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9 July 1971 ■

Prostaglandin E_1 in Platelet Harvesting: An in vitro Study

Abstract. *Prostaglandin E_1 (10^{-8} to 10^{-7} molar) is effective in improving the preparation of human platelet concentrates from plasma rich in platelets and from whole blood. A procedure has been developed for the use by blood banks, on a trial basis.*

The demand for platelet concentrates has increased steadily in view of their usefulness in treating hemorrhage due to thrombocytopenia, and their relation to chemotherapy and radiation and immunosuppressive therapy. Platelets are in short supply, however, because they can be stored only for a short time and because the cost, in time and materials, necessary to prepare them in therapeutic quantity is high. Usually, after centrifugal separation, platelets are suspended in one-tenth volume of autologous plasma. Until recently, this procedure was performed at low temperatures; however, the cold reduces the viability of platelets (*1*). This suggests that preparation and storage of platelet concentrates (PC) at room temperature will improve both the harvesting of the platelets and the maintenance of their function in vivo.

During the high-speed centrifugation of platelet-rich plasma (PRP), massive aggregates form because of the release of cell components or the exposure of sticky sites. Prostaglandin E_1 (PGE_1) is one of the most potent inhibitors of platelet aggregation known (*2*). It inhibits the rounding of platelets induced by adenosine diphosphate (ADP). This rounding is associated with a stickiness which then results in the aggregation of platelets when they come in contact with one another (*3*). The effect of PGE_1 on the aggregation and shape of platelets during their collection and storage was studied to provide a basis for the trials described by Becker *et al.* and Valeri *et al.* (*4*).

Blood was drawn from healthy volunteers into blood bags containing an anticoagulant: acid, citrate, and dextrose (ACD). Sterile polystyrene test tubes (Falcon) were used for studies on centrifugation and storage. Platelet-rich plasma was prepared by centrifugation (375*g*, 15 minutes) and portions of it (3 ml, *p*H 7.1) were transferred into the plastic test tubes. Prostaglandin E_1 (*5*) (0 to 60 ng/ml) was added and mixed well. After centrifugation at room temperature (1500*g*, 15 minutes) the shape of the resultant pellet was examined. The platelets which were originally centrifuged without PGE_1 or at low concentrations of PGE_1 ($\leqq$ 2 ng/ml) spread out in a thin film over the bottom of the test tube. However, platelets mixed with greater concentrations of PGE_1 ($\geqq$ 5 ng/ml) lay in a smooth, clearly defined round pellet (Table 1). Plasma supernatant (2.5 ml) was then removed by suction, and the platelets were suspended in the remaining plasma by gentle pumping with a plastic pipette.

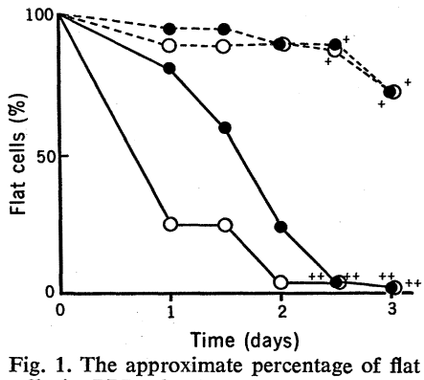

Fig. 1. The approximate percentage of flat cells in PRP after storage at room temperature (estimated with phase contrast microscopy); +, microscopic aggregates of less than ten cells; ++, macroscopic aggregates; open circles, at *p*H 6.5, closed circles, at *p*H 7.1; solid line, control (no PGE_1 added); dotted line, experimental [with addition of PGE_1 (60 ng/ml)].

The control sample (no PGE_1 added) showed visible aggregates. Low concentrations of PGE_1 ($\leqq$ 5 ng/ml) did not prevent microscopic aggregation, but higher concentrations of PGE_1 produced a smooth suspension of platelets. Microscopically, the shape of the free cells was flat in concentrations of PGE_1 greater than or equal to 10 ng/ml. These PC were then stored for 18 hours at room temperature (22°C). During storage, a gradual sedimentation of platelets occurred; however, when the PC's were shaken, a dispersion was observed in those with high PGE_1 concentrations ($\geqq$ 10 ng/ml). Since PGE_1 accelerates the dispersion of platelet clumps induced by ADP (*2*), we also studied the effects of adding PGE_1 immediately before suspension. When this method was used and the PC's were shaken, control samples showed only slight dispersion, even with 60 ng of PGE_1 per milliliter of PRP. Further aggregation during overnight storage was prevented, however. Acidic PRP (brought to *p*H 6.5 with excess ACD solution before centrifugation) showed far less aggregation when resuspended, even without PGE_1, although sphering of individual cells occurred rapidly.

