Inhibitor Effects of Three Adenosine Analogs on ADP Induced Platelet Aggregation

Barth H. Ragatz and Panayotis G. Iatridis
Northwest Center for Medical Education
Indiana University School of Medicine, Gary, Indiana 46408

Abstract

Adenosine and certain C-2 analogs are known to be potent *in vitro* inhibitors of platelet aggregation. Three synthetic nucleosides have been investigated and compared as inhibitors to adenosine. One of these three compounds, 1, N^6–ethenoadenosine (E–Ad) contains an additional ring fused to the adenine nucleus and is a poor inhibitor of adenosine 5–diphosphate (ADP) induced platlet aggregation even though the corresponding ADP analog is a stimulator of platelet aggregation. Two of these compounds 2′–0–methyladenosine (MeAd) and adenine arabinoside (A•Ara) are pentose modifications at position C–2′ and are also poor inhibitors of ADP induced platelet aggregation although A•Ara exhibits a time dependent improvement as an inhibitor. The effects of these compounds emphasize both the importance of structural integrity of the ribofuranosyl ring in adenosine and of the purine nucleus as the upper limit for size of a fused ring system in maintaining adenosine-like inhibitor functionality.

Introduction

Born (4) observed that adenosine is a potent inhibitor of ADP induced platelet aggregation *in vitro*. Mills *et al.* (8) reviewed the effects of various purine modified adenosine analogs and reported several of them with substituents at position C-2 to be even more potent inhibitors of ADP induced platelet aggregation than adenosine. Mester and Mester (7) found 2′-deoxyadenosine to be a weak inhibitor, and the 2′,3′-dialdehyde derived from adenosine by periodate cleavage to be devoid of inhibitor effect on ADP induced platelet aggregation.

The present investigation has been undertaken to study the correlation of the purine nucleoside chemical structure to inhibitor potency on ADP induced platelet aggregation.

Materials and Methods

Freshly collected whole blood from normal human subjects of both sexes was rapidly mixed with one-tenth volume of 3.2% sodium citrate. Blood donors were free of any medication for at least ten previous days. Platelet-rich plasma (PRP) was prepared by differential centrifugation (3) and platelet count (5) was adjusted to approximately 400,000/mm^3 by saline dilution. All nucleoside compounds used were obtained from P-L Biochemicals, Inc., Milwaukee, Wisconsin, and were dissolved in 0.155 *M* sodium chloride and adjusted to pH 7.0. The two compounds modified in the pentose moiety permit evaluation of the effects of adding a bulky substituent at position C-2′ in 2′-0-methyladenosine (MeAd) or of epimerization at this asymmetric center is adenine arabinoside (A•Ara). The purine modified compound, 1, N^6-ethenoadenosine (E-Ad) contains an additional fused heterocyclic ring which alters electronic charge distribution and hydrogen bonding potentials at nitrogen atoms N 1 and amino N^6. Platelet aggregation was studied by the turbidimetric

method of Born (2). Samples of PRP (0.5 ml.) were preincubated either with isotonic sodium chloride solution (0.05 ml.) or with test compound (0.05 ml.) for 30 seconds in the Payton Aggregometer and then stimulated with ADP (0.05 ml.) at a fixed concentration. In another series of experiments, 0.5 ml. PRP samples were treated with 0.05 ml. isotonic NaCl, followed by either 0.05 ml isotonic NaCl or compound after 30 seconds and by a fixed dose of ADP (0.05 ml.) after 5 minutes. Results were obtained using PRP from at least three donors for each compound and relative inhibitor potency was compared to adenosine over the same concentration interval.

Results

None of the three compounds examined (E-Ad, MeAd, A•Ara) showed any tendency to induce platelet aggregation when incubated alone with PRP and even in very high concentrations (100 fold above the effective dose of ADP which induced aggregation). Typical results are illustrated in Fig. 1 featuring the A•Ara system specifically.

