Antithrombin III, Plasminogen, Plasmin, and Alpha-2-Antiplasmin in Donor Blood and Plasma Components

D. K. McROYAN, M.D.,* C. J. McROYAN, MT(ASCP)SH,* K. L. SAUTER, MT(ASCP),* P. I. LIU, M.D., Ph.D.,† and S. J. DANIEL, M.D.†

*Hemostasis and Transfusion Divisions, Department of Pathology, University of South Alabama Medical Center  
†American Red Cross Blood Services, Gulf Coast Region, Mobile, AL 36617

ABSTRACT

The functional levels of antithrombin III, plasminogen, plasmin, and alpha-2-antiplasmin were evaluated in sequentially derived fresh frozen plasma, cryoprecipitate, and cryo-poor plasma aliquots from 20 registered blood donors. Antithrombin III is the major plasma inhibitor of the serine proteases of the procoagulant system. Plasminogen, the proenzymatic form of plasmin, is the primary endogenous profibrinolytic moiety; alpha-2-antiplasmin is the principal intermediate acting inhibitor of plasmin. As congenital or acquired deficiencies of antithrombin III and/or plasminogen predispose to thrombosis, and as these agents may be consumed in acute thrombosis, the goal of this investigation was to discern those plasma components which potentially might maximize both antithrombotic and fibrinolytic activities if used therapeutically.

Using enzyme specific synthetic substrate methods, it was determined that no spontaneous plasmin activity was evident through phlebotomy or component processing and storage. Analysis of variance showed antithrombin III to be significantly decreased in cryoprecipitate as compared to the other components (p < 0.0001). Furthermore, the level of antithrombin III or plasminogen in cryo-poor plasma and fresh frozen plasma was not statistically different. Also, the alpha-2-antiplasmin level was not statistically different among the specimen groups. Since fresh frozen plasma and cryo-poor plasma contain comparable total quantities of antithrombin III and plasminogen and as most of the activity of factors I, V, VIII, and XIII is diverted into cryoprecipitate, it is suggested that cryo-poor plasma may be preferable to fresh frozen plasma for the treatment of thrombosis associated with or complicated by antithrombin III and/or plasminogen deficiency.
Introduction

A growing number of disorders of coagulation and fibrinolysis have been recognized in recent years which predispose to thrombosis. Among these thrombotic coagulopathies are deficiency states involving antithrombin III (AT-III) and plasminogen (PLG). Physiologically, AT-III and PLG are the most important anticoagulant and profibrinolytic moieties, respectively. Deficiency states involving AT-III or PLG may be congenital or acquired on a chronic or acute basis. Further, superacutely either or both may be critically consumed in acute thrombosis. Hypothetically, thrombosis in the face of either or both of these lesions might not respond to pharmacologic intervention intended for anticoagulation or fibrinolysis.

The cardinal manifestation of a critical deficiency of AT-III is resistance to heparinization since heparin is physiologically non-functional in the absence of its cofactor. As the major plasma inhibitor of the serine proteases of the procoagulant system, AT-III possesses inherent antiprotease activity. This activity, however, is greatly enhanced and becomes therapeutically meaningful by the binding of heparin to lysine residues on the AT-III molecule. The resultant AT-III:heparin complex both inhibits the formation of activated serine proteases and rapidly inactivates those formed, principally factors IIa, Xa, IXa, and XIa. The net effect of this interaction is that clotting is inhibited at multiple critical points in the coagulation schema and anticoagulation is effected. It is well known that heparinization in patients with significant AT-III deficiency is suboptimal; therefore, vitamin K antagonists have been espoused as a means of achieving anticoagulation in these patients. However, the full antithrombotic effect of oral anticoagulants occurs only after a latent period and may not be efficacious acutely. Further, patients with AT-III deficiency unresponsive to oral anticoagulation have been reported.

Plasminogen, the proenzymatic form of plasmin, is the primary mediator of fibrinolysis. Plasminogen may be converted by limited proteolysis to plasmin by a variety of endogenous activators and by a number of increasingly important pharmacologic agents. Plasminogen that is affixed to fibrin within a thrombus by tissue-type plasminogen activators is converted to plasmin which acts locally and enzymatically releases fibrin degradation products which are themselves anticoagulant. Any plasmin which is formed free in the circulation is normally rapidly inactivated by alpha-2-antiplasmin (A2AP), thus delimiting systemic fibrinolysis. Thrombotic coagulopathy ascribable to disorders of the fibrinolytic system presently appear to be rare. However, acute thrombosis may be accompanied by a critical deficiency of PLG, hypothetically, leading to a failure of endogenous fibrinolysis or thrombolytic therapy.

