A Quicker Preparation Method for Autologous Fibrin Glue

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To establish a quicker preparation procedure for cryoprecipitate (Cryo) from a patient's autologous plasma, to be used as fibrin glue, we examined the effects of various conditions on the concentrations and yields of coagulation factors in Cryo. Human plasma from healthy volunteers was divided and treated under various freezing, shaking and defrosting conditions. The concentrations of fibrinogen, plasminogen, fibronecinct, and factor XIII in Cryo were then measured. Results were as follows: (1) concentrations and yields of plasma components in Cryo obtained from plasma stored at −20°C were significantly higher than those in Cryo from plasma stored at −80°C; (2) shaking at 70 cycles/min during the freezing process had a favorable effect on the concentrations and yields of coagulation factors in the Cryo; (3) a shaking thaw process in a cold water bath was a rapid method for obtaining adequate yields of coagulation factors; (4) shaking in the defrosting process did not affect the yields of coagulation factors. These results indicated that Cryo containing high concentrations of coagulation factors could be prepared easily and rapidly from a patient's autologous plasma (within 4–5 h).

Key words fibrin glue; fibrinogen; adhesive; autologous plasma; cryoprecipitate; rapid preparation

Fibrin glue consists of fibrinogen, coagulation factor XIII (F-XIII), thrombin, CaCl₂, and aprotinin as inhibitors of the fibrinolytic system.1) Fibrin glue reacts according to the physiological hemostatic mechanism and coagulates and adhears bleeding tissues.2) Several types of fibrin glue products are commercially available and are widely used as biological adhesives for use in surgical operations.3) However, these products have a number of problems.4) The fibrinogen, F-XIII, and thrombin used to make fibrin glue are produced from human plasma collected from healthy volunteers, and these products are heated at 60°C for more than 10 h to inactivate hepatitis B virus or human immunodeficiency virus-1.5) Patients in whom the fibrin glue is used are thus placed at risk of infection by the most common pathogens such as Parvovirus B19 and Prions.6,7) In addition, since each constituent of fibrin glue is classified into several subtypes, in which the amino acid sequences are partly different,8–11) its use is associated with the potential risk of allergic reaction. For the prevention of these adverse effects, cryoprecipitate (Cryo) produced from a patient's autologous plasma is recommended for making the fibrin glue.12,13) However, it takes about 2 d to obtain Cryo by the currently used preparation method, and thus autologous Cryo cannot be prepared quickly enough for emergency patients urgently needing surgery.14,15)

In the present study, we established a rapid preparation method for obtaining Cryo from a patient's autologous plasma, and we examined the effects of preparation conditions on the concentration and yield of coagulation factors in Cryo.

MATERIALS AND METHODS

Materials Human plasma from healthy volunteers, excluding alanine aminotransferase (ALT) test, was obtained from the Yamaguchi Red Cross Blood Center (Yamaguchi, Japan). The plasma was divided into plastic disposable test tubes (FQ2100, Eiken Kizai Co., Tokyo, Japan), and then used in the experiments. The assay kits used for fibrinogen, plasminogen, fibronecinct, F-XIII and fibrin degradation products were ILTest PT-Fibrinogen (Coulter Co., Tokyo), ILTest Plasminogen (Coulter), Fibronecinct (Ospson Protein) (Boehringer Mannheim Co., Tokyo), Latex Kit F-XIII (Hoechst Japan, Tokyo), and FDP reagent (ND) (International Reagent Co., Kobe, Japan), respectively. All chemicals used were obtained from commercial sources and were of analytical reagent grade.

Preparation of Cryo The divided plasma was treated under the following various conditions. The plasma was frozen in a −20°C or −80°C freezer (MDF-U536 and MDF-382, Sanyo Medica Systems Co., Osaka, Japan). Part of the divided plasma was shaken during the freezing periods by a horizontal shaker (NR-3, Taitec Co., Saitama, Japan). The shaking speeds were 30, 70, or 150 cycles/min. The plasma was then defrosted in (1) a cold water bath (4°C), (2) a refrigerator (4°C), or (3) at room temperature (the plasma was left until it was partially defrosted) and then in a cold water bath (until the plasma was completely defrosted). Part of each plasma sample was shaken during the defrosting period. Defrosted samples were centrifuged at 3000 rpm for 15 min at 0–4°C, and the supernatant was removed and the precipititation volume was adjusted to one-twentieth of the initial plasma volume.

The general preparation method for the Cryo was as follows; the plasma was frozen in a −20°C freezer. The plasma was then defrosted in a refrigerator (4°C) overnight. Defrosted plasma was centrifuged at 3000 rpm for 15 min at 0–4°C, and the supernatant was removed.

Analytical Methods The concentrations of fibrinogen and plasminogen in each plasma sample and Cryo were determined using commercial kits supplied with the automated coagulation analyzer-ACL3000 (Coulter Co., Tokyo). Fibronecinct was determined by the immunoprecipitation method. Factor XIII and fibrin degradation products were determined by the latex agglutination methods.

Measurement of the Temperature of the Plasma during the Freezing Period The sensor of a Thermo Recorder RT-10 (Tabai Espec Co., Osaka) was inserted into the plastic tubes containing plasma, and the tube was left in the −20°C or −80°C freezer. The temperature of the plasma was automatically measured and recorded every 15 s by the recorder.

