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PAS or plasma for storage of platelets? A concise review

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SUMMARY

Platelet additive solutions (PASs) are becoming increasingly popular for storage of platelets, and PAS is steadily replacing plasma as the storage medium of platelets. PASs are electrolyte solutions intended for storage of platelets, and they are used to modulate the quality of the platelets by adding specific ingredients. All currently available PASs contain acetate. Acetate reduces the amount of glucose that is oxidised into lactic acid and thereby prevents the lowering of pH, which decreases platelet quality. Furthermore, the oxidation of acetate leads to the production of bicarbonate, which serves as buffer. The presence of potassium and magnesium in PAS prevents the lowering of pH and reduces the degree of spontaneous activation of the platelets during storage. In the hospital, platelets stored in PAS result in about half of the number of allergic transfusion reactions as compared with platelets in plasma. Recovery and survival after transfusion, as well as corrected count increments, are at least as good for platelets in PAS as for plasma, and recent data suggest they may even be better. Therefore, with the current generation of PASs, PAS should be preferred over the use of plasma for the storage of platelet concentrates.

Key words: platelet additive solution, platelet concentrates, platelet storage.

WHAT IS A PLATELET ADDITIVE SOLUTION?

PAS is a balanced electrolyte solution intended for platelet storage. PASs were initially developed to remove plasma from platelet concentrates because plasma contains enzymes that have a negative effect on the quality of platelets (Rock *et al*., 1985). Moreover, buffer(s) could be added to keep the pH $>6{\cdot}0$, a level below which platelets are known to lose their clinical quality. In the ensuing years, further benefits were identified: more plasma becomes available for fractionation or transfusion; PAS has a standardised composition and can be steam-sterilised; it gives the ability to control the storage environment; the final platelet concentrate contains less protein, causing fewer allergic reactions; it has a lower ABO-titer; and possibly provides a reduction of antibody-mediated TRALI, although with the use of male-only plasma, that benefit is probably no longer there.

PAS is the generic term for any electrolyte solution for platelet storage. However, at some point, PAS was also used as a trade name (PAS-2, PAS-3), and the use of PAS-2 particularly resulted in decreased platelet quality (Turner *et al*., 1996). As a consequence, PAS became synonymous with decreased platelet quality. However, not all PASs are the same, and certainly with the current generations of PAS, as will be detailed later in this review, it is no longer true that PASs have decreased platelet quality. In 2010, a nomenclature was proposed to assign letters to each PAS (PAS-A, PAS-B and so forth), depending on the presence (not the concentration) of specific ingredients in the PAS to prevent confusion (Ashford *et al*., 2010). In this review, we will use that nomenclature.

THE DUTCH SITUATION

Sanquin is the national blood supplier for the Netherlands and, in 2014, provided about 433 000 red cell concentrates and about 59 000 platelet concentrates to roughly 100 hospitals. The majority of the platelet concentrates (~95%) are from buffy coats; the remaining 5% are collected by apheresis and are specifically collected as HLA-matched concentrates for refractory patients or as HPA-1a-negative concentrates for paediatric use. Of the buffy coat-derived platelets, approximately 80% were made and stored in plasma, and the remaining 20% of the units were stored in platelet additive solution (PAS, which in reality is a mixture of 30 – 40% plasma and 60 – 70% PAS due to the carryover of plasma from the buffy coats). Previously, when Sanquin had four processing sites, three made platelet concentrates in plasma and one in PAS. There was no specific rationale; it was just that hospitals in this one region requested platelets in PAS and that one Sanquin site promoted the use of PAS. In 2013, Sanquin switched from PAS-B with a 5-day outdating period to PAS-C (with phosphate as buffer) with a 7-day outdating period. Since 2015, all blood processing was consolidated in two processing sites, one of which still makes platelet concentrates in PAS for the hospitals in that specific region in the Netherlands.

