During the last two decades, there has been great interest in developing and using platelet additive solutions (PASs) for the storage of platelets. At present, such additive solutions are in use for transfusion in several countries. PAS is generally used as a substitute for plasma to (1) reduce the amount of plasma transfused with platelets and to recover plasma for other purposes, primarily fractionation into plasma products; (2) avoid transfusion of large volumes of plasma with possible adverse reactions and circulatory overload; (3) make possible photochemical treatment for the inactivation of bacteria and other pathogens in platelets; and (4) improve storage conditions as was discussed in a previous review.

A basic principle is that aging of platelets after in vitro storage at 22°C is significantly slower than aging of platelets in vivo at 37°C, a situation that may make extended storage of platelets possible. Three approaches were suggested to be of specific importance to improve storage conditions of platelets: (1) reducing the activation of platelets during collection of blood and the preparation and storage of platelets; (2) reducing the metabolic rate in terms of glucose consumption and lactate production; and (3) ensuring that glucose will be available in the storage medium during the entire storage period. The activation of platelets can be counteracted either by the addition of platelet activation inhibitors or of certain components such as magnesium to the PAS.

The use of PAS offers the possibility of including components with specific effects on platelets in the storage solution that are not present in plasma or in the anticoagulant. A number of effects have been observed that can be assigned to certain components. Reducing platelet activation and inclusion of key components in the platelet storage environment, such as acetate, citrate, glucose, potassium, and magnesium, were suggested to be useful tools to optimize platelet storage conditions. The compositions of some present PASs are presented in Table 1. The purpose of this review is to describe some events of the last several years as a complement to the knowledge presented in previous reviews.

In Vivo Characteristics

Results from a number of in vivo studies using PASs have been published during the last decade. In the first patient transfusion studies in the 1990s using PASs such as PAS-II (T-Sol, Baxter) or Plasma Lyte A (Table 1), the results were not consistent. In some studies, satisfactory CCIs were found in patient transfusion studies, in some studies significantly lower CCIs were observed than for platelets stored in plasma. In a recent study, encouraging platelet recovery and survival data were found, comparing platelets prepared by apheresis and stored in PAS-II for 1 versus 7 days. Mean recovery was 69 percent and 53 percent, and survival was 8.2 and 5.1 days at Days 1 and 7, respectively. The ratios of Day 7 to Day 1 were 0.80 and 0.65 for recovery and survival, respectively. A proposal by Murphy of a new standard of efficacy for the evaluation of platelets for transfusion has created considerable interest. This concept implies that acceptably stored platelets on the last day of storage would demonstrate at least two-thirds the recovery and one-half the survival of platelets collected from the same subject and then retransfused as reference “fresh” platelets. The ratios in this study met the proposed criteria for 7-day storage, although the use of a Day 1 control may not totally have fulfilled the requirements of the reference indicated.

Comprehensive patient transfusion studies using pathogen-reduced platelets have added significant knowledge about platelets stored in more recent PASs, particularly PAS-III (InterSol, Baxter). A photochemical treatment method using a novel psoralen, amotosalen HCl, in combination with ultraviolet light has been developed to inactivate viruses, bacteria, protozoa, and WBCs in platelets. Two major studies were conducted in Europe and in the United States, namely...
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the euroSPRITE trial in Europe and the SPRINT trial in the United States.18–21 In the euroSPRITE trial, comparable platelet CIs and CCIs and a comparable safety profile for photochemically treated and reference untreated pooled buffy-coat (BC) platelet units in 103 oncology patients with thrombocytopenia were demonstrated. In the SPRINT study, which included 645 transfused patients, the hemostatic efficacy of photochemically treated and reference untreated platelets was found to be equivalent.19 Transfusion reactions were significantly fewer, and adverse events and overall safety profiles were comparable for treated and reference platelets.20 In a second evaluation of data from the euroSPRITE patient transfusion study using BC-derived platelets, reference groups with untreated platelets were compared.22 Reference platelets were stored in either T-Sol (n=35) or plasma (n=16). The results indicated that 1-hour and 24-hour mean platelet CIs and posttransfusion hemostatic scores were not significantly different for patients receiving platelet components suspended in 100 percent plasma and patients receiving platelets in a T-Sol environment. In a review in 2003, the preliminary conclusion had been that additional in vivo data, from studies of clinical outcome after transfusion of platelets stored in either PAS or plasma, were needed/required.6 Today, comprehensive clinical data support the routine use of PAS as an equivalent alternative to plasma to suspend platelets.

