The Effect of Dextran-40 on Platelet Adenosine Triphosphate Release In Vitro

PAUL I. LIU, M.D., Ph.D.,*† KENT L. SAUTER, MT(ASCP),* CHERYL J. McROYAN, MT(ASCP)SH,* DENNIS K. McROYAN, M.D.,* and SARA JO DANIEL, M.D.*†
*Department of Pathology, University of South Alabama Medical Center and †American Red Cross Blood Services, Gulf Coast Region, Mobile, AL 36617

ABSTRACT

Studies of dextran's effect on platelet function have revealed inconclusive results; some reports have suggested it acts as an aggregating agent, others as an inhibitor to aggregation. This study demonstrates the inhibitory effect of dextran (40,000 MW) on in vitro platelet adenosine triphosphate (ATP) release when stimulated by conventional aggregatory reagents. These data support inhibition of platelet surface phenomena, possibly involving receptor sites, rather than intracellular phenomena as the mechanism of dextran's anti-aggregatory effect. Significant inhibition occurs in vitro at dextran levels approximately twice that normally attained in vivo.

Introduction

Platelet activation by exposure to subendothelial surface is characterized by a change in shape from a disc to a spiny sphere. Degranulation occurs with concomitant release of stored contents, including adenosine diphosphate (ADP), adenosine triphosphate (ATP), and serotonin. Continued interplatelet contact promotes platelet-to-platelet and platelet-to-fibrin adherence eventually forming a primary hemostatic plug.

In vitro simulation of this phenomena can be accomplished using an aggregometer, whereby platelet rich plasma may be subjected to a variety of stimulating agents. Their responses can then be measured by a change in light transmittance as platelets adhere to each other.

These stimulating agents differ in their mechanism of activation. A primary aggregating agent, ADP, promotes aggregation by its ability to cause platelet-to-platelet adherence in the absence of degranulation, termed primary aggregation. Subsequent liberation of intracellular contents, if present, is accompanied by secondary or irreversible aggregation. Collagen, a subendothelial polymer, promotes platelet aggregation by its attachment to a platelet surface receptor, stimulating shape change,
degranulation, and platelet adherence. Arachidonic acid, in contrast to these, exerts its effect in the intracellular environment. The cyclooxygenase system, if intact, then acts upon arachidonic acid, converting it to Thromboxane A2, which in turn promotes shape change, degranulation, and platelet aggregation.6

Recent advances allow simultaneous measurement of platelet secretion and aggregation, the secretion marker being released ATP. A photomultiplier quantitates ATP indirectly by measuring light emitted from a chemical reaction involving a luciferin-luciferase system which uses ATP as substrate.11

Dextran has been shown to interfere with platelet aggregation and adhesion.2,4,7,8,17,18 Reports of gingival bleeding and hematochezia13 as well as a prolonged bleeding time6,13 in some patients after the administration of dextran make elucidation of the mechanism of dextran’s role in anticoagulation a subject of interest.

Dextran has been shown to aggregate platelets spontaneously in vitro8,9 with an effectiveness proportional to molecular weight;17 however, no evidence of shape change or degranulation has been presented. Other reports have shown dextran to inhibit aggregation.4,18 Speculation of membrane alterations after surface absorption of dextran10,16 or interference of dextran with Factor VIII1 have been postulated as the mechanism of dextran’s effect.

The many reactions involved in platelet function5,6,12 make impairment of any one of these a predisposition to suboptimal activation and compromised hemostasis. An instrument which simultaneously monitors aggregation and ATP secretion allows some separation of surface phenomena from intracellular changes. Use of this method has lead to revision of the role of released substances in platelet aggregation.5 The focus of this study is the effect of dextran-40 on platelet secretion, as measured by dense granule ATP release.

Materials and Methods

Platelet Rich Plasma

Platelet rich plasma (PRP) was prepared from 13 volunteer donors who had abstained from known platelet inhibitory drugs for a period of at least 10 days. Blood was collected into 3.2 percent sodium citrate and centrifuged at 1600 g for two minutes to obtain platelet rich plasma. Platelet counts were adjusted to approximately 330,000 per μl with the donor’s platelet poor plasma (PPP).

