Hemostatic effects of three colloid plasma substitutes for priming solution in cardiopulmonary bypass

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Abstract

Objective: To evaluate the effects on hemostasis of three different plasma substitutes with special reference to a newly developed hydroxyethyl starch used as priming solution in an extracorporeal circuit as well as peri- and postoperative infusion fluid, we studied 36 patients randomly assigned to one of three groups, undergoing coronary artery bypass grafting. Methods: The compositions of the priming solutions were: 2.5% hydroxyethyl starch; 3% gelatin; and 4% human albumin. Platelet function tests and clotting assays were performed on blood samples collected during and after cardiopulmonary bypass. Results: We found that plasma von Willebrand Factor remained higher in the human albumin group. Hydroxyethyl starch preserved platelet agglutination as well as human albumin, whereas platelet aggregation induced by adenosine 5′-di phosphate (ADP) proved to be similarly affected during cardiopulmonary bypass in the three study groups. Prolongation of the in vitro bleeding constant during the bypass period and subsequent partial recovery showed an affected platelet function in all groups during cardiopulmonary bypass. The clotting times, activated partial thromboplastin time and prothrombin time were similar in the three groups. Bloodloss, peri- and postoperatively, showed also no differences. Hydroxyethyl starch appeared most cost-effective as priming solution in an extracorporeal circuit. Conclusions: We conclude that, with human albumin the golden standard, 2.5% hydroxyethyl starch is a suitable colloid plasma substitute to be used as priming solution in an extracorporeal circuit as well as peri- and postoperative infusion fluid, reasonably well maintaining hemostasis. © 1997 Elsevier Science B.V.

Keywords: Cardiopulmonary bypass; Hemostasis; Plasma substitute

1. Introduction

During heart surgery in adults, approximately 2 l of isotonic fluid is required to fill the extracorporeal circuit. Often, the priming solution which is used as a plasma substitute consists of crystalloids. However, colloid osmotic pressure and fluid balance is mainly negatively referred to with respect to pure crystalloid priming solutions [8,17]. Therefore, in order to preserve the colloid osmotic pressure a colloid priming solution is needed. Beside the natural plasma colloid human albumin, artificial colloids are also extensively used for this purpose.

An important reason for use of artificial colloids is their low cost as compared to the natural colloid human albumin. However, in contrast to human albumin, artificial colloids might have some negative effects on hemostasis. This is reflected by a disturbed hematological and hemostatic profile as evidenced by reported abnormalities of platelet number and function, clotting times, fibrinolysis, and coagulation factors (fibrinogen, Factor V, VII, VIII and IX) [10,12,14,17,21,22]. These negative effects of artificial colloids might be most apparent when high concentrations are used [12], as in priming solutions of extracorporeal circuits.
In order to study the influence on hemostasis of two different types of artificial plasma substitutes versus human albumin as priming solution in an extracorporeal circuit as well as infusion fluid, we studied three groups of patients who underwent a coronary bypass operation. The hemostatic functions of their blood components were measured by sensitive hematological and biochemical methods.

2. Patients and methods

2.1. Patients

This prospective study, approved by the Medical Ethics Committee, was conducted on 36 patients, who underwent an elective coronary bypass operation and were randomly assigned for cardiopulmonary bypass with one of the three priming solutions.

The patients were <75 years of age, had a body weight >65 kg, underwent a cardiopulmonary bypass time >30 min and had signed a written consent. Exclusion criteria were presence of severe heart failure, renal or liver dysfunction, bleeding diathesis, diabetes mellitus, and the use of platelet inhibiting drugs within five days before the operation.

Induction and maintenance of anaesthesia, surgical techniques and cardiopulmonary bypass procedures including anticoagulation with heparin and its neutralization with protamine, were performed in a standardized fashion [3].