Blood components or derivatives have rarely been advocated or used as adjuncts in the treatment of thrombosis or a thrombotic predisposition. In most cases, AT-III concentrates, which are investigational and not widely available, and fresh frozen plasma have been advocated as sources of AT-III prior to heparinization. Mintz et al in 1976 presented evidence to establish both fresh frozen plasma and cryo-poor plasma as potential replacement sources of AT-III. Further, it can be inferred from other accounts that fresh frozen plasma and cryo-poor plasma as potential replacement sources of AT-III. However, fresh frozen plasma contains significant amounts of factors I, V, VIII, and XIII, while cryo-poor plasma contains diminished amounts of these factors which are required for the formation of a stable clot. The effect of thrombosis caused by or
resulting in AT-III and/or PLG deficiency would be a window of patient vulnerability to rethrombosis and/or poor clot lysis, respectively. The goal of this investigation was to identify those plasma components which potentially might maximize both antithrombotic and fibrinolytic activities if therapeutically used as replacement sources of AT-III and/or PLG.

Materials and Methods

Blood from 20 registered, volunteer donors was evaluated; written, informed consent was obtained in each case. Immediate pre- and post-phlebotomy specimens of 4.5 ml each were collected into buffered sodium citrate in a ratio of 9:1, whole blood: citrate. Platelet poor plasma was promptly prepared by centrifugation at 1,500 × g for 10 min, aliquoted, and stored in polypropylene tubes at −70°C. The bank blood was collected in the usual manner into citrate-phosphate-dextrose-adenine-1 in a ratio of 7:1, whole blood: citrate. Fresh frozen plasma, cryoprecipitate, and cryo-poor plasma were prepared sequentially from each unit collected by conventional blood bank techniques; aliquots of each component were promptly stored in plastic tubes at −70°C.24

The functional AT-III, PLG, plasmin, and A2AP activity of each of the five groups of specimens were assayed using amidolytic, fluorogen-tagged, enzyme specific synthetic substrate methods.8 All assays employed Protopath methodologies and 5-aminoisophthalic acid dimethyl ester (AIE) tagged substrates.* The fluorescent substrates are AT-III, D-phe-pro-arg-AIE; PLG and plasmin, D-val-leu-lys-AIE; and A2AP, D-val-leu-lys-AIE. The aliquots were assayed in batch and in duplicate within 60 days. In addition, 10 aliquots of the sequentially derived fresh frozen plasma and cryo-poor plasma were assayed in batch for Factor I by thrombin activation,* and Factors V and VIII utilizing Factors V and VIII deficient plasma† factor assay methods.

Statistics were analyzed by analysis of variance and paired t-test with significance set at p < 0.05.

Results

In table I are shown the results of the functional assays for AT-III, PLG, and A2AP. As the volume of any component may vary considerably from donor to donor, the results and statistical treatment of these data are expressed on a milliliter to milliliter basis versus a total volume basis.24 The AT-III and A2AP data are expressed as percent activity per milliliter of normal human plasma; PLG is expressed as Committee on Thrombolytic Activity Units per milliliter of plasma.6

Plasmin activity is not included in the data as none was found in any specimen tested. The A2AP level was not statistically different among the specimen groups. Analysis of variance showed AT-III to be significantly decreased in cryoprecipitate (p < 0.0001) as compared to the other component. Also, the functional activity of AT-III did not differ significantly in fresh frozen plasma and cryo-poor plasma (p > 0.05). Further, these data suggest that PLG is a partially cryoprecipitable plasma protein and that cryoprecipitation results in a modest diversion of PLG into cryoprecipitate. However, despite this effect, the level of PLG is not significantly different in fresh frozen plasma and cryo-poor plasma (p > 0.05).

Given conventional component processing standards, fresh frozen plasma must have a minimum volume of 225 milliliters from which a maximum volume of

* American Dade, Miami, FL.
† General Diagnostics; Morris Plains, NY.
15 milliliters can be diverted into cryo-precipitate (precipitable protein plus supernatant plasma). The residual volume expressed after preparation of cryo-precipitate is cryo-poor plasma and, therefore, represents no more than a maximum 6.7 percent reduction of the original fresh frozen plasma volume. On this basis, the total amount of PLG or AT-III per single donor unit of fresh frozen plasma or cryo-poor plasma is comparable. The fresh frozen plasma units donated to this study had an average volume of 256 ml. Although neither the cryo-precipitate nor cryo-poor plasma volumes were determined, given the preceding standards, an average maximum reduction of 5.9 percent total volume and, therefore, total activity of PLG and AT-III would be expected proceeding from fresh frozen plasma to cryo-poor plasma.