When PRP was incubated for 30 seconds with any of the three compounds and then stimulated by a fixed dose of ADP, complete aggregation without significant time lag or diminution of magnitude occurred. There was no notable distinction among the three compounds and again, there was no dose dependent effect for any of these compounds over a 100-fold concentration range. Results for A•Ara are shown as a representative example in Fig. 2 along with the potent dose dependent inhibition produced within 30 seconds by adenosine for comparison.

With some adenosine analogs, potency of inhibition will change upon prolonged incubation with PRP (1). When PRP was preincubated with either E-Ad or MeAd for five minutes, no change in inhibitor

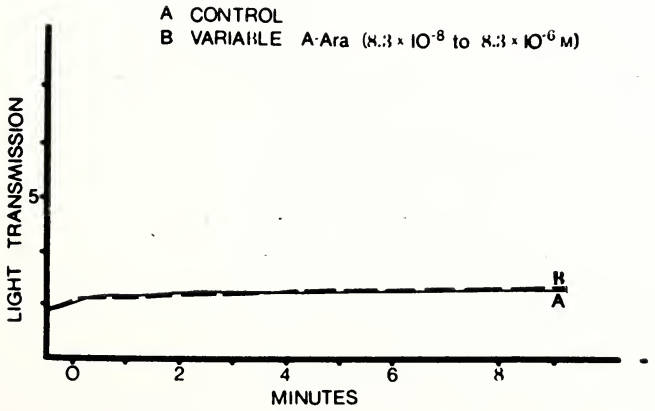

FIGURE 1. *Time dependent changes in light transmission through PRP suspensions after A•Ara is added. Curve A: control obtained by adding 0.05 ml. of 0.155M NaCl to PRP. Curve B: family of superimposed curves obtained by adding 0.05 ml. of A•Ara solutions; stock concentrations of A•Ara ranged from 1 x 10^{-5} M to 1 x 10^{-3} M.*

potency was observed when the platelets were stimulated by ADP. In contrast, a dose dependent improvement of inhibitor properties can be seen in Fig. 3 when PRP is preincubated with A•Ara for five minutes before ADP is added.

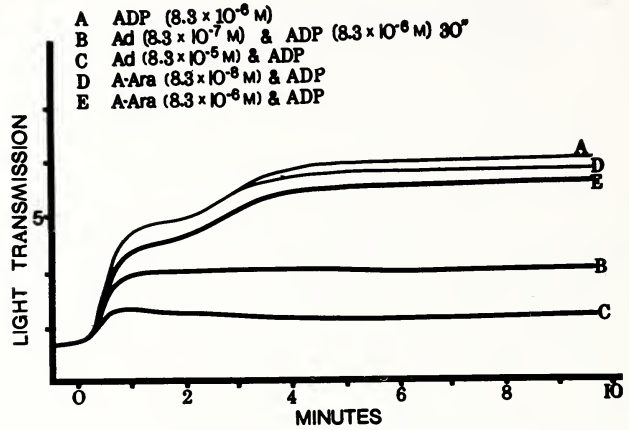

FIGURE 2. *Time dependent changes in light transmission through PRP suspensions preincubated with 0.05 ml. of variable concentrations of an inhibitor for 30 seconds. Zero minutes mark indicates the point at which the fixed (aggregation inducing) dose of ADP is added. Curve A: 0.05 ml. 0.155 M NaCl + 0.05 ml. 1 x 10⁻³ M ADP. Curve B: Low dose of adenosine challenged by aggregation inducing dose of ADP. Curve C: High dose of adenosine challenged by ADP. Curve D: Low dose of A•Ara + ADP. Curve E: High dose of A•Ara + ADP.*

