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Original Article

Influence of storage temperature/time for cryoprecipitate on concentration of coagulation factor VIII and pass rate

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Abstract: Objective: To investigate the changes in the concentration of coagulation factor VIII and the pass rate of cryoprecipitate at different storage temperatures and times. Methods: Ninety packs of 400 mL whole blood collected from January to August 2018 were prepared into 90 packs of 1U cryoprecipitate which were randomly divided into two groups (45 bags each) and stored in the refrigerator at -30°C and -80°C, respectively. After 3, 6, 9 and 12 months of storage, the concentration of coagulation factor VIII, activated clotting time (ACT) and time to maximum amplitude (TMA) and the pass rate of cryoprecipitate were compared. Results: Under same storage temperature, the concentration of coagulation factor VIII decreased significantly with the extension of storage time ($P < 0.05$); for same storage time, the concentration coagulation factor VIII at -80°C was significantly higher than those at -30°C ($P < 0.05$). Under same storage temperature, the ACT and TMA values were significantly longer with the extension of storage time ($P < 0.05$); for same storage time, the ACT and TMA values at -80°C were shorter than those at -30°C ($P < 0.05$). There was no significant difference in the reduction rate of concentration of coagulation factor VIII under combined influence of storage temperature and time. The difference in the pass rates of coagulation factor VIII was significant at different storage temperatures ($P < 0.05$) and was not significant at different times ($P > 0.05$). Conclusion: With the prolongation of storage time, the concentration of coagulation factor VIII will continue to decrease and the clotting time will continue to prolong. Storage temperature is the main factor affecting the concentration of coagulation factor VIII, ACT and TMA of cryoprecipitate. If the storage time is expected to exceed 6 months, ultra-low temperature storageis recommended for better quality.

Keywords: Cryoprecipitate, temperature, time, coagulation factor VIII, pass rate

Introduction

Cryoprecipitate specifically refers to white and opaque masses obtained after thawing fresh frozen plasma (FFP) at 2-4°C and centrifugation to remove the superficial plasma, which is mainly used to rescue patients with hemorrhage or massive transfusion in response to massive hemorrhage [1, 2]. It must be infused within 6 hours after thawing with the infusion rate not less than 200 ml/h. It is mainly used in children and adults with mild hemophilia A, patients with vascular hemophilia and congenital fibrinogen deficiency [3]. In addition, cryoprecipitate can be used as an alternative treatment for patients with post-surgical bleeding, severe trauma or DIC [4]. Cryoprecipitate contains coagulation factor VIII and fibrinogen, of which the concentration of fibrinogen is stable during storage, while the concentration of coagulation factor VIII is affected by many factors, leading to inactivation [5]. Gao [6] found that the concentration of coagulation factor VIII was affected by different storage temperatures and times. In order to further explore the changes of coagulation factor VIII and its pass rate at different storage temperatures and times, the present study was carried out to determine the concentration of coagulation factor VIII in 90 bags of 1U cryoprecipitate. According to relevant literature standard (coagulation factor VIII ≥80 IU/pack is qualified) [4], the concentration of coagulation factor VIII and its pass rate were determined.

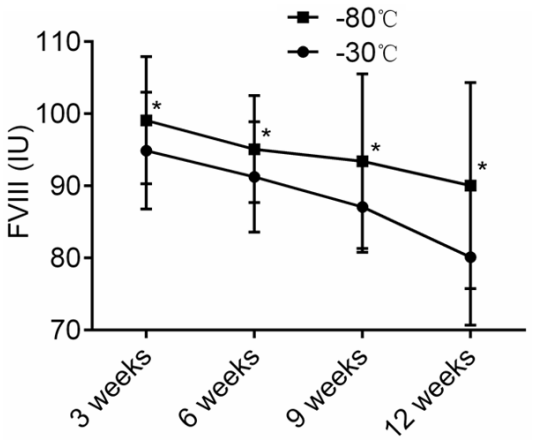

Figure 1. Comparison of coagulation factor VIII concentration (IU). Note: -80°C Vs. -30°C for the same storage time. Compared with the same time point at -30°C, $*P < 0.05$.

