Aggregability and Post-Transfusion Survival of Canine Platelets in Stored Whole Blood

Ryo TSUCHIYA1), Hisayo YAGURA1), Yoshio HACHIYA1), Toshihiko MOCHIZUKI1), Mitsuru FURUICHI1), Masaharu HISASUE1), Kosaku KOBAYASHI1) and Takatsugu YAMADA1)

1)Laboratory of Internal Medicine II, School of Veterinary Medicine, Azabu University, 1–17–71 Fuchinobe, Sagamihara, Kanagawa 229–8501, Japan

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ABSTRACT. The effects of whole blood storage time on platelet aggregation and on post-transfusion platelet survival time were assessed in dogs. Citrate phosphate dextrose adenine-1 (CPDA-1) was used as a blood cell preservative. Storage time dependent decay of platelet aggregability was assessed. Platelet aggregations to collagen and ADP were maintained for at least 8 hr at room temperature. During blood storage, immunoglobulin became nonspecifically bound to platelets, suggesting the potential for immune destruction of platelets by the mononuclear phagocyte system after transfusion. To assess this assumption, the survival times of infused platelets, which were stored for 0 to 8 hr in whole blood, were measured. Post-transfusion survival of platelets was not affected by these storage times. These results suggest that canine platelets maintain viability when stored at room temperature for up to 8 hr in CPDA-1 treated whole blood intended for transfusion.

KEY WORDS: canine, platelet transfusion, storage time, whole blood.

Blood transfusion is currently a routine therapeutic technique in small animal clinics [9]. Although the effect of platelet administration is transient [8], platelets are administered to dogs with severe thrombocytopenia or thrombocytopenia. Platelet rich plasma (PRP) and platelet concentrate (PC) are recommended for platelet administration in dogs because the separated blood components reduces the risk of adverse transfusion reactions and allows a more effective number of platelets to be transfused [9]. However, they are less available than other blood components (e.g., plasma, packed erythrocytes) because they cannot be stored long before use. Special equipment and technical skills are required for PC or PRP preparation. In particular, it is critical to maintain platelet viability during preparation and storage of platelets if platelet administration is to be effective. During whole blood storage, leukocytes decrease the functional biochemical pathways and post-transfusion survival time of canine erythrocytes [7]. Platelet viability in whole blood is possibly affected by leukocytes as well and decreases more rapidly than that in PRP or PC. Therefore limits of initial whole blood storage time should be clarified for effective PRP or PC transfusion.

It is also known that immunoglobulin (Ig) nonspecifically binds to the surface of canine platelets during blood storage [12, 21]. This phenomenon suggests the possibility that transfused platelets could undergo phagocytosis by the mononuclear phagocyte system (MPS) if the platelet bound Ig was involved in immune destruction. The non-radio labeling technique for canine platelet kinetic study is now available [11], and post transfusion survival of the platelet can be evaluated by using the technique.

In this study, the platelet aggregability and the survival of transfused platelets in stored whole blood were assessed to estimate the duration of the platelet viability in stored canine whole blood intended for transfusion.

MATERIALS AND METHODS

Eighteen beagle dogs, 3 to 10 years of age, weighing 7 to 13 kg, were used. Ten were males and 8 were females. All the dogs were clinically healthy, and had no abnormalities in blood tests including adult filaria antibody test (Clear Guide DIRO; Bio Vet Test Company, France), complete blood cell counts, and routine serum biochemical parameter tests. They were maintained in the Institute of Life Sciences or the Small Animal Facility in the Veterinary Clinical Center, Azabu University.

Blood was drawn into the 200 ml blood transfusion bags (Teruflex; Terumo Company, Tokyo, Japan) containing citrate-phosphate dextrose adenine 1 (CPDA-1) at the standard mixing ratio (1 part CPDA-1 to 6.5 parts blood) from the jugular vein through 17 gauge needles. After mixing the contents the bags were gently rocked at room temperature (24–26°C) until use.

