aggregation Induced by Polylysines, Adenosine Diphosphate, and Epinephrine

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Introduction

In the past there have been several confusing reports in the literature about the interaction of polylysine with human platelet-rich plasma (PRP) suspensions. Some reports indicate that this synthetic polycation can induce platelet aggregation and stimulate the release reaction when added to PRP suspensions (5). Other investigators have suggested that at most there is an electrostatic interaction between the positively charged polylysine and the sialic acid-rich negatively charged platelet surfaces (6). This polylysine effect has been reported to be independent of polymer molecular weight, with polymers in the molecular weight range 2500-400,000 Daltons being effective (4, 5, 6, 7, 8, 10). Once again, Metcalf and Lyman report that plasma cofactors may be required for the polylysine-platelet interaction but Massini et al. report that no plasma cofactor is required (4, 5). Published reports also indicate that conformational variations are possible and that extended left-handed polylysine helices effectively interact with platelets and that L, D, and D-L monomers can be present. Metcalf and Lyman indicate that the beta polylysine conformation interacts with the platelets, while the random coil conformation is ineffective (4).

It is reported also that the epsilon amino groups of the lysine monomers must remain intact for the interaction to occur. Succinylation of these groups abolishes activity as does deamination, N-acetylation, or N-dinitrophenylation. Various biological polyanions can also inhibit the polylysine-platelet interaction, presumably by forming electrostatic complexes with the added polylysine. Included in this category are heparin and chondroitin sulfates (7).

Since many reagents which induce platelet aggregation or the release reactions in platelets are dependent on the liberation of arachidonic acid from membrane bound phospholipids, and the subsequent generation of cyclic endoperoxides from the arachidonic acid, we decided to use a well known reagent to block the generation of these derivatives in the arachidonic acid cascade (13). We reasoned that if the polylysine-platelet interaction is primarily an electrostatic interaction, then impairment of the biochemical functionality of the platelets probably would not alter it.

It is well known that aspirin (acetyl salicylic acid) is a common pharmacologic agent which can block the generation of arachidonic acid derivatives (13). Published studies indicate that aspirin acetylates susceptible protein R groups on at least three platelet proteins, including the enzyme, platelet cyclo-oxygenase. This particular enzyme is involved in the generation of the cyclic endoperoxide intermediates (PGG_2, PGH_2) which are precursors to the potent platelet aggregator, thromboxane A_2 (2, 4, 9).

In our present study, we have taken platelets from human volunteer subjects who were either aspirin-free or well aspirinized at the time the platelets were collected, and we have then studied the interaction of various molecular weight, oligo-and polylysines with either aspirin-free or well aspirinized platelets in plasma suspension. We have also studied the interaction of these two kinds of platelet populations by incubating them with various molecular weight oligo-or polylysines and then adding low doses

of adenosine diphosphate to the suspensions 30 seconds later. The dose of adenosine diphosphate was selected to induce only a mild reversible primary aggregation when it is added alone. Finally, the two kinds of platelet populations were preincubated with various oligo-and polylysines and then epinephrine was added in strong aggregating 30 seconds later.

Materials and Methods

Potential platelet donors were recruited and each completed a questionnaire evaluating disease-free and drug-free state of the donor. Each donor also signed an informed consent statement developed and approved by the Committees for the Protection of Human Subjects within IUPUI and IPFW. Approximately 50 ml. of whole blood was collected into Becton-Dickinson 6419 Vacutainers, specifically designed for preparation of PRP. These evacuated containers were sterilized, silicone-coated, and contain 0.5 ml. of buffered 0.129M sodium citrate. The collected whole blood was centrifuged in a vibration-free Sorvall DuPont T6000 centrifuge at room temperature for ten minutes at 1000 RPM's. The PRP is carefully pipetted from the top of the tubes into a plastic container using a plastic Falcon 10 ml. pipet. Platelet poor plasma (PPP) was prepared by centrifuging the remaining blood components for ten minutes at 10,000 RPMs in a high speed refrigerated Sorvall centrifuge and the supernatant resulting was collected. Platelet counts were obtained on an automated Coulter counter at Veterans Administration Hospital in Fort Wayne, Indiana. All PRP typically had a platelet count greater than 350,000 platelets/mm^3.