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Calculation of % Yield: % yield of coagulation factors (CF) after treatment was calculated as follows: % yield = (concentration of CF in the Cryo) · (volume of the Cryo) / ((concentration of CF in the raw plasma) · (volume of raw plasma)) · 100.

RESULTS AND DISCUSSION

Effect of Freezing Temperature on the Yield of Plasma Components: To investigate the effect of the freezing conditions on the concentration and yield of CF in Cryo, plasma was frozen at −20 and −80°C. As shown in Fig. 1, the concentration of each plasma component in the Cryo prepared from plasma stored at both temperatures was higher than that in the raw plasma. The concentrations and yields of fibrinogen, fibronectin and F-XIII in the Cryo prepared from plasma stored at −20°C (Cryo-20) were significantly higher than those in the Cryo prepared from plasma stored at −80°C (Cryo-80), while there was no difference in the plasminogen concentration and yield.

Compared to the raw plasma, the concentrations and yields of each component in the Cryo prepared from plasma frozen at −20°C and then at −80°C (Cryo-20 and 80) were significantly higher. The concentrations and yields of each component in the Cryo-20 and 80 were also significantly higher than those in Cryo-80, while there was no difference between those of Cryo-20 and those of Cryo-20 and 80 (Fig. 1). These results indicate that the optimal preparation of Cryo does not require the cooling of plasma to −80°C.

Time Course of Plasma Temperature in the Freezer: To clarify the reason why the concentrations of plasma components in Cryo-20 were significantly higher than those in Cryo-80, the temperature of plasma in each freezer was continuously measured by a Thermo Recorder. As shown in Fig. 2, the temperature of the plasma kept in the −20°C freezer quickly dropped to −4°C (liquid phase), stayed at −0.7 to −4°C for about 25 min (liquid phase to solid phase), and then gradually dropped to −20°C (solid phase). The temperature of the plasma kept in the −80°C freezer dropped quickly, stayed at −1 to −4°C for about 5 min, and then rapidly fell to −80°C. Thus, the plasma cooled slowly in the −20°C freezer and rapidly in the −80°C freezer. It is well known that the size of ice crystals depends on the cooling speed, and that the more rapid the cooling speed, the smaller the crystals formed. This is known as the Tammann phe-
nomina. If large ice crystals are formed in plasma, liquid layers containing higher concentrations of salts and proteins are generated among the ice crystals. In this condition, proteins such as CF may easily associate with each other. In addition, if the layers between ice crystals are located at one side of the vessel, the association of CF might be accelerated. The shaking procedure is a simple method to gather these layers.

**Effect of Shaking on the Yield of CF in the Freezing Process** To clarify the effect of shaking during the freezing process on the concentrations and yields of CF in Cryo, plasma was frozen and shaken at various speeds using a horizontal revolution shaker in the –20°C freezer. As shown in Fig. 3, the concentrations of fibrinogen, fibronectin, and F-XIII in the Cryo obtained with shaking were significantly higher than those in Cryo obtained without shaking (general method). The concentrations in Cryo obtained with shaking at 70 cycles/min were the highest, but no significant difference was revealed among the various speeds. Moreover, the effect of the shaking method on the yield of fibrinogen was studied. We could not detect a difference in the concentration and yield of CF between the horizontal revolution method and the horizontal reciprocation method. These results indicate that shaking in the freezing process has a favorable effect on the concentration and yield of CF in Cryo.

**Effect of Defrosting Conditions on the Yield of CF** To establish a rapid preparation procedure for Cryo, the optimum defrosting procedure for frozen plasma was determined. The defrosting time and yield of CF in Cryo prepared by various defrosting procedures are shown in Fig. 4. The defrosting time with the room temperature and cold water bath method was the shortest, although the yields of CF in Cryo obtained by this method were the lowest. The defrosting time obtained by shaking in a cold water bath was short and the yields of CF were adequate. The defrosting time in the refrigerator might be prolonged, since a layer of cold air formed around the bag containing frozen plasma. On the other hand, the % yield of CF when using the room temperature and cold water bath method, may be decreased since an unstirred layer of warm plasma was formed around the partially frozen plasma, and a part of the Cryo might be dissolved in warm plasma. These results indicate that shaking-defrosting in a cold water bath is a quick and useful method for producing CF to be used in Cryo.

**Effect of Shaking on the Yield of CF in the Defrosting**
Process  Next, to clarify the effect of shaking during the defrosting process on the yields of CF in Cryo, plasma that was frozen and shaken at 30 or 70 cycles/min in the −20 °C freezer was defrosted in a cold water bath and shaken at various cyclic speeds using a horizontal revolution shaker. As shown in Fig. 5, the yield of fibrinogen in plasma shaken at 70 cycles/min during defrosting was slightly higher than that obtained at other shaking speeds, but no significant difference was recognized among them. These results indicated that shaking during the defrosting process does not affect the yield of CF.

In summary, in a study of the effects of various conditions on the preparation procedure of Cryo, as autologous fibrin glue, we obtained the following results. (1) The concentrations and yields of plasma components in plasma frozen at −20°C were significantly higher than those in plasma frozen at −80°C. (2) Shaking at 70 cycles/min during the freezing process had a favorable effect on the concentrations and yields of CF. (3) The shaking thaw in a cold water bath was a rapid method for obtaining adequate yields of CF. (4) Shaking during the defrosting process had no effect on the yield of CF. These results indicated that Cryo containing high concentrations of CF could be prepared easily and rapidly from a patient’s autologous plasma (within 4—5 h).

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