Effects of gelatin-based resuscitation fluids on platelet aggregation

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Summary
Fluid resuscitation aims to maintain intravascular volume without significant effects on haemostasis. Several different types of i.v. fluid are available for use in a patient who has suffered trauma, but there is evidence that some resuscitation fluids may affect primary haemostasis. We have compared the effects of two resuscitation fluids, Haemaccel and Gelofusine, on platelet aggregation in vitro. These resuscitation fluids are both based on gelatin but Haemaccel contains a high concentration of Ca\(^{2+}\) whereas Gelofusine does not. Their effects on platelet aggregation in whole blood, induced by a range of different agents, were determined using a platelet-counting technique. Both Haemaccel and Gelofusine prevented platelet aggregation induced by ristocetin (P < 0.05, Mann–Whitney). In addition, Haemaccel proved to be a potent inhibitor of the platelet aggregation that occurred in response to all of the other agonists investigated: adenosine diphosphate, platelet-activating factor, collagen, a thromboxane A\(_2\) mimetic (U46619) and epinephrine. The additional inhibitory effects of Haemaccel were largely, but not completely, attributable to its high Ca\(^{2+}\) content. Inhibition of platelet aggregation by ristocetin may indicate a mechanism by which Haemaccel or Gelofusine may contribute to impaired haemostasis. The presence in Haemaccel of high concentrations of Ca\(^{2+}\), which is largely responsible for inhibition of the aggregation induced by other agents, may provide an additional means by which haemostasis could be impaired. (Br. J. Anaesth. 1998; 81: 198–202)

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Treatment of hypovolaemic shock requires immediate restoration of the circulating volume by rapid infusion of i.v. fluid to restore and sustain blood volume, cardiac output, stroke volume, blood pressure, urinary output and oxygen delivery. Some resuscitation fluids contain the colloid gelatin; some also contain Ca\(^{2+}\), which is intended to correct the decrease in ionized calcium that follows traumatic hypovolaemia.\(^1\) The aim of adding calcium is to improve cardiac output by increasing and maintaining the force of contraction and also by increasing peripheral vascular tone.\(^2\) The amount of calcium in the fluids is variable, and one gelatin-based solution, Haemaccel, contains a high concentration, 6.25 mM. Gelofusine is a gelatin-containing solution that does not contain Ca\(^{2+}\).

There is concern that Haemaccel may interfere with primary haemostasis. In a previous study it was noticed that Haemaccel infusion to traumatized patients was associated with an increased bleeding time and pronounced blood loss.\(^3\) An increased bleeding time in animals receiving Haemaccel has been documented,\(^4\) and this was associated with significantly reduced adenosine-diphosphate-induced platelet aggregation in platelet-rich plasma derived from these animals. Haemaccel infusion to trauma patients can lead to hypercalcaemia, because of its high Ca\(^{2+}\) content.\(^5\) It is also known that increasing the concentration of Ca\(^{2+}\) in blood plasma \textit{in vitro} can impair the platelet aggregation that is a determinant of primary haemostasis.\(^6\)\(^7\) It was therefore speculated that impaired haemostasis might be a consequence of the high concentration of Ca\(^{2+}\) in Haemaccel.\(^5\) In the present study we have investigated the effects of Haemaccel and Gelofusine on the platelet aggregation induced by several different agonists. The agonists chosen were adenosine diphosphate (ADP), platelet-activating factor (PAF), collagen, a thromboxane A\(_2\) mimetic (U46619), epinephrine and ristocetin. The effects of Haemaccel and Gelofusine on platelet aggregation were determined in whole blood using a platelet-counting technique.\(^5\) The fluids were added to achieve a final concentration of 40% in blood, equivalent to an infusion of about 2 litres into a 70 kg person. As hypercalcaemia is also known to affect the platelet aggregation induced by some agents,\(^8\)\(^9\) we also compared the effects of Haemaccel and Gelofusine with those of Ca\(^{2+}\) alone. We also carried but experiments in which Ca\(^{2+}\) had been added to Gelofusine. The whole blood was prepared using hirudin as the anticoagulant rather than the conventional citrate. This is because hirudin, unlike citrate, does not reduce the physiological level of ionized calcium in plasma, and additional Ca\(^{2+}\) can be added without affecting its ability to act as an anticoagulant.

Materials and methods

BLOOD COLLECTION

Venous blood was obtained from normal subjects who denied taking any medication in the 2 weeks before sampling. Blood was taken into a polypropylene
syringe using a 19-gauge needle, and dispensed into a polypropylene tube containing Revasc (recombinant hirudin, final concentration 50 g ml\(^{-1}\)). The sample was routinely kept for 30 min at 37°C before testing.

