

# **Interactions of Two ADP Analogs, Xanthosine-5'-Diphosphate and 8-Bromoadenosine-5' Diphosphate, and ADP with Citrated Human Platelet Rich Plasma**

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## **Introduction**

It has been well known for twenty years that adenosine-5'-diphosphate (ADP) will cause aggregation of a stirred suspension of platelets in citrated human platelet-rich plasma (PRP) (1, 3). A number of other naturally occurring nucleotides have also been examined for this potential and only deoxy adenosine 5'-diphosphate was shown in early reports to aggregate platelets (3). Later investigations also revealed that two additional ADP analogs could induce platelet aggregation: 3'-deoxy adenosine-5'-diphosphate and the 1-N-oxide of ADP (4).

Tentative conclusions were made that the C-6 amino group of adenine and the pyrophosphate moiety attached to the ribose were essential for these activities to be observed (3, 4). Several investigators have suggested that this event is triggered by the binding of ADP to a discrete platelet membrane receptor (2). The present and earlier investigations in this laboratory have been aimed at evaluating the stereochemical nature of this hypothetical receptor by systematic variation of structural features of the ADP molecule. The two current ADP analogs, xanthosine-5'-diphosphate (XDP) and 8-bromo-adenosine-5' diphosphate (BrADP) represent structural variants with purine ring modifications.

## **Materials and Methods**

Whole blood freshly collected from normal human subjects was rapidly mixed with one-tenth total volume of 3.2% sodium citrate. Platelet-rich plasma was prepared by differential centrifugation and the microscopically determined platelet count was adjusted to approximately 400,000/mm<sup>3</sup> by dilution with physiological saline (0.155 M NaCl). Both of the present test nucleotides were supplied by P-L Biochemicals Inc., Milwaukee, Wisconsin, and each was dissolved in physiological saline and adjusted to a final pH of 7.

Platelet aggregation was evaluated by the turbidometric method of Born in a Payton Assoc. dual channel aggregometer (1). Samples of PRP (0.5 ml) were placed in cuvettes and stimulated after 30 seconds by a) nucleotide solutions only, b) nucleotide solutions followed in 30 seconds with an aggregating dose of ADP, and c) nucleotide solutions followed in 5 minutes with an aggregating dose of ADP. The longer incubation intervals were used to maximize opportunities for binding of the analogs to a platelet membrane receptor and also to emphasize any additional events caused by metabolites of these nucleotides under study.

## **Results**

A typical result of additions of BrADP over a 100 fold concentration range to platelet rich plasma suspensions is shown in Figure 1. It can be noted that when physiologic saline (control) only added to PRP that no change occurs in light transmission. If aggregation had occurred, a rapid increase in the light transmission through the solution would have been observed. Similarly when 0.05 ml ali-

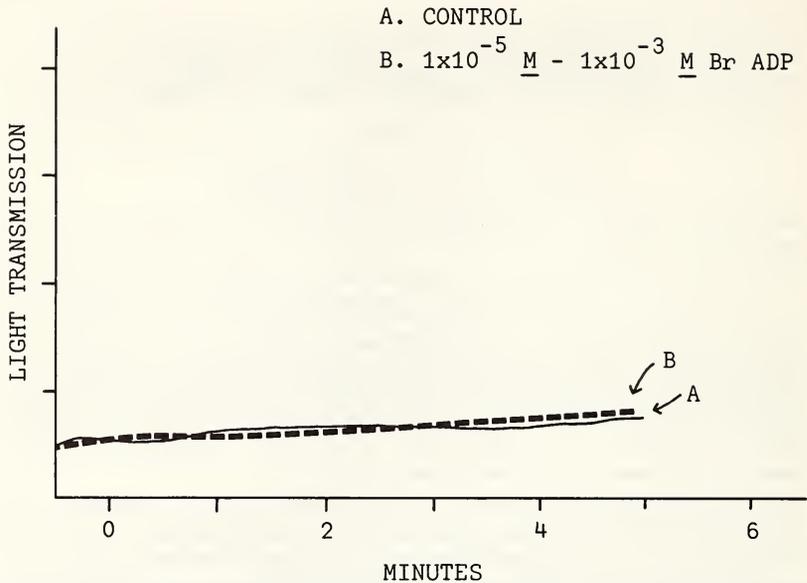


FIGURE 1: *Effects of direct addition of various concentrations of BrADP to human platelet rich plasma.*

quots of BrADP in the concentration range  $1 \times 10^{-5}$  M -  $1 \times 10^{-3}$  M are added, no change in light transmission occurs. Thus, one can conclude that this nucleotide over a 100 fold concentration range failed to stimulate any aggregation of platelets in the PRP suspension for periods of time observed up to 5 minutes. An analogous collection of curves were also obtained when aliquots of XDP were added to PRP over this same concentration range.