Adenosine diphosphate and most of the polylysines were obtained from Sigma Chemical Company. Some of the polylysines and all oligolysines were obtained from Vega Chemical Company. Epinephrine was obtained from Bio Data Corporation. Stock solutions were prepared at appropriate concentrations by diluting the respective reagent with 0.85% sodium chloride. Dilutions were prepared also using this sodium chloride solution and all solutions were adjusted to pH7 with an Orion 501 pH meter. The test reagent solutions and standards were stored in plastic culture tubes at $-20^{\circ}C$ in 5 ml. aliquots. Polylysines and oligolysine were dissolved at the appropriate concentration on the day of usage.

Platelet-rich plasma was stored at room temperature and promptly utilized within 4-6 hours after the whole blood was drawn. All aggregation tests were done in a Payton 300 Dual Channel Aggregometer at $37^{\circ}C$. with a constant stirring speed of 900 RPM's. These conditions are optimal for efficient aggregation and do not cause sufficient shearing forces to disaggregate platelet clumps. The chart recorder ranges are established on the two pen recorder system using aliquots of platelet-rich and platelet-poor plasma. Baseline stability is periodically checked and aggregation standards are added to samples periodically to insure that platelets are remaining viable. If obvious erythrocyte sediments or hemolysis is detected in the PRP, it is discarded promptly.

Results

Typical results evaluating the interaction of platelet suspensions with the oligo- and polylysines at 1 mg./1 ml. concentrations are shown in Table 1. It can be seen that if small oligolysines (lysyl-lysine, pentalysine) or intermediate molecular weight lysines (molecular 4000-14,000 Daltons) are added to suspensions of normal platelets or aspirinized platelets, no aggregation effect is observed when monitored for at least five minute periods. It can be seen, however, when large molecular weight polylysines (25,000-240,000 Daltons) are added to normal or aspirinized platelet suspensions that a prompt, complete aggregation occurs. The experimental results suggest that a larger polylysine (molecular weight = 55,000 Daltons) is required to bring about initial

TABLE 1. Interaction of Platelet-Rich Plasma Suspensions with Oligo- and Polylysines (at lmg/ml concentrations)

Compound Added	Aspirin Free Platelets	Aspirinized Platelets
Lysyl-Lysine	–	–
Pentalysine	–	–
4K Polylysine	–	–
14K Polylysine	–	–
25K Polylysine	+	–
55K Polylysine	+	+
90K Polylysine	+	+
150K Polylysine	N.D.	+
240K Polylysine	N.D.	+

(+) = Complete, Irreversible Aggregation
(–) = No Effect
N.D. = No Data Collected

aggregation of platelets, but basically it can be concluded that the polylysine-platelet interaction is independent of the usual functioning arachidonic acid cascade leading to the production of cyclic endoperoxides and thromboxane A_2.

Table 2 shows the effects of pre-incubating normal platelets or aspirinized platelets with various oligo- or polylysines for 30 seconds before a low dose of adenosine diphosphate (ADP) is added. This table indicates once again that there is no difference in responsiveness between the normal platelets and the aspirinized platelets. Furthermore, this series of experiments shows that there is a cooperative interaction between the larger polylysines (molecular weight greater than 25,000 Daltons) and adenosine diphosphate. This cooperative interaction between polylysine and ADP can be explained by a linkage of a discrete polycation receptor with the adenosine diphosphate receptor, or by the fact that platelets preincubated with polylysines are drawn in closer proximity to one another and are more readily stimulated by low doses of ADP than is the case when polylysines are absent. It can certainly be seen that aspirin does not impair in any way this polylysine and adenosine diphosphate interaction with platelets.