The extracorporeal circuit consisted of an integrated microporous plate membrane oxygenator (Cobe-Excell, Cobe, CO) and polyvinyl chloride tubing with silicon rubber tubing in the pump head. The volume of the circuit was 2 l and the three priming solution compositions were:

1. Human albumin (20%) 400 ml (CLB, Red Cross, Amsterdam) + 1600 ml Ringer’s lactate (human albumin final concentration = 4%) (HA group). The concentration of this natural colloid was chosen to obtain a physiological concentration. Therefore this human albumin group was considered as reference.

2. Gelatin 2000 ml (Geloplasma (3%), Rhône-Poulenc, Amstelveen, Netherlands). The dose limitation was 3000 ml/24 h (GEL group). This regimen was based on the routine practice in our hospital.

3. Hydroxyethyl starch 500 ml (HAES-steril 10%, Fresenius AG, Oberursel, Germany) + 1500 ml Ringer’s lactate (hydroxyethyl starch final concentration = 2.5%). The dose limitation was 2 mg/kg·24 h (20 ml/kg·24 h) (HES group). For practical reasons the volume was distributed to allow the use of each 500 ml of hydroxyethyl starch as peri- and postoperative plasma substitute and as priming solution.

After reaching these defined study colloid dose limits, another colloid as plasma substitute was free to infuse peri- and postoperatively.

To all priming solutions 1500 I.U. heparin was added.

2.2. Methods

During the operative day and on the first postoperative day nine blood samples were taken for hematological and biochemical study. Sample schedule: after anaesthesia induction (baseline), just before cardiopulmonary bypass but after heparin, 5 min after the start of cardiopulmonary bypass, 30 min after the start of cardiopulmonary bypass, just before the end of cardiopulmonary bypass, 30 min after the end of cardiopulmonary bypass and protamine administration, and on the first postoperative day in the intensive care unit (day 1). Blood samples were obtained from the radial artery catheter and were mixed with 3.06% citrate or with 0.1 mM ethylenediaminetetraacetic acid (EDTA) solution, with a ratio of 9:1. The samples were put on ice during storage.

Platelet agglutination and aggregation (Chrono-Log, Haverton) was performed by means of an (electrical) impedance measurement in citrated whole blood samples [4,9]. Platelet agglutination was induced by the agonist ristocetin (Sigma, St. Louis, MO), final concentration 0.75 mg/ml. Platelet aggregation was induced by the agonist ADP (Sigma, St. Louis, MO), final concentration 5 μM. All blood samples were 50% diluted with saline and agonist prior to aggregation. This impedance method to induce platelet agglutination and aggregation was started by adding the agonist to the sample, after a stabilisation period with adhesion of platelets onto the electrodes. The increase of impedance is proportional to the platelets capacity to agglutinate and aggregate. This time related change in agglutination and aggregation impedance was recorded on paper from which the angle of the curve was calculated.

The in vitro bleeding test, another evaluation of platelet function, was performed using a platelet function analyser (Thrombostat 4000, von der Goltz, Seeon, Germany). In the Thrombostat citrated whole blood is sucked through a 200 μm teflon capillary and successively through a 150 μm hole in a collagen type I coated filter to which ADP (40 nmol) as agonist was applied. Platelets adhere and aggregate within the filter causing a reduction in blood flow until the filter becomes obstructed. From the in vitro bleeding test the initial blood flow and the bleeding volume were measured, whereas the bleeding constant was calculated by the Thrombostat built-in computer program. The bleeding constant was expressed as the total collected bleeding volume divided by the initial blood flow [11,25], which corrects for hemodilution and thus expresses platelet function best.
The remaining blood was centrifuged at 1100 × g for 12 min to obtain platelet poor plasma which was stored at −20°C until further determinations of biochemical assays and clotting times.

Factor VIII—von Willebrand Factor complex (plasma) concentration was determined with a fluoroimmuno assay. First a plate was coated with rabbit anti human Factor VIII (Behring, Marburg, Germany) and secondly a diluted citrated platelet poor plasma sample was added. After incubation at room temperature and washing, the third step of incubation with rabbit anti human von Willebrand Factor (Dako, Glostrup, Denmark) labeled with europium–diethyleneetriaminetetraacetic acid (DTTA) medium (Pharmacia, Uppsala, Sweden) was performed. After incubation the unbound antibody was washed off from the plate, enhancement fluid (Pharmacia, Uppsala, Sweden) was finally added to disconnect the europium–DTTA and measuring the fluorescence (LKB Wallac, Turku, Finland). The complex was expressed as percentage of the average baseline concentration.