Materials and methods

Objects of study

Ninety bags of 400 mL whole blood were collected from January 2018 to August 2018, and prepared into 200 mL FFP after centrifugation for 10 min and immediately transferred to quick-freezing machine. After 25 min, when the temperature reached -30°C, the samples were transferred to a cryogenic refrigerator at -35°C and stored for 2 days. Then the sample was transferred to a refrigerator at 4°C for thawing. After melting, it was placed in a centrifuge for centrifugation for 10 min, followed by treatment with the automatic blood components separator for preparation of cryoprecipitate. The cryoprecipitate volume of each bag was set to 25 mL. All cryoprecipitate was randomly labeled, i.e., No. 1-90, and then divided into two groups by random number table method. Each group consisted of 45 bags, and was finally placed in the refrigerator at -30°C and -80°C for storage. Every procedure was approved by the Animal Care and Use Committee of Hangzhou Fuyang Hospital of Traditional Chinese Medicine. All patients voluntarily signed informed consent.

Instruments and reagents

Sysmex CA-6200 automated coagulation analyzer (Beijing Stago Diagnostic Products Trading Co., Ltd.); APTT kit (Shanghai Yuanye Biotechnology Co., Ltd.); the coagulation factor VIII assay test (Shanghai Qincheng Biotechnology Co., Ltd.). All operations were performed in strict accordance with the kit instructions.

Methods

The cryoprecipitate stored at -30°C and -80°C were kept for 3, 6, 9 and 12 months respectively. Ten bags of each group were taken out and put into the plasma thawing box (JTRJ-6D, Hangzhou Julai Instrument Co., Ltd.) for adequate thawing less than 10 min, and finally 2 mL of the thawed cryoprecipitate was retained using a Sterile Tubing Welder (Terumobct TSCD II, Beijing Exxon Science and Technology Co.).

Observations indicators

Coagulation factor VIII concentration and pass rate in the two groups were calculated and compared. Activated clotting time (ACT) and time to maximum amplitude (TMA) values were analyzed and compared. ACT referred to the time required from the beginning of the blood sample detection to the appearance of blood clotting, and TMA indicated the clot dynamics, reflecting the time required to form a stable clot.

Statistical analysis

SPSS 23.0 software was used to process the data in this study. Graphpad Prism 8 was used to draw the graphs. The coagulation factor VIII concentration, ACT and TMA were expressed as $\bar{x} \pm s$, and examined by *t* test. The pass rate of coagulation factor VIII was expressed as a percentage, and χ^2 test was used. $P < 0.05$ indicated that the difference was statistically significant.

Results

Comparison of coagulation factor VIII concentration at different storage temperatures and times

Under the same storage temperature, the concentration of coagulation factor VIII decreased significantly over time ($P < 0.05$). Under the same storage time, the content of coagulation factor VIII at -80°C was significantly higher than that at -30°C ($P < 0.05$). This indicated that storage temperature and time influenced the concentration of coagulation factor VIII (**Figure 1**).

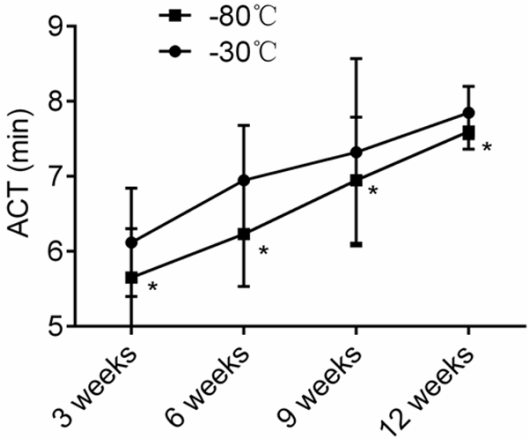

Figure 2. Comparison of ACT value at different storage temperatures and times (min). Note: ACT at -80°C < ACT -30°C. Compared with the same time point -30°C, *P < 0.05.