In Figure 2, the results of adding ADP alone to platelet rich suspensions are shown by curve A. It can be noted that within 30 to 60 seconds, a dramatic increase in light transmission has occurred and as more platelets are involved in the aggregation, this increase in light transmission begins to slow and eventually levels off in a plateau. If various doses of XDP varied over a 100 fold concentration range are preincubated with PRP for 30 seconds before this aggregating dose of ADP is added, one obtains curves B, C and D. It can be noted in examining these curves that various amounts of XDP failed to yield any competitive inhibition of the ADP induced platelet aggregation. If inhibition were observed, a lag time in the ADP induced aggregation would have occurred and also the magnitude at which the plateau would be observed would have been smaller also. Completely analogous results were observed for BrADP.

In Figure 3, the results of preincubating PRP with BrADP for 5 minute periods before adding an aggregation inducing dose of ADP can be seen. Curve A shows the result of adding a 0.05 ml aliquot of physiological saline only to a stirred suspension, and then adding an aggregating dose of ADP after 5 minute preincubation. It can be seen that the initial light transmission change which is caused by a morphologic change in platelet shape causes a slight increase in light transmission immediately after the ADP is added. Once again, the early phase of aggregation proceeds rapidly with a dramatic decrease in light transmission followed by a slow-

- A.  $5 \times 10^{-4}$  M ADP
- B.  $1 \times 10^{-5}$  M XDP +  $5 \times 10^{-4}$  M ADP 30" LATER
- C.  $1 \times 10^{-4}$  M XDP +  $5 \times 10^{-4}$  M ADP 30" LATER
- D.  $1 \times 10^{-3}$  M XDP +  $5 \times 10^{-4}$  M ADP 30" LATER

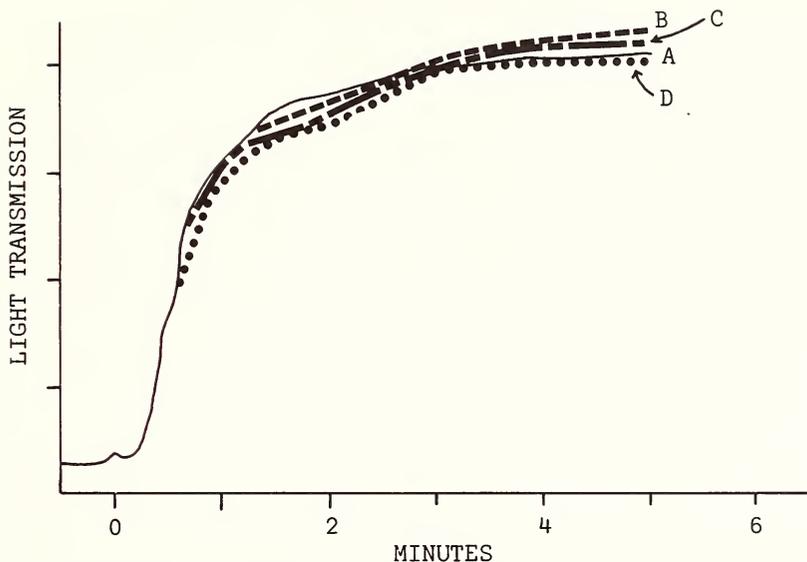


FIGURE 2: *Effects of additions of various concentrations of XDP to human platelet rich plasma, followed by an aggregating dose of ADP after 30 seconds.*

- A. Saline +  $1 \times 10^{-3}$  M ADP 5' LATER
- B.  $1 \times 10^{-5}$  M Br ADP +  $1 \times 10^{-3}$  M ADP 5' LATER
- C.  $1 \times 10^{-4}$  M Br ADP +  $1 \times 10^{-3}$  M ADP 5' LATER
- D.  $1 \times 10^{-3}$  M Br ADP +  $1 \times 10^{-3}$  M ADP 5' LATER