In table II are shown the results of the functional assays for factors I, V, and VIII. The factor V and factor VIII data are expressed as percent activity per milliliter of normal human plasma, and fibrinogen is expressed in milligrams per deciliter of plasma. Factors I, V, and VIII all showed a highly significant decrease from fresh frozen plasma to cryo-poor plasma (p < 0.0001).

**Discussion**

The absence of plasmin activity in all of the component groups assayed indicates that phlebotomy and conventional component processing and storage methods do not activate the fibrinolytic system. This finding suggests that the PLG content and, therefore, the potential fibrinolytic activity of these components is not diminished by present modes of preparation. This observation is consistent with those of Nilsson et al.

The functional level of AT-III did not vary significantly in fresh frozen plasma and cryo-poor plasma. Further, analysis of variance showed AT-III to be significantly decreased in cryo-precipitate (p < 0.0001) as compared to fresh frozen or cryo-poor plasma. This is in contradistinction to the findings of Mintz et al; this divergence in findings is undoubtedly attributable to the greater sensitivity and specificity of enzyme specific synthetic substrate assays as compared to biological assays. These data suggest that PLG is a partially cryoprecipitable plasma protein which results in a modest diversion of PLG into cryo-precipitate and away from cryo-poor plasma. The magnitude of this effect, however, is not sufficient enough to cause the PLG contents of fresh frozen plasma and cryo-poor plasma to vary significantly from fresh frozen plasma to cryo-poor plasma.
to be significantly different. Therefore, equal volumes of single donor fresh frozen plasma and cryo-poor plasma contain comparable quantities of AT-III or PLG (see table I). Also, the total quantity of AT-III or PLG per single donor unit of fresh frozen plasma and cryo-poor plasma is comparable (see Results). Further, it is well known that significant amounts of factors I, V, VIII, and XIII are diverted from fresh frozen plasma into cryoprecipitate, consequently, rarefying the clottability of cryo-poor plasma; these data are consistent with a significant diversion of Factors I, V, and VIII (see table II). The practical application of these data is that the potential anticoagulant and fibrinolytic properties of cryo-poor plasma are preserved in processing while its clottability is greatly diminished. This suggests that cryo-poor plasma may be a valuable adjunct in the treatment of thrombosis associated with AT-III and/or PLG deficiency.

These data suggest that cryoprecipitate is an enriched source of PLG. Cryoprecipitate as a source of PLG cannot be recommended because the total units of PLG activity per single donor unit are low. Achieving a meaningful therapeutic dose of PLG would probably necessitate the use of multiple units which might expose the recipient to an inordinately high hepatitis risk. Also, the fibrinolytic potential of cryoprecipitate may be negated by its thrombogenic potential.

Congenital and acquired deficiencies of AT-III are associated with a predisposition to thrombosis. It is suggested that AT-III levels between 50 to 75 percent of normal confer a moderate risk for thrombosis, and levels less than 50 percent indicate a significant risk. Congenital AT-III deficiency is the most common known heritable state associated with thrombosis and may be as prevalent as one per 2000 to 5000. Acquired conditions which may be associated with chronic AT-III deficiency and thrombosis include hyperestrogenic states and the nephrotic syndrome, while disseminated intravascular coagulopathy and the post-operative state may be associated with an acute deficiency. Further, AT-III and other coagulation proteins may be consumed and critically decreased in acute thrombosis.

Although congenital abnormalities of PLG are associated with a predisposition to thrombosis, they presently appear to be rare. Acquired hypoplasminogemia may be associated with disseminated intravascular coagulopathy, postoperative states, and acute thrombosis.

Life-threatening thrombosis caused by or resulting in deficiencies of AT-III and/or PLG might not respond to therapeutic intervention intended for anticoagulation or fibrinolysis. As AT-III concentrates are investigational and not widely available, fresh frozen plasma has been espoused as a source of AT-III. This approach to replacement therapy might be more effective if cryo-poor plasma were used. These data indicate that cryo-poor plasma contains quantities of AT-III and PLG comparable to fresh frozen plasma. Further, as a result of processing the clottability of cryo-poor plasma is rarefied by the diversion of factors I, V, VIII, and XIII into cryoprecipitate, whereas fresh frozen plasma is not. A model is currently being developed to test the efficacy of cryo-poor plasma in thrombosis.

References

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