Haemaccel (3.5% w/v polygeline) was purchased from Behring, and Gelofusine (4% w/v succinylated gelatin) from B. Braun Medical. Calcium chloride (CaCl\(_2\cdot6\)H\(_2\)O) was obtained from Fisons, and sodium chloride (0.9% w/v) from Baxter Health Care. All platelet agonists were obtained from Sigma Chemicals except collagen (Hormon-Chemie) and ristocetin A sulphate (AL). Revasc (recombinant hirudin) was a gift from Ciba Geigy. The platelet fixative solution contained 150 mM NaCl, 4.6 mM Na\(_2\)EDTA, 4.5 mM Na\(_2\)HPO\(_4\), 1.6 mM KH\(_2\)PO\(_4\) and 0.16% w/v formaldehyde, pH 7.4.

**PLATELET AGGREGATION**

Platelet aggregation in whole blood was measured using the platelet counting technique described by Fox and colleagues.\(^8\) A blood sample (292 \(\mu\)l) was mixed with 200 \(\mu\)l of the test solution (Haemaccel, Gelofusine or isotonic saline, previously warmed to 37°C) in a small polystyrene tube. After removing a 12 \(\mu\)l aliquot to 30 litre of fixative solution, 20 \(\mu\)l of a solution of an aggregating agent was added, and the sample placed in a water bath (37°C) and stirred (1000 r.p.m.). At appropriate time-points, 12 \(\mu\)l aliquots were removed from the sample and fixed as described. The number of single platelets in the fixed samples was measured using an Ultra-Flo 100 Whole Blood Platelet Counter. Platelet aggregation was expressed as the percentage fall in the number of single platelets compared with the starting count. The results are expressed as medians and interquartile ranges; in each case \(n=7\), using blood from different volunteers. When the effects on platelet aggregation of additional Ca\(^{2+}\) were investigated, 10 \(\mu\)l of an isotonic solution of 100 mM CaCl\(_2\) was added to saline (190 \(\mu\)l) or Gelofusine (190 \(\mu\)l) before adding the blood and aggregating agent. In the case of saline and Gelofusine this resulted in a concentration of ionized calcium in the blood of 2.6 mM and 2.5 mM respectively. This compared with a concentration of 2.8 mM when Haemaccel was used alone, and with 0.7 mM and 0.6 mM respectively when saline and Gelofusine were used alone. These measurements were performed using an AVL 988–4 analyser.

Statistical analysis was performed using a Mann–Whitney test. \(P<0.05\) was considered statistically significant.

**Results**

The effects of the various fluids on the platelet aggregation induced by a range of aggregating agents are shown in figs 1–6. The agonists used were ristocetin (1 mg ml\(^{-1}\), fig. 1) adenosine diphosphate (ADP, 0.3 \(\mu\)M, fig. 2), collagen (1 \(\mu\)g ml\(^{-1}\), fig. 3), platelet-activating factor (PAF, 0.3 \(\mu\)M, fig. 4), the thromboxane A\(_2\) mimetic U46619 (0.3 \(\mu\)M, fig. 5) and epinephrine (100 \(\mu\)M, fig. 6). The timepoint after stimulation at which aggregation was measured was dependent on the agonist used. With ADP and PAF, aggregation was determined at 30 s (figs 2a and 4a) and 2 min (fig. 2b and 4b), as aggregation to low con-
centrations of these agonists tends to be reversible. The response to the remaining agonists was measured when aggregation would normally be complete (collagen and U46619, 2 min; epinephrine and ristocetin, 4 min).

In the presence of Haemaccel or Gelofusine the platelet aggregation induced by ristocetin was totally prevented (fig. 1). Adding Ca\(^{2+}\) to the blood also inhibited the aggregation but this inhibition was only partial. The abolition of platelet aggregation in response to ristocetin by Gelofusine was not affected by increasing the Ca\(^{2+}\) concentration.

Haemaccel significantly inhibited the platelet aggregation that occurred in response to all of the other agonists (figs 2–6). Haemaccel also appeared to make the aggregation in response to ADP and PAF reversible.

*P<0.05 compared with saline, **P<0.05 compared with saline + Ca\(^{2+}\)).
more reversible (figs 2 and 4). Adding CaCl₂ to the whole blood also resulted in a significant inhibition of platelet aggregation in response to all of the agonists studied. The inhibition seen with Haemaccel was always significantly greater than that seen with CaCl₂ alone, the only exception being when collagen was used as the agonist (fig. 3).

Except when ristocetin was used as the agonist, Gelofusine had only a slight effect on platelet aggregation. Significant inhibition of aggregation was seen in response to ADP at 30 s (fig. 2A), but this effect was lost by 2 min (fig. 2B). Gelofusine appeared to slightly potentiate the aggregation brought about by epinephrine (fig. 6).

Generally, there was less platelet aggregation with Gelofusine containing added CaCl₂ than with Gelofusine alone. This was the case in response to ADP (fig. 2), collagen (fig. 3), PAF (at 30 s, fig. 4A) and epinephrine (fig. 6). The results obtained for U44619 (fig. 5), and PAF (2 min, fig. 3b) show a trend towards inhibition but this did not reach statistical significance. The degree of inhibition seen with Gelofusine containing CaCl₂ was significantly greater than that observed with CaCl₂ alone for ADP (30 s, fig. 2A) and PAF (30 s, fig. 4A). Overall, the effect of Ca²⁺ on platelet aggregation was similar whether it was used alone or in combination with Gelofusine.

