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THE ANTITHROMBIN III CONTENT OF CRYOPRECIPITATE PREPARED FROM B--ETC(U)  
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The Antithrombin III Content of Cryoprecipitate Prepared from Blood  
Collected with and without Heparin

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Running title: Cryoprecipitate and Antithrombin III

Abstract

Antithrombin III (AT III) is a plasma protein which acts as the principal inhibitor of thrombin and is a major modulator of intravascular coagulation. Hereditary deficiency of AT III leads to recurrent episodes of thromboembolism. Acquired deficiency of AT III occurs in persons with a variety of conditions, including severe liver disease and disseminated intravascular coagulation. Replacement of AT III may be important in some deficient persons. To determine if cryoprecipitate is a useful source of AT III, we measured the AT III content of cryoprecipitate prepared from citrate phosphate dextrose (CPD) blood using coagulation, fluorogenic, and immunoassays. Using the fluorogenic assay, we also determined the effect of adding heparin to blood on cryoprecipitation of AT III. Functional and antigenic AT III levels were similar to those of normal plasma in all CPD units tested, indicating that AT III is not concentrated in cryoprecipitate. Heparin had no effect on the cryoprecipitation of AT III.

Key Words: Cryoprecipitate, Antithrombin III, Heparin

### Introduction

Antithrombin III (AT III) is an  $\alpha_2$ -globulin of plasma which acts as the principal inhibitor of thrombin<sup>1</sup> and other activated procoagulants, such as factor X<sub>a</sub>.<sup>2</sup> As heparin cofactor, it accounts for the anticoagulant effect of heparin.<sup>3</sup> Hereditary deficiency of AT III leads to recurrent thromboembolism.<sup>4</sup> Acquired deficiency occurs in a number of conditions, including severe liver disease<sup>5</sup> and disseminated intravascular coagulation (DIC).<sup>6</sup> The latter has been successfully treated by infusion of an AT III concentrate.<sup>7</sup> Such concentrates, however, are not generally available and have not been approved for clinical use.

The purpose of this study was two-fold: first to determine whether cryoprecipitate could serve as a useful source of AT III for replacement therapy in persons with conditions such as DIC, and second to determine whether the addition of heparin to blood at the time of collection would increase the AT III content of cryoprecipitate.

### Methods

Blood from ten donors was collected in citrate phosphate dextrose and cryoprecipitate prepared by standard blood bank methods.<sup>8</sup> Blood from three donors was collected into sodium citrate (3.8%), and sodium citrate plus heparin (169 U/mg of protein) (Sigma Chemical Co., St. Louis, MO.). Blood from one of these donors was also collected into heparin alone.

AT III activity was measured by coagulation (Ortho Diagnostics, Raritan, NJ.), and fluorogenic (Dade Division, American Hospital Supply Corporation, Miami, FL.) assays. AT III antigen level was measured by radial immunodiffusion (Behring Diagnostics, American Hoechst Corporation, Somerville, NJ.). Factor VIII procoagulant activity was measured by a one-stage coagulation assay.<sup>9</sup>

## Results

The AT III concentration as determined by the three different assay methods for each of the ten cryoprecipitates is shown in Table 1. The values for the three assays are comparable and are similar to those found in normal human plasma. Addition of heparin to blood at the time of collection had no effect on the AT III content of cryoprecipitate as measured by the fluorogenic assay (Table 2).

## Discussion

In addition to its use as a source of factor VIII, cryoprecipitate is often used to replace fibrinogen.<sup>10</sup> More recently, it has been used as a source of fibronectin to correct opsonic deficiency in patients with trauma and sepsis.<sup>11</sup> Such patients frequently have DIC as well, and might benefit from AT III replacement. Without adequate levels of AT III, heparin is ineffective.

The results of the present study show that AT III is not concentrated in cryoprecipitate in contrast to factor VIII, fibrinogen and fibronectin. The AT III concentration of cryoprecipitate is essentially the same as that in plasma. The fact that AT III activity and antigenic levels are comparable indicates that the AT III that is present is active. These results extend those of Mintz et al<sup>12</sup> who found similar AT III levels in fresh-frozen plasma, cryoprecipitate and cryoprecipitate-depleted plasma from five normal donors.

Heparin has been reported to increase the precipitation of fibronectin<sup>13</sup> and factor VIII.<sup>14</sup> Because heparin is known to bind to AT III,<sup>3</sup> it was reasonable to determine if it might increase the AT III concentration of cryoprecipitate as well. The results of the present study indicate that this is not the case. The AT III concentration of



cryoprecipitate remained the same as that of plasma when heparin was added to blood at the time of collection in concentrations ranging from 1 to 1000 U/ml.