Stability of platelet aggregability: Platelet aggregability in whole blood stored in the blood transfusion bags was assessed by platelet aggregometry. Blood samples (10 ml each) were collected from the bags at 0, 4, 8, 12 and 24 hr after blood drawing respectively. Then PRP was separated by 2,000 g/1 min centrifugation and the platelet counts were adjusted to approximately 25 × 10^4/µl by adding autologous platelet poor plasma. After 30 min resting of platelets, optical platelet aggregometry was performed by using a Chrono-log C550 dual-channel aggregometer (Chrono-log Corp. Havertown, PA, U.S.A.). Collagen (Chrono-collagen, Chrono-log) and adenosine diphosphate (ADP;
Chrono-ADP, Chrono-log) were used as the agonists. Because CPDA-1 is not an ideal anticoagulant for aggregometry [15], the stimulating conditions were modified as follows to evaluate the platelet responses against a single agonist: Final concentrations of collagen and ADP were 50 µg/ml and 50 µM respectively. In addition, 4 mM of CaCl$_2$ (using 100 mM solution) was added prior to ADP stimulation.

Nonspecific Ig binding on platelet surface with whole blood storage: Platelet surface bound Ig was measured by flow cytometry following the method by Lewis et al. [13] except that staphylococcus protein A (SPA) was used as the Ig detector [12, 21]. PRP was separated from whole blood stored at room temperature for 0, 4, 8 and 24 hr. The platelets were washed 3 times with 500 µl of 10 mM phosphate buffered physiological saline, pH7.2 (PBS), containing 1 µM prostaglandin E$_1$ (Sigma Chemical Company, Saint Louis, MO) by 2,000 g/5 min centrifugation. They were resuspended in 285 µl PBS with 3% bovine serum albumin (Fraction V, Sigma), and then 15 µl of 50 mg/ml biotin-conjugated SPA (Sigma) was added and incubated at room temperature for 30 min. After 3 times washing, platelets were resuspended with 100 µl of 50 mM bicarbonate buffer, pH 8.5, and then 5 µl of 5.0 mg/ml streptavidin conjugated fluorescein isothiocyanate (avidin-FITC; Funakoshi, Tokyo, Japan) was added. The tubes were kept in the dark and incubated for 15 min before being washed 3 times. Platelet surface fluorescence was measured by using an Epics XL flow cytometer (Beckman Coulter, Fullerton, CA). Linear and logarithmic amplification was used for light scatter and for fluorescent signal respectively. DNA-check Beads (Beckman Coulter) was used to calibrate the fluorescence. A minimum of 10,000 platelets were analyzed. Platelets were initially identified by forward and side light scatter and identity was further documented by using the FITC conjugated anti-canine platelet monoclonal antibody 2F9 [11]. The monoclonal antibody was kindly provided by Dr. G.L. Dale, University of Oklahoma Health Sciences Center.

Post transfusion survival of platelets in stored whole blood: Platelet survival was investigated by the biotin labeling technique [11]. Blood was drawn from the jugular vein into 200 ml CPDA-1 bags from 15 dogs. PRP was separated after the different periods (0, 4 and 8 hr respectively; 5 blood samples each time) by 1,000 g/3.5 min centrifugation. The bags containing blood were allowed to rock gently at room temperature until PRP separation. Then the platelet-survival was assessed as follows: N-hydroxysuccinimido biotin (0.2 mg/ml PRP) dissolved at 20 mg/ml in dimethylsulphoxide was added into PRP, and then mixed well but gently. After 10 min incubation at room temperature, the biotin-treated PRP was infused to the same individuals through a cephalic vein over 30 min. Two ml of blood was drawn 10 min after reinfusion (Day 0) and at Days 1, 2, 3, 4, and 6 to assess the survival of the reinfused platelets. These samples were stored at 4°C until the final sample was collected. Then the percentage of biotin positive platelets was measured following the Heilmann’s technique [11]. FITC-streptavidin was used instead of phycoerythrin-streptavidin and flow cytometry was performed immediately after the fluorescein-stain. Platelet survivals were calculated using the least squares fitting of the data to gamma functions [16].

RESULTS

Stability of platelet aggregability: Figure 1 shows platelet aggregability in stored whole blood collected in the transfusion bags. Time-dependent change was statistically analyzed by using the Friedman’s one-way analysis of variance test. Most of collagen-induced aggregation did not decrease for at least 12 hr and no significant time-dependent change

![Fig. 1. Changes of collagen and ADP-induced platelet aggregation following various whole blood storage times. The blood samples were collected at each time from the transfusion bags containing CPDA-1 treated whole blood which were rocked at room temperature. PRP was separated by 2,000 g/1 min centrifugation. Final concentrations of collagen and ADP were 50 µg/ml and 50 µM respectively. Four millimolar CaCl$_2$ was added prior to adding ADP.](image-url)
was found until 24 hr after blood drawing. ADP-induced aggregation somewhat randomly changed, especially at 4 hr after drawing; however, the maximum aggregation ratio of ADP-induced aggregation decreased to 0% between 8 and 12 hr after blood drawing in 2 cases. Significant time-dependent decrease was found by the statistical analysis (P<0.01).