The activated partial thromboplastin time and prothrombin time were performed in citrated plasma samples. To determine the activated partial thromboplastin time, platelet poor plasma and rabbit brain cephalin (Sigma, St. Louis, MO) suspended in ellagic acid was incubated for 3 min at 37°C; after adding CaCl₂ (final concentration 10 mM), the clotting time was recorded in s (Coagulometer, Amelung, Lieme, Germany).

The prothrombin time was determined by means of rabbit thromboplastin (Kordia, Leiden, Netherlands) and CaCl₂ (final concentration 10 mM) added to platelet poor plasma.

Blood loss was registered until the first postoperative day. Blood loss included the total amount of vacuum suction and swab fluid loss perioperatively and wound drainage blood collected in the intensive care unit.

2.3. Statistics

Before data analysis all the individual sample points were tested and found normally distributed according to the Kolmogorov-Smirnov goodness-of-fit test. To detect possible differences in effect of each priming solution on a dependent variable, one way analysis of variance (ANOVA) was used to compare groups. If differences between the three groups were significant ($P < 0.05$), post hoc multiple comparisons were performed to quantify any differences among groups using the Tukey HSD test with a level of significance $P < 0.05$. A Bonferroni correction was made for multiple testing. The variables are expressed as mean ± S.E. Correction for hemodilution was only made for the Factor VIII–von Willebrand Factor complex concentration.

3. Results

Between the three groups no significant differences were measured with regard to age, sex, weight, body surface area, cardiopulmonary bypass and aortic cross clamp time, number and origin of grafts. Three patients were considered as drop-outs from the study because the (post)operatively administered colloids caused violation of the study protocol. Therefore a group of 12, 11 and 10 valid and evaluable patients remained in the HA, GEL and HES group, respectively.

3.1. Ristocetin induced platelet agglutination

After heparinisation a decline of ristocetin induced platelet agglutination was observed (Fig. 1). After the dilutional effect at the onset of cardiopulmonary bypass, in the HA and HES group the platelet agglutination capacity recovered. On the first postoperative day the ristocetin induced agglutination was remarkably reduced in all groups as compared to the initial agglutination (mean reduction of 38%).

3.2. ADP induced platelet aggregation

ADP induced platelet aggregation reduced in all groups by half during cardiopulmonary bypass (Fig. 2). Protamine infusion had further negative repercussions on ADP aggregation in all groups. The capacity to aggregate restored in all groups postoperatively but remained about 39% affected compared to the initial sample.

Fig. 1. Ristocetin induced platelet agglutination by means of the whole blood impedance method. The 33 evaluable patients were assigned for cardiopulmonary bypass (CPB) with one of the three priming solutions: ■, Human albumin; △, gelatin; and ○, hydroxyethyl starch.
3.3. In vitro bleeding test

Immediately after the onset of cardiopulmonary bypass, the in vitro bleeding constant increased twice in all groups and recovered after neutralisation of heparin, in the HA group to near baseline (Fig. 3). In the intensive care unit a further recovery of the in vitro bleeding constant was observed only in the HA and HES group but not in the GEL group.

3.4. Factor VIII–von Willebrand Factor complex

Just after the start of cardiopulmonary bypass the circulating concentration of Factor VIII–von Willebrand Factor complex decreased (Fig. 4), however, this was more pronounced in the GEL and HES group than in the HA group although no significance was measured. During cardiopulmonary bypass a more pronounced and significant difference appeared in the HA versus GEL group (\( P = 0.002 \)). After protamine administration a further decrease was observed for all groups. Factor VIII–von Willebrand Factor complex concentration returned to baseline values at day 1.