Until AT III concentrates become available for clinical use fresh-frozen plasma can be used as a convenient source of AT III.

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Table 1. AT III Activity and Antigen Level and VIII<sub>c</sub> Activity in 10 Units of CPD Cryoprecipitate.

| Cryoprecipitate  | AT III Activity   |         |                   |         |                |       |                |         |                            |      | VIII <sub>c</sub> Activity |      |
|------------------|-------------------|---------|-------------------|---------|----------------|-------|----------------|---------|----------------------------|------|----------------------------|------|
|                  | Coagulation Assay |         | Fluorogenic Assay |         | AT III Antigen |       | AT III Antigen |         | VIII <sub>c</sub> Activity |      | U/ml                       | U/ml |
|                  | %                 | U/ml    | %                 | U/ml    | %              | mg/dl | %              | mg/dl   | %                          | U/ml |                            |      |
| 1                | 89                | 0.9     | 100               | 1.0     | 86             | 24    | 792            | 7.9     |                            |      |                            |      |
| 2                | 85                | 0.9     | 85                | 0.9     | 86             | 24    | 400            | 4.0     |                            |      |                            |      |
| 3                | 111               | 1.1     | 84                | 0.8     | 89             | 25    | 480            | 4.8     |                            |      |                            |      |
| 4                | 99                | 1.0     | 100               | 1.0     | 93             | 26    | 560            | 5.6     |                            |      |                            |      |
| 5                | 98                | 1.0     | 90                | 0.9     | 93             | 26    | 60             | 0.6     |                            |      |                            |      |
| 6                | 71                | 0.7     | 78                | 0.8     | 89             | 25    | 400            | 4.0     |                            |      |                            |      |
| 7                | 98                | 1.0     | 96                | 1.0     | 93             | 26    | 240            | 2.4     |                            |      |                            |      |
| 8                | 98                | 1.0     | 105               | 1.1     | 93             | 26    | 220            | 2.2     |                            |      |                            |      |
| 9                | 89                | 0.9     | 90                | 0.9     | 86             | 24    | 396            | 4.0     |                            |      |                            |      |
| 10               | 101               | 1.0     | 95                | 1.0     | 96             | 27    | 392            | 3.9     |                            |      |                            |      |
| Mean             | 92.3              | 1.0     | 94.0              | 0.9     | 90.0           | 25    | 394            | 3.9     |                            |      |                            |      |
| SD               | 8.4               | 0.1     | 10.9              | 0.1     | 3.7            | 1.1   | 199.7          | 2.0     |                            |      |                            |      |
| Normal Range for |                   |         |                   |         |                |       |                |         |                            |      |                            |      |
| Human Plasma     | 82-120            | 0.8-1.2 | 80-120            | 0.8-1.2 | 74-104         | 17-30 | 50-150         | 0.5-1.5 |                            |      |                            |      |

Values are given as percent of normal for each assay. U/ml are also given for assays measuring activity, and mg/dl for the immunoassay. Normal activity for AT III and VIII<sub>c</sub> are taken as 1 U/ml of human plasma.

Table 2. AT III Activity in Cryoprecipitate Obtained from Blood with and without Addition of Heparin

| Donor           | Sodium Citrate Only |        | Sodium Citrate + Sodium Heparin |          |           | Sodium Heparin Only |        |
|-----------------|---------------------|--------|---------------------------------|----------|-----------|---------------------|--------|
|                 | 1 U/ml              | 5 U/ml | 10 U/ml                         | 100 U/ml | 1000 U/ml | 1 U/ml              | 5 U/ml |
| 1               |                     |        |                                 |          |           |                     |        |
| Cryoprecipitate | 115                 | 120    | 120                             | 96       | 94        | 95                  | 95     |
| Plasma          | 120                 |        |                                 |          |           |                     |        |
| 2               |                     |        |                                 |          |           |                     |        |
| Cryoprecipitate | 114                 |        | 107                             |          |           |                     |        |
| Plasma          | 103                 |        |                                 |          |           |                     |        |
| 3               |                     |        |                                 |          |           |                     |        |
| Cryoprecipitate | 92                  | 82     |                                 |          |           |                     |        |
| Plasma          | 96                  |        |                                 |          |           |                     |        |

Values are percent of normal activity for the fluorogenic assay. The normal range for human plasma is 80-120%. Sodium heparin concentration is in units/ml blood.

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