Dextran Stock Solutions

Dextran-40 in 0.9 percent NaCl was obtained from Travenol as a 10 percent solution. Concentrated solutions of 30 percent and 50 percent were prepared by ultraconcentration using a YM10 membrane.*

Platelet Aggregation and ATP Release

Platelet aggregation and ATP release were studied at 37°C using a Chrono-log Lumiaggregometer Model 500.† Measurements were recorded on a dual pen recorder.‡ Measurement of platelet aggregation is made by an infra-red light source and a photodiode that determine the difference in light transmission between PRP and PPP. Platelet ATP release is detected by light emitted from a luciferin-luciferase system, in which platelet released ATP is the limiting agent. Measurement of light produced by this reaction is accomplished via a sensitive high voltage photomultiplier placed at a right angle to the infra-red

* Amicon Corp., Danvers, MA.
† Chrono-log Corp., Havertown, PA.
‡ Linear Instruments, Reno, NV.
optical light source to eliminate interference.

Additions of 50 μl of a concentrated dextran solution (one percent, three percent, or five percent) or 50 μl of normal saline as control and 50 μl of luciferin-luciferase at 40 mg per ml† were made to 450 μl PRP and allowed to stir at 1,100 rpm for one minute. Because of the density of high dextran concentrations, manual agitation was found to enhance mixture of the PRP-dextran suspension prior to placement in the aggregometer. Adequate amounts of PRP permitted the use of all three inducing reagents at final concentrations of one percent, three percent, and five percent dextran. Aggregation was induced using either ADP (10⁻⁵M), Arachidonic Acid (10⁻³M), or Collagen (2 μg per ml) and allowed to proceed for a minimum of three minutes. All optical aggregation measurements were taken at exactly three minutes, whereas all ATP release measurements were made at peak response, usually within five minutes. An internal standard of 2 μM ATP was added at the end of each aggregation to facilitate platelet-released ATP quantitation. Paired t-test was used for statistical analysis.

Results

Aggregation and ATP release of each concentration of dextran are compared with a control containing saline and presented in figures 1, 2, and 3.

Dextran's effect was most pronounced in the degree of aggregation inhibition utilizing ADP as the stimulus. Significant inhibition is seen with one percent dextran (p = 0.03) and became progressively more pronounced as the concentration increased to five percent (p < 0.0001) in a dose dependent fashion. Stimulation with collagen or arachidonic acid required higher dextran concentrations before aggregation inhibition was observed. Nevertheless, inhibition was significant at dextran concentrations of both three percent and five percent with collagen stimulation (p = 0.006, p < 0.0001) or arachidonic acid stimulation (p = 0.03, p = 0.0005).

A significant decrease (p < 0.02) in platelet ATP release occurred only in ADP and collagen stimulated aggregation and then only at dextran concentrations of three percent and greater. These secretion decreases paralleled the aggregation response. In contrast, ATP secretion by arachidonic acid stimulation actually increased although not significantly (p > 0.05). Dextran concentrations of three percent and greater also inhibited secondary wave aggregation induced by epinephrine in limited trials.

Release of ATP occurred concurrently with platelet aggregation in collagen or arachidonic acid stimulated PRP, but only near completion in ADP induced
aggregation. Similar responses have been observed by others in platelet aggregation without dextran.5

Discussion

It is generally assumed that secretion is not a prerequisite to secondary aggregation5 but a parallel event; thus, aggregation may not be dependent on secretion. However, secreted substances, particularly ADP and thromboxane A2,6 are well known inducers of aggregation and possibly provide a mechanism for maximal aggregation.

As seen in the results, dextran produces an inhibitory effect on platelet aggregation, whether stimulated by aggregation-mediated (ADP), surface receptor (collagen), or direct-trans-membrane (arachidonic acid) activation. Secretion, however, is unaffected when membrane associated activation is bypassed as observed in arachidonic acid stimulation. These data support previous studies which suggest dextran manifests its anti-aggregatory effect at the membrane or in the extracellular environment.2

Binding of dextran to the platelet membrane may block receptor sites for ADP or other membrane associated activating systems. This would explain the maximal inhibitory effect against activation by ADP and other membrane associated activators. Submaximal aggregation by arachidonic acid, while not compromising the observation of secondary aggregation and secretion as parallel events, may be the result of blocked membrane receptors or membrane enzyme systems. Other explanations might include interference with platelet-to-platelet or platelet-to-fibrin binding,15 equally plausible from the observed data. Increase in viscosity owing to dextran may affect platelet aggregation, although this is not well documented. Viscosity changes were not measured in this study. These findings show in vitro platelet secretion in the presence of Dextran-40 to be decreased only when activation occurs via membrane receptors, and then only at levels higher than usually attained in vivo.

References