3.5. aPTT, PT

The clotting time expressed as activated partial thromboplastin time in all groups prolonged after cardiopulmonary bypass to about 119% compared to pre cardiopulmonary bypass levels. The activated partial thromboplastin time almost returned to initial values at day 1 (+14%). The prothrombin time prolonged by about 88% in all groups and returned to near initial values at day 1 (+13%).

3.6. Blood loss

None of the artificial colloids, hydroxyethyl starch and gelatin had a statistically significant different effect on the amount of blood loss during operation or in the intensive care unit, as compared to the natural colloid albumin (Fig. 5).
4. Discussion

Preservation of a good hemostasis after cardiopulmonary bypass is a major concern in open heart surgery. Due to improved techniques and use of more biocompatible materials, hemostasis in routine cardiopulmonary bypass has been greatly improved.

A variety of priming solutions for extra corporeal circuits are used including human albumin as natural plasma colloid. Although human albumin is expensive, human albumin priming solutions are often used, amongst others because it has no adverse effects on hemostasis.

In the present paper the HA group maintained hemostasis for the investigated variables also reasonably well, indicated by a modestly reduced ristocetin induced aggregation at the end of bypass and in vitro bleeding constant recovery after cardiopulmonary bypass and preservation of Factor VIII–von Willebrand Factor complex concentration. On the contrary, the GEL group scored low overall on these variables, emphasized most clearly after protamine administration. Hydroxyethyl starch priming solution (HES group) appeared to be almost as good for the tested hemostatic variables as human albumin priming solution, and showed of all groups the lowest blood loss, albeit not statistically significant, possibly as a result of the limited patient numbers.

Hydroxyethyl starch was first clinically used in the 1960s, is a well established substitute in plasmapheresis and was described as an effective priming solution by 1975 [13]. It shares chemical similarities with dextrans. Both are hydrolysed polysaccharides, with different glycoside linking. Dextrans are dose limited since they cause platelet disaggregation by coating the platelet membranes and the endothelial wall, facilitate fibrinolysis [6] and also have a synergetic effect with heparin [1]. Due to the similarity, a dose limitation of 2 g/kg 24 h is also recommended for hydroxyethyl starch.

Hydroxyethyl starch seems not to be associated with excessive postoperative bleeding [19], which is also supported by the present study. Blood loss in the HES group was similar to the HA group, therefore hydroxyethyl starch can not be labeled as an antithrombotic agent like dextrans [1].

We used in our study a low molecular weight hydroxyethyl starch, a pentastarch with a mean molecular weight of 240 kD (bottom (10%–top (90%) fraction: >13–<780 kD) with a molar substitution ratio (the number of glucose molecules which have been replaced by hydroxyethyl groups) of 0.5 (range: 0.43–0.55). Its analog was first used as a priming solution in 1992 [14], and its behaviour could be quite different from the high molecular weight hydroxyethyl starch (hetastarch) [5,10,13,18,19,21,22]. Low molecular weight hydroxyethyl starch with a low degree of molar substitution ratio, can be faster hydrolysed and hence be more rapidly cleared by the kidneys, although the high molecular fraction may have prolonged tissue storage (4% remainder after 3 days, as shown in animal studies by the manufacturer). A comparative study between low and high molecular weight hydroxyethyl starch showed a tendency for higher postoperative bleeding in the high molecular weight HES group [12].

Gelatins have been clinically used since 1915 and have never been indicated to compromise hemostasis, so there is no dose limitation [16]. Only sludging has been described as a side effect of gelatins [16], but in a recent study evidence for platelet receptor function impairment is described in the presence of a gelatin priming solution [23]. This paper shows that gelatin, and to a lesser extent hydroxyethyl starch, can affect some variables of hemostasis and mainly the platelet adhesive function.