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PLATELET STORAGE LESION

Platelets undergo numerous changes during their time outside the human body, isolated from whole blood, in a plastic container, and stored at room temperature. The changes are complex and multifactorial and are therefore referred to as the 'platelet storage lesion'. Briefly, it is 'all things bad happening to a platelet', and can manifest itself at many levels in the platelet, ranging from changed biochemistry resulting in increased metabolism; increased baseline activation; increased signals for removal from circulation; and poorer responses to stimuli to reduced adhesion at a wound site. In the patient, the platelet storage lesion can translate to a lower recovery after transfusion or a shorter survival time, and ultimately, platelets with the storage lesion are less able to stop or prevent bleeding upon transfusion.

The platelet storage lesion is likely caused primarily by an elevated glucose consumption rate of the platelets, resulting in the generation of lactic acid (Dekkers *et al*., 2007). Lactic acid causes the lowering of the pH of the storage medium, and this in turn leads to further platelet activation. Platelet activation gives an elevated glucose consumption, completing the self-sustaining circle of the platelet storage lesion. Over the years, various generations of PAS were formulated to counteract the platelet storage lesion. Citrate, acetate, potassium, magnesium, phosphate, bicarbonate, calcium and glucose, to name a few, were tested in an effort to maintain the platelet quality during storage in the blood bank. It is outside the scope of this review to go into detail about the effect of all the various ingredients on platelet quality (for more details, see Van der Meer & De Korte, 2013), but some key findings in the development of the optimal PAS will be discussed here.

MODULATION OF PLATELET CHARACTERISTICS BY PAS

Platelets are extremely metabolically active cells and consume about 3 μmol glucose 10^{-10} platelets h^{-1}, which, to put it into perspective, is six times as fast as resting muscle and 30% as fast as the mammalian brain (Guppy *et al*., 1990). Platelets use glucose to make ATP. Full oxidation of glucose to carbon dioxide and water gives about 30 ATP, whereas incomplete (anaerobic) oxidation of glucose into two molecules of lactic acid gives only two ATP (Guppy *et al*., 1990). Nevertheless, platelets almost exclusively use the pathway that yields the *least* ATP; of the glucose consumed, $0{\cdot}05 \pm 0{\cdot}01$ μmol glucose 10^{-11} platelets h^{-1} is fully oxidised versus $3{\cdot}13 \pm 0{\cdot}44$ μmol glucose 10^{-10} platelets h^{-1} converted into lactic acid (Guppy *et al*., 1990). *In vivo*, lactic acid is neutralised by the liver, but outside the body, platelets quickly acidify their storage medium. To maintain the pH and the quality of the platelet transfusion product has been one of the major challenges in the past decades.

The early versions of PAS were based on infusion fluids that contained acetate. Acetate had been added to some infusion fluids because it is converted into bicarbonate by the liver and therefore can correct the acid–base balance of the patient. It was serendipity that platelets also metabolised acetate *ex vivo*; one study indicated that if only glucose was present during platelet storage, $2{\cdot}4 \pm 0{\cdot}5$ μmol lactate 10^{-11} platelets day^{-1} was produced. When a PAS with 23 mM acetate was used (in the presence of potassium and magnesium), the platelets started to produce much less lactate, namely $1{\cdot}3 \pm 0{\cdot}3$ μmol 10^{-11} platelets day^{-1} (Shimizu & Murphy, 1993). This indicates that platelets start using acetate for their metabolism and consequently need less glucose. Platelets take up acetate into their mitochondrion, where it is oxidised into carbon dioxide and water. In order for acetate to enter the mitochondrion, it needs to be neutral in charge, and a hydrogen ion is taken up to convert acetate into acetic acid (Murphy *et al*., 1995). Consequently, acetate metabolism results in a lower hydrogen ion concentration, that is, it increases pH. Due to the pH-dependent equilibrium between water and carbon dioxide on one hand and hydrogen ions and bicarbonate on the other, the uptake of hydrogen ions are, in practice, observed as an increase in the bicarbonate concentration during platelet storage. In other words, the presence of acetate in PAS induces a switch from glucose to acetate metabolism, whilst the acetate metabolism itself leads to the formation of its own buffer. Since the early 1990s, all PASs are based on acetate.