In Vitro Platelet Aging at 22°C Versus In Vivo Aging at 37°C

Because the average survival of human platelets in the circulation, after release from megakaryocytes, is known to be 9 to 10 days, it may be questioned whether it is at all meaningful to extend the shelf life of platelet concentrates up to or even beyond 7 days.23 Removal of platelets from the circulation is believed to be age-dependent and is probably associated with failure to maintain normal hemostasis. Transfusion of aging platelets may be of little help to stop bleeding because they would be removed from the circulation.

Studies comparing aging of platelets in vivo with in vitro storage at 22°C, using isotope labeling, suggested that aging of platelets after in vitro storage for 5 days at 22°C was similar to aging of platelets during 2.1 days in vivo at 37°C.7 The relative aging factor was found to be 0.42 (2.1 days divided by 5.0 days). In a subsequent study, a similar relative aging factor was found (0.44).7 These differences were associated with a much higher turnover rate of ATP, the major energy carrier in platelets, at 37°C than at 22°C. The calculated ATP turnover ratio of 0.48 at those two temperatures was of the same magnitude as the calculated relative aging factor, indicating that the relative decrease in aging of platelets at 22°C compared with that at 37°C is similar to the relative decrease in metabolic rate at this temperature. The rate of aging of platelets during storage at 22°C may be less than half of that found in vivo at 37°C. If this ratio is applied to the normal lifespan of platelets, 9 to 10 days in the circulation would correspond to at least 18 to 20 days of in vitro storage at 22°C. These data may provide meaningful objectives to develop methods and storage environments for extended storage of platelets.

The Use of PAS in Combination With Different Methods for the Preparation of Platelets for Transfusion

In general, the various PASs can be used for apheresis as well as for BC platelets. The storage medium normally is composed of a mixture of plasma (generally 20–40%) and PAS (60–80%). The main difference is that in apheresis platelets, ACD is often preferred to CPD anticoagulant, because resuspension of platelets after preparation is facilitated. In some apheresis equipment, platelets are kept in the centrifugation chamber during the entire apheresis cycle; in other equipment, platelets are continuously transferred to a platelet storage container. These differences may result in significant variation in platelet activation. Although apheresis equipment originally may have been designed for the preparation of platelets suspended in plasma, most equipment can be

| Table 1. Composition of some current PASs (in mmol/L) including commercial designations* |
|---------------------------------|---------------------------------|-----------------|-----------------|---------------------|-------------------|
| Plasma Lyte A                  | PAS-II (T-Sol, Baxter)          | PAS-III (InterSol, Baxter) | Composol (Fresenius) | PAS-III M (SSP+, MacoPharma) |
| NaCl                            | 90                              | 116                          | 77                           | 90                  | 69               |
| KCl                             | 5                               | –                            | –                            | 5                   | 5                |
| MgCl₂                           | 3                               | –                            | –                            | 1.5                 | 1.5              |
| Na₃citrate                      | –                               | 10                           | 10                           | 11                  | 10               |
| NaH₂PO₄/NaH₂PO₄                 | –                               | 26                           | –                            | 26                  |                  |
| Na-acetate                      | 27                              | 30                           | 30                           | 27                  | 30               |
| Nagluconate                     | 23                              | –                            | –                            | 23                  |                  |

*The compositions of commercial solutions may be slightly different from basic compositions.
used for platelet collection in a small final resuspension volume of plasma, which is necessary to allow the addition of PAS. Satisfactory results were obtained by Ringwald et al.\textsuperscript{24,25} when evaluating the suspension of high concentrations of apheresis platelets (4000–5000 \times 10^9 platelets/L) in PAS.

BC-derived platelets suspended in PAS are generally prepared in pools from several donors. There are primarily variations in the number of BCs included in the pools (generally 3–7 BCs) and in the time of holding of whole blood preceding the preparation of BCs. Either BCs can be prepared on the day of collection, generally within 8 hours, and stored overnight for the preparation of platelets on the following day; or whole blood can be stored overnight for the preparation of BCs and platelets on the following day.

The platelet rich plasma (PRP) method is generally not used for the routine preparation of platelets in PAS. However, in a recent study by Sweeney et al.,\textsuperscript{26} platelets were prepared from individual donors by the PRP method in a first step and then pooled as platelets from several donors and suspended in either plasma or PAS. Platelets were stored for 7 days for in vitro evaluation. Good preservation of platelet quality in both environments was reported.