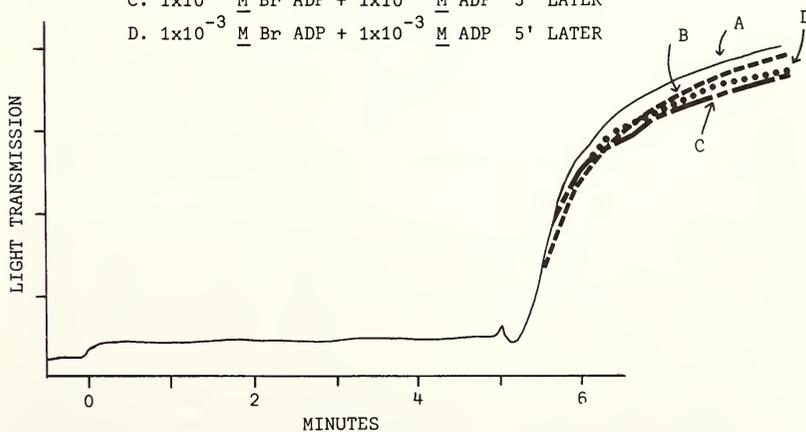


FIGURE 3: *Effects of additions of various concentrations of BrADP to human platelet rich plasma, followed by an aggregating dose of ADP after 5 minutes.*

ing to a plateau after an additional 1 minute period. The shape of this curve A is quite similar to the curve A shown in Figure 2. Finally it can be seen that when BrADP is systematically varied over the selected 100 fold range of concentration preincubated with PRB for 5 minute periods, no inhibition of ADP aggregation is observed. In analogous fashion if XDP is preincubated for 5 minute periods in order to permit membrane linked or metabolic effects to occur, once again, no inhibition of ADP induced aggregation is observed.

### Discussion

Several purine modified derivatives of ADP have been reported to be mildly active in the direct aggregation of PRP or in the inhibition of ADP induced aggregation of PRP. In 1972, Gough *et al.* reported that 2-methoxy adenosine-5'-diphosphate or 2-methylthio adenosine-5'-diphosphate was actually more active on a comparative molar basis than ADP. These authors concluded that substituents at C-2 of the adenine moiety are nicely tolerated with respect to platelet aggregation induction and they also suggest that the C-6 amino group must be present for aggregation to occur. They state that their results are compatible with Born's hypothesis of a specific ADP receptor on the platelet membrane. Unfortunately, their results may not be directly comparable to the present results since they were using sheep PRP and they also did not report any competition studies as has been reported in this present paper (5, 6).

Stone *et al.* report three thiol analogs of ADP with the sulfhydryl groups being attached at either position C-2, C-6 (in place of the amino group) or C-8 (8). When these compounds are added directly to human PRP the following relative activities compared to similar doses of ADP were observed: with the C-2 analog, 74% activity; with the C-8 analog, 0.65% activity; and with the C-6 analog, 0.08% activity. They concluded that substitutions at C-2 of the adenine ring are tolerated but substitutions at position C-8 lead to steric hinderance of binding to the platelet ADP receptor. Finally they conclude that removal of the C-6 amino group of ADP causes complete loss of activity.

Our present results are in agreement with theirs in that substituents at position C-2 and C-6 as is seen with XDP are not tolerated at the platelet ADP receptor and furthermore our C-8 substituted compound (BrADP) is also not tolerated at the platelet ADP receptor. Robey *et al.* produced one additional C-2 analog to be used in electron spin resonance studies of the interaction of ADP-like substances with platelet membrane receptors (7). This particular compound with a bulky group attached to C-2 was once again found to be active in inducing aggregation of human PRP. This is a rather striking example that very bulky groups can be tolerated at position C-2 of the adenine ring.

Our present results support the concept that there is a discrete ADP receptor on the platelet membrane and that this receptor is sensitive to certain structural changes in the purine moiety of ADP. The lack of effect of XDP on PRP suggests that a substituent similar in size and change to the C-6 amino group of ADP must be present for recognition of the compound by the ADP platelet membrane receptor.

The present results for 8-bromoadenosine-5'-diphosphate also support the conclusion that a bulky group (such as bromine atom) cannot be tolerated at position C-8 of the purine ring either for correct alignment of the compound on the platelet membrane receptor. The search continues for an ADP-like compound of negligible toxicity which can inhibit the pathologic aggregation of platelets *in vivo*.

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