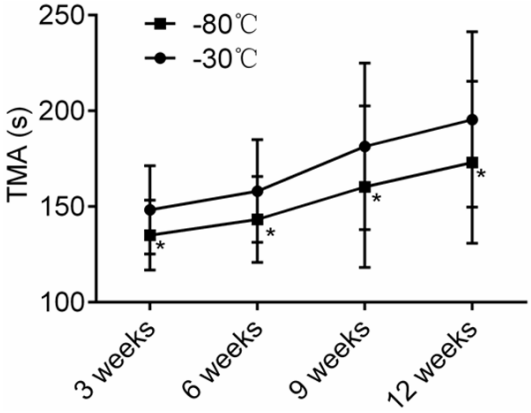

Figure 3. Comparison of TMA at different storage temperatures and times (s). Note: TMA at -80°C < TMA -30°C for the same storage time. Compared with the same time point at -30°C, *P < 0.05.

Comparison of ACT at different storage temperatures and times

From 3, 6, 9 to 12 months, the ACT became significantly longer under the same storage temperature (P < 0.05). For the same storage time, the ACT at -80°C was shorter than that at -30°C (P < 0.05). Storage temperature and time had an effect on ACT (**Figure 2**).

Comparison of TMA at different storage temperatures and times

With the extension of storage time (i.e., 3, 6, 9, and 12 months), the TMA was significantly longer under the same storage temperature (P < 0.05). Under the same storage time, TMA at -80°C was shorter than that at -30°C (P < 0.05). This suggested that storage temperatures and times altered TMA (**Figure 3**).

Effects of combination of storage temperatures and times on concentration of coagulation factor VIII

There was no interaction between different storage temperatures and times (F = 0.47, P > 0.05), namely, there was no significant difference in the reduction rate of concentration of coagulation factor VIII under combined influence of storage temperature and time (**Table 1**).

Comparison of the pass rate of coagulation factor VIII at different storage temperatures and times

The differences in the pass rate of coagulation factor VIII was not significant with regard to different storage temperatures and times (P > 0.05), suggesting storage temperature and time had no significant effect on the pass rate of coagulation factor VIII concentration (**Table 2**).

ANOVA analysis of the pass rate of coagulation factor VIII at different storage temperatures and times

The pass rates of coagulation factor VIII at different storage temperatures were significantly different (P < 0.05), but the pass rate of coagulation factor VIII at different storage times was not significantly different (P > 0.05). The storage temperature had a limited effect on the pass rate of the coagulation factor VIII concentration, while storage times had no significant effect on the pass rate (**Table 3**).

Discussion

Cryoprecipitate contains a certain amount of coagulation factor VIII and fibrinogen, which is mainly used clinically in the treatment of hemophiliacs or patients with persistent bleeding due to lack of coagulation factor VIII and fibrinogen in the body [7]. It was found that the combined infusion of cryoprecipitate and FFP was better than the infusion of FFP alone in the rescue of patients with acute gastrointestinal bleeding, and it could also improve the coagulation function of patients more effectively and achieve a better hemostatic effect [8]. In addition, in patients with severe bleeding and coagulation abnormalities, the infusion of cryoprecipitate combined with platelets had a better hemostatic effect than the infusion of platelets

Table 1. ANOVA analysis of coagulation factor VIII at different storage temperatures and times

Source of variation	Degree of freedom	SS	MS	*F*	*P*
Total variation	89	13098.362	-	-	-
Main effects of different storage temperature	1	649.733	649.733	4.42	< 0.05
Main effects of different storage time	4	1547.830	649.733	3.48	< 0.05
Two-factor interaction	4	230.123	73.347	0.47	> 0.05
Error	81	10673.353	150.124	-	-

Table 2. Comparison of the pass rates of coagulation factor VIII [n (%)]