TABLE 2: Effects of 30 Sec. Pre-Incubation of Oligo- and Polylysines on ADP-Induced Platelet Aggregation

ADP Added 30" After:	Aspirin Free Platelets	Aspirinized Platelets
Lysyl-Lysine	–	N.D.
Pentalysine	–	–
4K Polylysine	–	N.D.
14K Polylysine	–	–
25K Polylysine	+	+
90K Polylysine	+	+
150K Polylysine	+	+
240K Polylysine	+	+

(–) = No Effect
(+) = Rapid, Complete, Irreversible Aggregation
N.D. + No Data Collected

TABLE 3. Effects of 30 Sec. Pre-Incubation of Oligo- and Polylysines on Epinephrine-Induced Platelet Aggregation

Epi Added 30" After:	Aspirin Free Platelets	Aspirinized Platelets
Lysyl-lysine	–	N.D.
Pentalysine	–	–
4K Polylysine	–	–
25K Polylysine	+	+
55K Polylysine	+	+
90K Polylysine	+	+
150K Polylysine	+	+
240K Polylysine	+	+

(–) = No Effect
(+) = Enhances Aggregation in Magnitude or Onset
N.D. = No Data Collected

Finally Table 3 shows the effect of pre-incubating normal platelets or aspirinized platelets with various oligo-or polylysines and then adding a vigorous aggregating dose of epinephrine 30 seconds after the polycation addition. Once again, the data in Table 3 shows there is no difference in response between the normal and aspirinized platelets. It can be seen that a minimal sized polylysine (molecular weight = 25,000 Daltons) is required for this cooperative effect and again, there is a positive interaction between polylysine pre-incubation and epinephrine addition. As was the case for secondary ADP induced aggregation, this phenomenon could be explained by the linkage of a discrete polycation receptor to a discrete membrane epinephrine receptor on the platelets, or it could be explained alternatively by an electrostatic interaction of the polylysines with the platelets initially bringing them into spatial proximity to enhance the effect of epinephrine. Again, it is obvious that the aspirinized, biochemically impaired platelets yield equally good responses in these polylysine-epinephrine experiments.

Discussion

The present results indicate that polylysine polymers in the molecular weight range 25,000 to 240,000 Daltons are effective in inducing aggregation of platelet rich plasma when added at concentrations of 1 mg./1 ml. In contrast to earlier published studies with normal platelets, we have observed no aggregation with polylysines of molecular weight lower than 14,000 Daltons. Many of the earlier studies were done with citrate addition to whole blood in which the relative concentration of citrate was less carefully controlled and not standardized as has been the case in the present study using the B-D liquid citrate Vacutainers (4, 5, 6, 7, 10). Control of relative citrate concentration has been shown to be an important parameter in obtaining good platelet aggregation results in clinical studies (11).

In both normal and aspirinized platelets, we have seen a cooperative interaction between polylysines and classical aggregating agents, such as adenosine diphosphate or epinephrine. These positive interactions could be explained by a coupling between discrete receptor sites on the platelet surface and by an electrostatic interaction of platelets with polylysines initially. These present results do demonstrate that biochemically impaired platelets with an inability to generate the cyclic endoperoxides PGG_2 or PGH_2, or the potent aggregating substance, thromboxane A_2, give equally good responses to polylysines or combinations of polylysines with either adenosine diphosphate or epinephrine. Although the present results alone do not prove conclusively that polylysines are without biochemical or metabolic effects on the platelets, they are certainly sug-

gestive that polylysine-platelet interactions are largely electrostatic in nature. Earlier published reports by Guccione et al. in which platelets were pretreated with adenosine, EDTA, or prostaglandin E_2 also suggest that platelet functionality is not altered when polylysines are finally added (3).

In conclusion, there is no difference in response to populations of normal or aspirinized platelets to any of the tests mentioned above. There is a minimum molecular weight for polylysines that is required for induction of the aggregation reaction with B-D Vacutainer prepared platelet-rich plasma. However, since lysine and oligolysines have been shown to inhibit adenosine diphosphate or thrombin induced aggregation, it is obviously relevant for us to learn more about this polycation-platelet interaction (1, 12). The next phase of our research will include transmission electron photomicrographic studies to determine if the polylysine interaction causes any of the classic morphologic changes seen in platelets when various conventional aggregating agents are added.

The authors wish to acknowledge the preparation of this manuscript by Ms. Elaine Wilson and the platelet counts which were provided by Mr. Zane Smith at the Veterans Administration Hospital in Fort Wayne.

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