The change in shape of platelets in PRP at *p*H 7.1 and *p*H 6.5 (made with excess ACD solution) was studied in sterile plastic test tubes with PGE_1 (60 ng/ml) or without PGE_1. Test tubes were kept in the vertical position at room temperature (22°C) for 0 to 3 days. Individual test tubes were inverted gently before examination to suspend the platelet sediment. Control samples (those without the addition of PGE_1) yielded massive aggregates at both *p*H values after 2 to 3 days of storage and subsequent suspension. PGE_1 again prevented the macroscopic aggregation and made suspension possible; however, a few fine aggregates were observed microscopically (Fig. 1). After 1 to 2 days of storage at acidic or neutral *p*H, most platelets became spherical when subsequently suspended. Acidic conditions were more destructive to cells than were neutral *p*H environments. The PGE_1 (60 ng per milliliter of PRP) slowed the course of cell sphering during storage at acidic or neutral *p*H. After storage for 3 days, more than 60 percent of the free cells were flat at both *p*H values, in spite of microscopic aggregation.

Cold-induced sphering of platelets is reversible during the first few hours (*6*); at low temperature, platelet cells may become sticky and, consequently,

suspend poorly. Also, the cells may have a shorter life-span. At neutral *p*H, PRP was chilled to 4°C and then warmed to 37°C without stirring. This procedure caused sphering, but the flat shape was restored on immediate warming. No aggregates were seen when the chilling-warming cycle occurred within 30 minutes; however, when the warming occurred after 60 minutes, visible aggregates were observed during continuous stirring. Rapid chilling did not induce aggregation even with stirring, in spite of the sphering of individual cells. Thus, aggregation takes place during the process of shape change induced by warming (*7*).

The effects of long-term storage at 4°C was studied with PRP (*p*H 7.1). The PRP was prepared at room temperature and divided into 6-ml portions. The test tubes were rotated (1 rev/2 min) to prevent platelet sedimentation. Aggregation induced by cold storage and warming was measured quantitatively (a process called aggregatometry) (*3*, *8*) at 1 hour, and during 1 to 4 days of storage.

In control samples, the amount of aggregation (maximum change in absorbancy at 600 nm) increased rapidly during the first day of storage. On the other hand, the sample containing PGE_1 (20 ng/ml, added before cooling) showed markedly less aggregation than the controls did before stirring started on the second day, and, although the amount of aggregation increased with time, it was always less than that of the control (Fig. 2). Under these conditions, PGE_1 (20 ng per milliliter of PRP) improved the ability of platelets to resume the flat shape to some extent. After only 3 hours of cold storage, samples were warmed without shaking at 37°C (water bath). The sphering of platelets in samples where no PGE_1 had been added was already irreversible at this time. In the samples containing PGE_1 though, the cells resumed their flat shape within 1 hour of warming. However, the change in the shape of the cell in the PGE_1 sample also became irreversible after 1-day cold storage.

Platelet aggregation takes place also in whole blood. Thus we confirmed the observation (*9*) that PGE_1 prevents ADP-induced aggregation in whole blood. Therefore, the effect of PGE_1 on platelet aggregation of blood during cold storage was examined. A mixture of ACD and whole blood was divided into portions and stored without shaking in sterile plastic test tubes at 4°C.

Table 1. Effect of PGE_1 on harvesting of platelets from PRP. The appearance of platelet pellets was examined after centrifugation of PRP (1500*g* for 15 minutes). Platelet aggregates and platelet shape were determined after suspension in one-sixth original volume of plasma. ++, Macroscopic aggregates; +, microscopic aggregates (less than ten cells).