Another important finding was the addition of potassium and magnesium to PAS. A step-wise addition of potassium, magnesium and the combination of the two to PAS-B revealed that pH was better preserved and that the activation markers (which should be kept as low as possible during blood bank storage) remained low (De Wildt-Eggen *et al*., 2002). Storage of platelet units during 7 days in PAS-B with potassium and magnesium gave a pH of $7{\cdot}15 \pm 0{\cdot}10$, which was significantly higher than in units in PAS-B in the absence of potassium and magnesium that had a pH of $6{\cdot}94 \pm 0{\cdot}05$. A third control arm of platelets stored in plasma showed a pH of $7{\cdot}03 \pm 0{\cdot}06$ by day 7. CD62P expression of the stored platelets was $23 \pm 6\%$ when stored in PAS-B with potassium and magnesium versus $50 \pm 8\%$ when potassium and magnesium were absent. Platelets stored in plasma showed an average CD62P expression rate of $35 \pm 8\%$. The mechanism of action is multifactorial and not entirely elucidated (Van der Meer *et al*., 2004), whereas it is clear from this and subsequent studies that there is a strong favourable effect of adding potassium and magnesium to PAS. Because potassium and magnesium have always been investigated in acetate-based PASs, it is difficult to determine what beneficial effect is caused by specific acetate metabolism and what effect is caused by potassium and magnesium. A large paired comparative study by our group, evaluating platelets in plasma, PAS-B, PAS-C, PAS-D and PAS-E, showed for the two PASs with potassium and magnesium unequivocally superior results with better preservation of pH, a lower production rate of lactic acid, a lower expression of CD62P and a lower expression of the apoptotic marker phosphatidylserine (which is detected with annexin A5) than compared with the PASs without potassium and magnesium (Van der Meer *et al*., 2010). In the absence of potassium and magnesium, PASs always performed worse than plasma. Phosphatidylserine expression was better

preserved on platelets in potassium- and magnesium-containing PAS than in plasma, but for the other parameters, there were no differences.

RESIDUAL PLASMA OR RESIDUAL GLUCOSE?

Except probably for PAS-E (Gulliksson *et al*., 2003), the current generations of PAS still require at least 30% of residual plasma. This percentage was established following a study where platelets were stored in PAS-B with 20% plasma/80% PAS-B or in 100% plasma. This study showed that with 20% plasma carryover, nearly all platelets had a bloated appearance, with the loss of many of their granules by day 8 of storage (Klinger *et al*., 1996). When stored in 100% plasma, only about one-fifth of the platelets showed a changed appearance. These results thus indicate that a certain percentage of plasma is needed to preserve structural integrity of the platelets. Plasma contains all kinds of proteins, enzymes, growth factors, vitamins, etc., and our group was interested whether it was one of these factors or glucose that preserved the platelet quality. A paired study was set up, with one group of platelet concentrates in 35% plasma/65% PAS-E and a matched unit with 100% PAS-E that was supplemented with a similar amount of glucose as the other unit. That as yet unpublished experiment revealed no differences in the quality of the platelets, indicating that glucose is enough to preserve platelet quality. Thus, plasma carryover was necessitated by the glucose requirement, and the presence of protein or other plasma factors is not essential.

CLINICAL OBSERVATIONS OF PLATELETS STORED IN PAS

In addition to the optimising of the platelet quality during storage, the reduction of allergic transfusion reactions has been a main driver for the development of PASs. Multiple studies have shown that the replacement of plasma for a non-protein solution results in about a 50% reduction of the number of allergic reactions (Bertolini *et al*., 1989; Oksanen *et al*., 1994; De Wildt-Eggen *et al*., 2000; Kerkhoffs *et al*., 2006; Andreu *et al*., 2007; Cohn *et al*., 2014; Tobian *et al*., 2014).