Discussion

One of the platelet agonists chosen for this investigation was ristocetin, which induces platelet aggregation through a mechanism involving von Willebrand factor and its receptor glycoprotein Ib on the platelet surface. Von Willebrand factor is also important in mediating adhesion of platelets to damaged vascular tissue, by forming a link between glycoprotein Ib and the damaged tissue. The aggregation induced in whole blood by this agent was prevented by Haemaccel and Gelofusine. Inhibition of ristocetin-induced aggregation by Haemaccel confirms earlier observations and extends the finding to the aggregation that occurs in whole blood as well as that in platelet-rich plasma. Platelets from patients with von Willebrand disease do not respond to ristocetin and this lack of response is associated with markedly reduced platelet adhesion to damaged vascular tissue and increased bleeding. There are indications that other, non-Ca²⁺-containing, gelatin resuscitation fluids, as well as Haemaccel, may impair haemostasis. In a retrospective analysis of patients who had received oxypolygelatin before cardiopulmonary bypass, use of high doses of the fluid was associated with increased postoperative blood loss. A recent prospective study, in which Gelofusine was infused into healthy volunteers, reported a significant increase in bleeding time. Interference with haemostatic mechanisms involving von Willebrand factor could therefore be one way in which Haemaccel and Gelofusine, and presumably other gelatin-containing fluids, could increase bleeding and blood loss. An in vitro test of the effects of Haemaccel or Gelofusine on “clot quality” revealed that the presence of either agent led to production of clots that were more friable than normal clots.

The other platelet agonists used in this investigation were ADP, PAF, collagen, U46619 and epinephrine. All these agents activate glycoprotein IIb/IIIa receptors on the platelet surface, leading to fibrinogen binding and platelet aggregation—another mechanism through which platelets contribute to primary haemostasis. Haemaccel inhibited the aggregation that occurred in response to all these agonists and increased the reversibility of the response. Gelofusine did not produce the same effect. The effect of Gelofusine on the aggregation induced by all agents other than ristocetin was slight, and although there was significant inhibition of the early aggregation induced by ADP this was matched by a slight potentiation of the aggregation induced by epinephrine. The different results obtained for the two gelatin preparations appeared to be largely, but not entirely, due to the presence of a high concentration of Ca²⁺ in Haemaccel.

There are reports that gelatin-containing resuscitation fluids can inhibit platelet aggregation but, until now, there have been no direct comparative evaluations of the antiplatelet effects of Ca²⁺-containing vs non-Ca²⁺-containing preparations. Infusion of Gelofusine during cardiopulmonary bypass in man can lead to a reduced platelet response to Gelofusine, but it is not known how the fluids affect the different pathways leading to platelet aggregation.

Stibbe and colleagues studied the effects of several gelatin plasma substitutes on the platelet aggregation in platelet-rich plasma induced by ADP, collagen and epinephrine. Haemaccel always inhibited the aggregation induced by all these agents whereas aggregation was enhanced by some other gelatin preparations. Our own experiments extend these observations to whole blood, emphasize that different results may be obtained with different types of gelatin colloids, and demonstrate that the presence of high concentrations of Ca²⁺ in Haemaccel increases the inhibition of aggregation seen using a range of platelet agonists. The inhibitory effects of Haemaccel on platelet aggregation induced by agents that act through glycoprotein Iib/IIIa and fibrinogen seem largely to be attributable to its high Ca²⁺ content, but some of the inhibitory effect may lie with its gelatin component, polygeline, which differs from succinylated gelatin, the gelatin component of Gelofusine.

As Haemaccel inhibits the platelet aggregation induced by a range of agonists in addition to ristocetin, its administration could have an additional detrimental effect on primary haemostasis. On the other hand, agents that inhibit platelet aggregation can reduce the tendency to thrombosis, and in an animal model, platelet thrombus formation was significantly impaired after Haemaccel infusion.

Agents that specifically block platelet aggregation by interfering with the ability of fibrinogen to bind to glycoprotein Iib/IIIa receptors are being developed for use as antithrombotic agents in man.

We believe that further studies of gelatin-containing colloids are warranted to determine their potential for increasing bleeding after large-volume blood replacement. It might be important to balance this effect against the perceived need to restore calcium lost as a result of hypovolaemic traumatic shock. There may also be a case for further study of circumstances in which the inhibitory effects of such fluids on platelet aggregation could be beneficial. For example, an i.v. fluid with antiaggregatory properties might benefit trauma patients who have underperfused hypostatic
capillary beds, by promoting blood flow and enhancing organ perfusion and tissue oxygenation. Similarly, inhibition of platelet aggregation could provide a degree of protection from thrombosis. However, whether these possible benefits would outweigh the potential for an increase in bleeding in the trauma patient remains to be proven.

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References