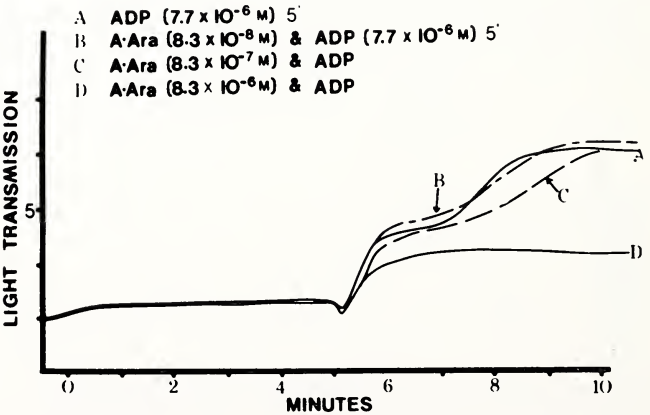

FIGURE 3. *Time dependent changes in light transmission through PRP suspensions preincubated with 0.05 ml. of 0.155 M NaCl, followed by 0.05 ml. of NaCl or A•Ara at 30 seconds (zero minute mark). A fixed ADP dose (0.05 ml.) is ultimately added at five minute mark. Curve A: Control; NaCl + NaCl (30") + ADP (5'). Curve B: NaCl + Low dose A•Ara (30") + ADP (5'). Curve C: NaCl + Mid-range dose A•Ara + ADP. Curve D: NaCl + High dose A•Ara + ADP.*

Discussion

It is unusual to find an adenosine analog with platelet aggregation stimulating activity; even the nucleoside phosphates, adenosine 5′ monophosphate and adenosine 5′ triphosphate, are inactive in this respect. Mester and Mester (7) postulate that the 5′ aldehyde product formed by chemical oxidation of adenosine is capable of inducing a mild and reversible aggregation since it possesses a partial negative charge at this essential molecular site. It is a necessary control in the screening of new compounds for platelet functionality to check for any aggregating properties, and the three reported compounds are devoid of this effect as shown in Fig. 1 with A•Ara as an example.

Other pentose variants of adenosine have been shown to be weak inhibitors of ADP induced platelet aggregation. The naturally occurring nucleoside, 2′ deoxyadenosine, is a very weak inhibitor as is 3′ deoxyadenosine (1). The 2′3′ dialdehyde product obtained from the periodate cleavage of adenosine has been shown to be devoid of inhibitor effect when incubated up to three minutes with PRP before ADP is added (7). It remains to be determined if loss of inhibitor effect in the dialdehyde compound is caused by loss of hydroxyls in a critical molecular region necessary for interaction with the platelet receptor or if loss of the rigid pentose ring conformation is the important structural determinant. In any case, the present pentose variants (A•Ara, MeAd) are shown to be poor inhibitors of ADP induced platelet aggregation when preincubated for 30 seconds with PRP.

Adenosine analogs which bear some similarity to E-Ad are reported by Kikugawa *et al.* (6) to be almost as inhibitory as is adenosine. Included in this series are compounds with bulky rings attached to the adenosine amino group (N^6-cyclohexyladenosine, N^6-phenyladenosine) (6). The present N^6 compound, E-Ad, is not completely analogous since the amino group is now part of an additional fused imidazole ring system rather than representing only the attachment of a bulky substituent at N^6 which is subject to relatively unhindered rotation. To account for the observation that E-Ad is not inhibitory while the compounds of Kikugawa *et al.* (6) are, it may be necessary to consider differences in pK of the amino group or differences in electronic charge at the various purine ring atoms in addition to substituent bulkiness.

Finally, it can be noted in Fig. 3 that only A•Ara improves as an inhibitor of the ADP induced platelet aggregation in experiments when the test compound is preincubated with PRP for five minutes before ADP is added. This is supported also by published A•Ara results of Agarwal and Parks (1) which report 20% inhibition of maximum aggregation for preincubation intervals of ten to sixty minutes. The time dependent change of inhibitor magnitude of an adenosine analog may be explained by slow conformational changes at the platelet membrane or by penetration of a compound and its entry into the metabolic or stored nucleoside phosphate pools within the platelet.

The present experiments suggest that the conformation of both the purine ring and the pentose moiety of adenosine are important de-

terminants of inhibitor properties. Further investigation of structure-function relationships with nucleoside analogs may be of some value in the future design of antithrombotic drugs.

Acknowledgements

The authors wish to acknowledge the technical assistance of Miss Georgina Modrak and Mrs. Sarah Stoehr in the present project.

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