PGE_1 (ng/ml)	Platelet pellet	Suspension aggregation	Platelet shape
0	Spread, filmlike	(++)	Spherical
1	Spread, filmlike	(+)	Spherical
2	Spread, filmlike	(+)	Spherical
5	Smooth, round	(+)	Flat, spherical
10	Smooth, round	No aggregation	Flat
20	Smooth, round	No aggregation	Flat
60	Smooth, round	No aggregation	Flat

The screen filtration rate (SFR) (*10*) was measured before storage (day 0), and at 1 and 5 days' storage time (day 1 and day 5). The stored samples were then brought to room temperature with as little shaking as possible. After storage, the control blood sample (without PGE_1) had an extremely low filtration rate under these conditions. When PGE_1 (20 ng per milliliter of whole blood) was added before storage, the aggregation was reduced; however, after 5 days, the aggregates in the control samples were not dispersed by treatment with PGE_1 (up to 60 ng/ml) and gentle shaking for 10 minutes at room temperature. The SFR of the experimental samples at days 0, 1, and 5 were 1.80, 1.75, and 1.45, respectively. The SFR of the control samples at days 0, 1, and 5 were 1.75, 1.55, and 0.25, respectively.

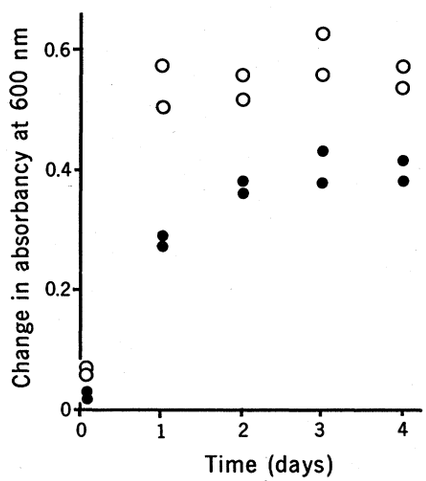

Fig. 2. Warming-induced aggregation after cold storage and effect of PGE_1. Samples of PRP were stored in 6-ml portions at 4°C on a rotator. After storage (at 1 hour and at 1 to 4 days), PRP was transferred directly into the aggregometer chamber which was maintained at 37°C. Maximum absorbancy change from original absorbance of PRP was taken as an index of aggregation. Measurements were taken in duplicate. Open circles, controls (no PGE_1 added); closed circles, experimental [PGE_1 (20 ng/ml) added before storage].

The decreased effectiveness of platelet concentrates in therapy when compared with the effectiveness of PRP seems to be due to aggregation during preparation of the PC (*11*). When PGE_1 was added at low concentrations ($2.8 \times 10^{-8}M$), it prevented the aggregation and change of shape in PRP that was centrifuged at neutral *p*H in plastic test tubes. Since the material of the container may affect platelet behavior, these results cannot be applied directly when blood bags are used. Nevertheless, the preservation of the normal shape of platelets by PGE_1 indicates an improvement in harvesting of the cells. A concentration of $10^{-7}M$ PGE_1 also slowed the time course of undesirable shape change during 3 days' storage of the blood at room temperature. During cold storage, the effect of PGE_1 was less than that at room temperature; however, the aggregation occurring during the cooling-warming cycle was significantly reduced during 4-day storage at 4°C, and the recovery of the flat shape from cold-induced sphering was improved to some extent. The effect of PGE_1 on cold-induced aggregation was also seen in mixtures of ACD and whole blood.

These results, therefore, indicate a use of PGE_1 in obtaining platelets for transfusion; however the platelets treated with PGE_1 may be resistant to aggregation in the body of the recipient. Using rat PRP, we observed a transient effect of PGE_1 on reduction of ADP-induced aggregation in vitro (*3*). It has also been suggested (*12*) that the PGE_1 effect on platelet behavior in vivo is reversible.

The amount of PGE_1 infused during platelet transfusion must be considered. Since platelets do not take up PGE_1 from plasma (*13*), the PGE_1 remaining in PC will be proportional to the volume of the plasma used for suspension.

In other words, if 10 ng of PGE_1 per milliliter of plasma is used in 250 ml of PRP, only 0.25 μg, a negligible amount, will be transfused into the recipient, where it is known to be rapidly metabolized (*14*).

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17 December 1971

Prostaglandin E_1 in Preparation and Storage of Platelet Concentrates

Abstract. *The addition of prostaglandin E_1 to blood collection bags improved platelet harvesting; platelets were easily suspended immediately after centrifugation. The treatment with prostaglandin E_1 did not affect the survival time of these platelets after infusion into a recipient.*

At the recommendation of Shio and Ramwell (*1*), we studied the effects of prostaglandin E_1 (PGE_1) in the preparation of and short-term preservation of platelets.