Storage temperature	3 months	6 months	9 months	12 months
-30°C	41 (91.11)	41 (91.11)	36 (80.00)	32 (71.11)
-80°C	45 (100.00)	41 (91.11)	36 (80.00)	36 (80.00)
t	2.355	0.000	0.000	0.963
P	> 0.05	> 0.05	> 0.05	> 0.05

Table 3. ANOVA analysis of the pass rate of coagulation factor VIII concentration

Source of variation	Degree of freedom	SS	*MS*	*F*	*P*
Total variation	8	0.06	-	-	-
Different storage temperatures	1	0.05	0.05	14.80	< 0.05
Different storage times	3	0.03	0.01	9.32	> 0.05
Errors	3	0.01	0.0033	-	-

alone [9, 10]. If the prothrombin time in DIC patients is 1.5 times higher than the normal time, cryoprecipitate or FFP should be infused, and if the fibrinogen level in DIC patients is less than 1.0 g/L, only cryoprecipitate should be administrated, especially for those with low fibrinogen level, cryoprecipitate therapy must be performed in the early stage [11, 12]. The fibrinogen in cryoprecipitate is a stabilizing factor during long-term cryopreservation, while the coagulation factor VIII is a relatively unstable factor that is easily inactivated by many factors [13]. Therefore, clinical preservation requirements should be adhered, *i.e.* specimens that cannot be measured immediately for coagulation factor VIII concentration should be immediately stored at -30°C instead of -20°C, suggesting that storage temperature could influence the components (coagulation factor VIII, fibrinogen), *i.e.* storage temperature is an important factor affecting the concentration of coagulation factor VIII [9, 14]. It was previously reported [15, 16] that the average concentration of coagulation factor VIII was 90.70 IU/bag after keeping 20 bags of cryoprecipitate at -20°C for 1 day, and dropped to 55.58 IU/bag after 12 months of storage under same temperature, indicating that the change in the concentration of coagulation factor VIII could reflect the quality of cryoprecipitate.

The results of this study showed that under the same storage temperature, the content of coagulation factor VIII decreased significantly over time; under the same storage time, the concentration of coagulation factor VIII was significantly higher at -80°C than that at -30°C. There was no interaction between storage temperatures and times, indicating that there was no significant difference in rates of decrease of concentration of coagulation factor VIII under the influence of both storage temperature and time. The pass rates of coagulation factor VIII were significantly different at different storage temperatures and were not significantly different at different storage times. The pass rates of coagulation factor VIII stored were 87.78% and 83.33% at -80°C and -30°C, respectively. Zhang et al. [17] found that the concentration of coagulation factor VIII decreased continuously as the storage time increased, which is basically consistent with the results of this study. This showed that there was a certain difference in pass rate at different storage temperatures, thus indicating that storage temperature was a factor affecting the concentration of coagulation factor VIII.

In addition, the present study also observed and compared the dynamic process of blood coagulation, and the results showed that under

the same storage temperature, the ACT and TMA values were significantly extended over time, and were shorter at -80°C than at -30°C, indicating that coagulation function declined progressively with increasing storage time. The lower storage temperature indicates the less impact on coagulation function. The reason may be that ACT and TMA could be used to assess the speed and stability of blood clot formation, which is helpful to understand the interaction between the clotting cascade and the relationship among blood components such as platelets, white blood cells and red blood cells, which could help comprehensively assess the process of blood clotting and is an effective tool to assess the whole process of blood coagulation [18-20]. ACT and TMA values provide early warning signs for the bleeding risk of infused subjects as well as heparin use [1, 21, 22].

In summary, with the extension of storage time, the coagulation factor VIII level continued to decrease; storage temperature affected the level of coagulation factor VIII. If the storage time may exceed 6 months, ultra-low temperature storage was recommended. However, due to the small sample size of this study, the results may be biased, and the sample size should be expanded in the future studies.

Disclosure of conflict of interest

None.

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