Von Willebrand Factor is responsible for platelet adhesion during (high) flow conditions in the vicinity of the injured subendothelial vessel wall. In vitro interaction of von Willebrand Factor with platelets is accomplished by the agonist ristocetin. In this paper a marked reduction of platelet agglutination was observed at the onset of cardiopulmonary bypass. This affected platelet response could be due to reduced functional plasma von Willebrand Factor fractions and/or to synergistically impaired platelet function [23].

Another platelet function test, performed by ADP stimulation, revealed remarkably reduced response in all groups after heparinisation and during the onset of cardiopulmonary bypass. However, no significant dif-

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Fig. 5. Blood loss included the total amount of suction and swab fluid loss perioperatively and fluid collected via drains in the intensive care unit (ICU). Blood loss was no different between groups during the total operating room (OR) and intensive care period. Black column, human albumin; hatched column, gelatin; and white column, hydroxyethyl starch.
ferences were observed between the three study groups. No human albumin coating effect [20] and subsequent platelet preservation by human albumin was manifest. Others [2] obtained data in disagreement with ours. They found with ADP induced stimulation a small increase of platelet aggregation during bypass and a small decrease after bypass, both with comparable hydroxyethyl starch and human albumin priming solutions like ours. But they used the optical density method in platelet rich plasma and worked with hemodilution corrected platelet numbers, whereas we used the electrical method in whole (diluted) blood. Under these sample measuring conditions, they found low dose gelatin priming solution (final concentration = 0.7%) even enhanced ADP induced aggregation.

Despite systemic heparinisation and citrated samples, platelets (3–4 μm) are capable of blocking the 150 μm capillary of the in vitro bleeding test measurement [24]. Although all patients had obvious increased in vitro bleeding constants during cardiopulmonary bypass, the HA and HES groups recovered after cardiopulmonary bypass and had in vitro bleeding constants of near baseline on the first postoperative day. Simultaneously the GEL group persisted in substantial prolonged in vitro bleeding constants. Because ADP induced aggregation was similarly affected in all the study groups, this in vitro bleeding constant implicates an impaired platelet adhesive function in the GEL group, in agreement with the lowest Factor VIII–von Willebrand Factor complex concentration and ristocetin induced platelet agglutination.

In vivo bleeding times, as determined by others, revealed no significant differences [12,14,15,17,22], indicating that in vivo both the platelet and the endothelial wall fractions of the von Willebrand Factor are of primary importance.

Although in all the study groups the Factor VIII–von Willebrand Factor complex concentration was reduced at the start of the cardiopulmonary bypass, a persistent negative effect in the perioperative period was only seen in the GEL group. Others [12] observed a significant reduction of Factor VIII and von Willebrand Factor, with high (5%) concentration hydroxyethyl starch (low and high molecular weight type) compared to Ringer’s acetate both used as priming solution. If the concentration hydroxyethyl starch (low molecular weight type) compared to human albumin and Ringer’s lactate, all used as cardiopulmonary bypass priming solution, was reduced to 3.75% no longer significant differences of Factor VIII existed [14].

Standardized clotting times like activated partial thromboplastin time and prothrombin time demonstrated no significant differences between the three study groups. Some [2,14] found effects on the activated partial thromboplastin time or the prothrombin time [10,19] by artificial colloids. These effects were not supported by others [7,13,18].

HA and HES groups had similar blood loss, in accordance with the literature [2,12–14]. In our study we restricted gelatin administration, since it might affect hemostasis, resulting in increased bloodloss [23].

In conclusion, our study revealed in the measured variables impaired in vitro hemostasis like reduced platelet agglutination, increased in vitro bleeding constant and reduced plasma Factor VIII–von Willebrand Factor complex concentration with all priming solutions.

In this paper hydroxyethyl starch maintained the studied hemostatic variables relatively well. However, clinical significance remains to be determined, amongst others because the data is restricted by limited patient numbers.

Hydroxyethyl starch as compared to gelatin and human albumin as a priming solution was in our set-up respectively a factor three and thirteen less expensive, therefore it is also a cost effective plasma substitute to be used as priming solution additive in cardiopulmonary bypass circuits as well as peri- and postoperative artificial colloid infusion fluid.

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