Non-specific Ig binding on platelet surface: Figure 2 shows a change of fluorescence histograms detecting platelet surface Ig with 8 hr storage of whole blood. Platelet surface Ig increased in CPDA-1 treated blood with storage time. Similar results were repeatedly obtained in 2 other samples. Some of the nonspecific bound Ig on platelets was removed by incubation at 37°C for 30 min and three times washing, however a major part of it was not removed (data not shown) as Scott et al. [21] reported using the other anticoagulants and radioimmunoassay.

Post transfusion survival of platelets in stored whole blood: Figure 3 shows the kinetics of reinfused/biotin-labeled platelets. Regardless of the storage time, the decreasing curves of administered platelets were similar among groups given platelets from fresh, 4 and 8 hr stored whole blood. Platelet survival was 5 to 6 days in each group as shown in Table 1. No significant differences were found among the survivals of each group by the unpaired student-t test. These results indicate that the nonspecific bound Ig on the platelet surface does not affect post transfusion platelet survival.

### DISCUSSION

In human medicine, PCs are usually used for platelet administration and PCs are transfused within 72 hr after blood drawing [6, 15, 20]. Even the lyophilized dry technique of platelets was investigated to extend storage time [19]. In veterinary clinics, successful canine PC administration has been reported [1]. Allyson et al. [3] reported that canine platelet function in PC stored at room temperature in polyolefin bag with gentle rocking was well maintained for 5 days. However, whole blood units are initially held for a while after blood drawing and the time limit of whole blood storage prior to PRP or PC preparation should be clarified for effective platelet administration. Norte and Mischke [18] reported changes of canine platelet aggregability in whole blood after long-term storage at 4–6°C. They reported that 24 to 46 percent of platelet aggregability decreased during the first 6 hr, and the remaining aggregability was maintained during the next 3 to 4 weeks. However, whole blood is preferably stored at room temperature...
for platelet administration in dogs [4, 5, 14] to obtain the highest effect.

Changes of platelet aggregability were assessed in this study also. Because platelet aggrega- tion in CPDA-1 treated PRP is much weaker than that in 0.38% sodium citrate treated PRP [15], CaCl₂ was added to counteract the excess citrate and therefore enhance aggregation. Groh et al. [10] also performed the re-calcification technique for platelet aggregometry using PRP treated with acid citrate dextrose, also a blood cell preservative but not an ideal anticoagulant for platelet aggregometry. They added heparin to avoid plasma coagulation after re-calcification. In this study, heparin was not added, but no coagulation occurred during aggregometry.

Collagen and ADP-induced platelet aggregatability were better maintained at room temperature than at 4–6°C, as reported by Nolte and Mischke [18]. ADP-induced platelet aggregation markedly decreased between 8 and 12 hr after blood drawing in 2 of the 7 samples. Similarly, sporadic platelet samples for diagnostic platelet aggregometry are sometimes refractory to ADP, even with fresh samples. This seems to be related to technical aspects of blood withdrawal and PRP preparation. Platelets release small amount of the granular pool ADP during platelet processing and storage. The amount of ADP released may depend on the special handling techniques and conditions. Platelets continuously exposed to low concentration of ADP specifically lose ADP-induced aggregation responses [17]. Although the in vivo effect of transfusing platelets with decreased ADP aggregability is unclear from this study, canine platelets maintain aggregability for at least 8 hr in whole blood after blood drawing. Careful blood/platelet preparation may extend the storage time without severe loss of platelet aggregability.

During storage, nonspecific Ig binding occurred on platelet surfaces. This finding is consistent with past reports in which different anticoagulants including ethylenediamine-tetraacetic acid and trisodium citrate were used [12, 21] and it seems to be principally IgM [21]. The presence of platelet surface-associated Ig indicates the potential for immune mediated destruction of administered platelets in stored whole blood. To assess this possibility, the survival times of infused platelets, which were stored for 0 to 8 hr in whole blood, were measured. Ig increased on the platelet surface during whole blood storage but did not affect post transfusion survival in this study. Therefore, decay of the platelet function was the only problem we identified when assessing whole blood storage for canine platelet administration. In conclusion, we estimated the storage time limit of CPDA-1 treated canine whole blood for platelet administration is at least 8 hr at room temperature.

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REFERENCES