One milligram of PGE_1 (*2*) was dissolved in 0.2 ml of ethanol and diluted with isotonic saline to 20 ml (final concentration, 50 ng/μl). The solution was sterilized by filtration through a 0.2-μm filter (Millipore) and stored in a sterile container at 4°C until use. Its potency was checked periodically by testing its ability to inhibit adenosine diphosphate (ADP)–induced aggregation of fresh normal platelets. Platelet morphology was studied by phase microscopy of wet platelet suspensions and by electron microscopy (*3*) of platelets fixed with glutaraldehyde and osmium tetroxide. The amount of platelet aggregation was determined with an aggregometer (Chrono-Log) and platelet viability in vivo was determined by survival studies with ^{51}Cr (*4*). Blood was collected in Fenwal triple packs (FP-297). Platelet-rich plasma (PRP) was prepared by centrifuging whole blood at 1500*g* for 6 minutes. Plasma was then transferred to a second bag and centrifuged at 4000*g* for 10 minutes to compact the platelets; all but 30 ml of plasma was expressed into the third pack. The platelet concentrate was suspended and stored on a rotator which maintained a state of gentle agitation (*5*).

In 24 studies in which PGE_1 (4 to 200 μg per bag) was added to either whole blood or PRP, clumping of platelets was totally prevented in concentrates that were prepared either at room temperature or at 4°C, and either with acid, citrate, and dextrose (ACD) or with citrate, phosphate, and dextrose (CPD) anticoagulants (Fig. 1, a and b). Concentrates thus prepared could be suspended with only a minimum of manipulation immediately after rapid centrifugation. The smallest quantity of PGE_1 used (12 ng of PGE_1 per milliliter of plasma) was about one-fourth the amount needed to produce 50 percent inhibition of aggregation (induced by $2 \times 10^{-6}M$ ADP) of fresh platelets.

Platelet viability and function were

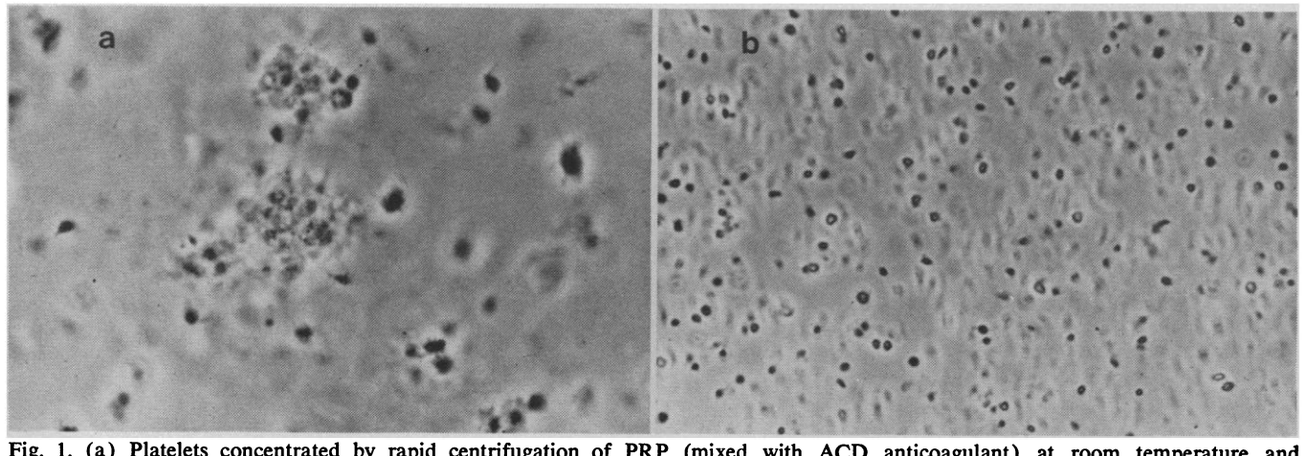

Fig. 1. (a) Platelets concentrated by rapid centrifugation of PRP (mixed with ACD anticoagulant) at room temperature and then immediately suspended. Many clumps of platelets were present which did not disperse completely even after 2 days of gentle agitation at room temperature. About 50 percent of the total platelets were contained in the aggregates. At 5°C, or when CPD anticoagulant was used, platelet clumping was even more pronounced (× 980). (b) Platelets prepared as in (a) but with the addition of 8 μg of PGE_1 to PRP before rapid centrifugation (final PGE_1 concentration, 24 ng/ml). A smooth suspension of platelets was obtained within 15 to 30 seconds (× 400).