**Effects on Metabolism and Platelet Function Associated With Components in PAS**

The results of early studies by Holme et al.\textsuperscript{27} and Gulliksson et al.\textsuperscript{28} on the effects of PAS indicated that the presence of glucose during the entire platelet storage period is crucial for platelet metabolism. Effects observed after depletion of glucose involved rapid decrease in adenine nucleotide levels, cessation of lactate production, and finally disintegration of platelets. Depletion of glucose is generally associated with an increased rate of platelet metabolism and fall in pH levels. The results suggested that depletion of glucose, not the pH level alone, is detrimental to platelets during storage. In contrast to plasma, the fall in pH during storage of platelets in PAS will stop at a significantly higher level than pH 6.0, often at about pH 6.5 as a result of the limited amount of glucose generally available in PAS.\textsuperscript{28} Because the buffering capacity of PAS is approximately half that of plasma, PAS is more susceptible to increased production of lactic acid by platelets.\textsuperscript{29,30} On the other hand, metabolism of acetate present in PAS significantly stabilizes the pH level. In parallel with glucose, acetate is used as a substrate for the oxygen-dependent platelet metabolism, and enters into the tricarboxylic acid cycle, and is further oxidized in the respiratory chain.\textsuperscript{29–32} The end products are carbon dioxide from the first step and water from the second step. By formation of bicarbonate from the carbon dioxide produced by acetate, very stable pH levels are maintained during platelet storage.\textsuperscript{29,30,32} A possible third substrate for platelet metabolism may be fatty acids.\textsuperscript{33}

Generally, phosphate has two possible roles during storage of platelets, as a stimulant of platelet glycolysis to increase production of lactic acid and as a buffer to prevent a fall in pH. These two effects theoretically compete and may neutralize each other. There are no indications of net utilization or production of phosphate during storage of platelets.\textsuperscript{29}

The new PASs designated Composol and PAS-IIIM as well as the early Plasma Lyte A all contain magnesium and potassium. Composol and PAS-IIIM also contain citrate, in contrast to Plasma Lyte A. The three components: magnesium, potassium, and citrate are associated with complexity of effects and interdependence. Effects on platelet membrane function and platelet activation as well as rate of glycolysis have been described and the various effects may even be combined.

Increased concentration of extracellular magnesium ions significantly inhibits exposure of P-selectin, decreases binding of fibrinogen to ADP-activated platelets, and significantly decreases ADP-induced platelet aggregation.\textsuperscript{34} Magnesium may also modify calcium influx into the platelets. In addition, magnesium, calcium, and the concentration of citrate have an effect on potassium permeability of the platelet membrane and the intracellular concentration of potassium.\textsuperscript{35} Citrate also heightens platelet responsiveness to some activating agents such as ADP.\textsuperscript{36} In addition, effects on the rate of glucose consumption and lactate production related to the concentration of citrate and the presence of potassium and magnesium in PAS have been observed. Platelets stored in medium with a citrate concentration of 8 mmol/L produced only half the quantity of lactate produced by platelets in a similar medium with a citrate concentration of 14 to 26 mmol/L.\textsuperscript{37} Inasmuch as no negative effects on adenine nucleotide levels were observed, the results suggested that synthetic media preferably should include citrate at low concentrations to avoid excessive lactate production and an acid pH. On the other hand,
increased production of lactate associated with higher concentrations of citrate can be neutralized by the addition of acetate.\textsuperscript{37} Again, this situation illustrates the complexity of effects and interdependence associated with those components. In parallel, the combination of magnesium and potassium ions present in PAS has a similar effect on platelets, i.e., significantly reduced metabolic rate in terms of glucose consumption and lactate production and also reduced platelet activation.\textsuperscript{38-40}

When platelets are shipped between different centers, agitation may be interrupted for a considerable period of time. A previous study using a plasma environment suggests that possible storage time without agitation is strongly affected by platelet concentration.\textsuperscript{41} The level of pH should be kept above 6.5 to avoid negative effects on functional in vitro factors such as hypotonic shock response (HSR).\textsuperscript{41} In a recent study, the effects of interruption of agitation on platelets stored in two PAS alternatives, namely PAS-IIIM and Composol, were evaluated.\textsuperscript{42} At a platelet concentration of approximately 1000 × 10\textsuperscript{9}/L, platelets could be stored for 4 days in PAS-IIIM with maintenance of HSR and pH. This was not possible with Composol, suggesting that the presence of phosphate is of importance to maintaining pH and other in vitro characteristics during interruption of agitation. Similar effects were not observed during continuous agitation.

Future Perspectives

To conclude, present knowledge and experience support platelet storage only at 22°C. The use of PAS instead of plasma as the platelet storage environment would provide benefits to patients and facilitate the inclusion of certain components with proven favorable effects on platelets that are present in neither plasma nor anticoagulant. The present knowledge of effects associated with different approaches discussed above suggests that reducing platelet activation in combination with the inclusion of key components in the platelet storage medium, such as acetate, citrate, glucose, potassium, magnesium, and additional possible future ingredients, should be the tools to optimize the storage conditions and maintain the function of platelets. Additional in vivo studies of recovery and survival as well as count increments in patients with thrombocytopenia will be needed.

References


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