Some of the early PASs showed decreased platelet increments after transfusion (Turner *et al*., 1996), and this reputation has stuck to PASs in general. However, two decades have since passed, and in this respect, we know more about PASs, and large clinical improvements have been made. Unfortunately, the literature is scarce when it comes to published data. Currently, the most that is known about PAS-C is that one does not contain potassium or magnesium but has phosphate as additional buffer. This PAS shows slightly lower increments than platelets stored in plasma. In a pathogen reduction trial, where platelets stored in plasma and stored in PAS-C both were the control groups, the 1- and 24-h corrected count increments (CCIs) were 9 and 7% lower than when stored in plasma, respectively (Kerkhoffs *et al*., 2010). Tobian *et al.* (2014) retrospectively studied clinical patients receiving platelets in plasma or in PAS, and the platelet concentrates in PAS-C showed 24 and 19% lower 1- and 24-h CCIs than when stored in plasma, respectively. For platelets stored in PAS-E, with potassium and magnesium, only published data as an abstract is available. In that one clinical study, platelets stored in PAS-C and PAS-E were compared with those stored in plasma. CCIs were calculated from platelet counts measured anywhere between 1 and 24 h after transfusion. Platelets stored in PAS-C showed CCIs that were 16% lower than those stored in plasma, whereas platelets in PAS-E had CCIs that were only 2% lower than stored in plasma (Tardivel *et al*., 2012). Slichter *et al.* (2014) published data on platelets stored in PAS-F (also containing potassium and magnesium). They determined the recovery and survival using re-transfusion of radioactive-labelled apheresis platelets in healthy volunteers. When platelets in plasma had been stored for 7 days, the recovery was $44 \pm 5\%$ (it never exceeds 70% due to clustering of platelets in the spleen), and survival was $4{\cdot}9 \pm 0{\cdot}7$ days. Platelets stored for 7 days in PAS-F had superior values of $52 \pm 3\%$ and $6{\cdot}0 \pm 0{\cdot}3$ days. Interestingly, platelets stored in PAS-F for 13 days still had a recovery of $49 \pm 3\%$ and a survival time of $4{\cdot}6 \pm 0{\cdot}3$ days; in other words, this is rather similar to the values found for 7-days-stored platelets in plasma. These studies were performed with platelet concentrates obtained with apheresis, and further studies with some optimisation steps are needed to show whether similar encouraging results can be obtained with buffy coat-derived platelet concentrates. The attempt should not be to store platelets *longer*, but these data indicate that there are opportunities to store platelets *better*, which will ultimately help us administer a superior transfusion product to our patients when using a PAS.

DISCUSSION

Over the past decades, a variety of PASs were developed, and the 'newer' PASs certainly show an *in vitro* quality that is not worse than when stored in plasma and probably even better. The key to maintaining good platelet quality is the use of acetate as fuel for the platelets, which limits the formation of lactic acid due to a lower glucose metabolism, and it provides its own bicarbonate buffer, thereby preventing a pH drop and the platelet storage lesion. Various further modifications have been carried out to optimise platelet quality, and the addition of potassium and magnesium (in acetate-based PASs) has particularly shown great improvement, both in *in vitro* quality as well as in recovery and survival studies and in CCI studies. When going back to the question of 'PAS or plasma' for storage of platelets, there have been many recent developments, all supporting the benefits of using a PAS. Therefore, in summary, it is the author's opinion that with the current generation of PASs because (i) the storage quality of platelets, judged *in vitro* and *in vivo*, is at least as good, if not better and (ii) the number of allergic reactions are fewer, the use of PAS for the storage of platelet concentrates should be preferred.

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CONFLICT OF INTEREST

The author has no competing interests.

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