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AN EXPERIMENTAL STUDY ON THE EFFECT OF PLASMA EXPANDERS ON BLOOD COAGULABILITY

BY

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ABSTRACT

The effect of various plasma expanders on blood coagulation was studied using the thromboelastography (TEG). Solutions of 5, 10, 20, and 50% concentration of each expander (6% low molecular weight hydroxyethyl-starch (6% Hespander®)[*2], 3% dextran-40, 6% high molecular weight hydroxyethyl-starch (6 HES®)[*3], and 6% dextran-70) or solvent (lactated Ringer solution and normal saline))) in blood were prepared and their coagulability was examined by TEG.

In the low molecular weight plasma expanders (6% Hespander® and 3% dextran-40), in general, the coagulability decreased when the concentration was increased. In the high molecular weight plasma expanders (6 HES® and 6% dextran-70), the coagulability increased slightly at low and high concentrations and the coagulability was reduced. As a conclusion, at clinically used concentrations of less than 20% in blood, changes in the TEG of the plasma expanders are minimum and have no clinical significance.

INTRODUCTION

Recently, the amount of blood available for transfusion is becoming insufficient, and the possibility of post-transfusion hepatitis creates problems. New plasma expanders have been developed and are used in clinical practice. Commonly, plasma expander infusion is used for slight to moderate hemorrhage or an emergency infusion for massive hemorrhage in order to maintain circulating blood volume.

Plasma expanders increases plasma volume but they also cause blood coagulability disorders when large quantities are used.[1)]

There have been many studies on changes in individual blood coagulation factors after plasma expander infusion, but the changes in the whole process of blood coagulation, including the platelets and other coagulation factors, have not been studied. Moreover, coagulability disorders in clinical concentrations of plasma expanders have not been studied *in vitro*.

The thromboelastography (TEG) explores the whole blood coagulation process and provides objective values for each part of the coagulation and fibrinolysis processes.

METHODS

The following plasma expanders were studied.

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[*2] 6% Hespauder® (Morishita Seiyaku, Ôsaka) is the trade-mark of the low molecular weight hydroxyethyl starch (M.W. 30,000-40,000 and D.S. 0.50-0.55).

[*3] 6 HER® (Kyôrin Seiyaku, Tokyo) is the high molecular weight hydroxyethyl starch (M.W. 200,000 and D.S. 0.69-0.66).

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Table 1. Ingredients of the low and high molecular weight plasma expanders and control solutions (in 100 ml)

	Lactated Ringer	low molecular weight		Normal Saline	high molecular weight	
		6 % Hespander®	3 % Dextran 40		6 HES®	6 % Dextran 70
M.W.	—	40,000	40,000	—	200,000	70,000
$CaCl_2$	0.02 (g)	0.02 (g)	0.02 (g)	—	—	—
NaCl	0.4 (g)	0.4 (g)	0.6 (g)	0.9 (g)	0.9 (g)	0.9 (g)
$NaHCO_3$	0.31 (g)	0.224 (g)	0.31 (g)	—	—	—
KCl	0.03 (g)	0.03 (g)	0.03 (g)	—	—	—
Glucose	—	1.0 (g)	—	—	—	—
Hydroxy-ethyl-starch	—	6.0 (g)	—	—	6.0 (g)	—
Dextran	—	—	3.0 (g)	—	—	6.0 (g)

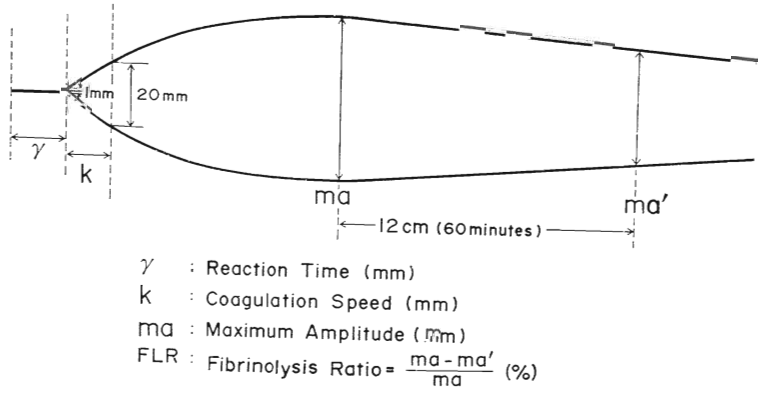

Fig. 1. Thromboelastogram and its components. Reaction time (γ), coagulation speed (k), maximum amplitude (ma), and fibrinolysis ratio (FLR).

As a low molecular weight solution, 6% low molecular weight hydroxyethyl-starch (6% Hespander®) in lactated Ringer solution and 3% dextran-40 in lactated Ringer solution (3% dextran-40) were used, and the control was the lactated Ringer solution (LRS). As high molecular weight solutions, 6% high molecular weight hydroxyethyl-starch in normal saline (6% HES®) and 6% dextran-70 in normal saline (6% dextran-70) were used, and the control was normal saline. The ingredients of these solutions are shown in Table 1.

Blood was collected directly from 26 healthy adult volunteers. The plasma expander or control solution was mixed with the blood in 10-m*l* silicone-coated syringes to make concentrations of 5, 10, 25, and 50%. The volume of plasma expander used clinically is 400–1,200 m*l*, which constitutes 10–30% of the circulating blood volume, and this concentration was simulated.

As shown in Fig. 1, reaction time (γ), coagulation speed (k), and maximum amplitude (ma) of the thromboelastogram (TEGm) were measured.

Table 2. Comparison of actual γ, k, and ma values x ± S.D. (mm)

	6% Hespander®			3% Dextran 40			L R S		
	γ	k	ma	γ	k	ma	γ	k	ma
5%	22.4±2.7	13.8±1.8	47.6±5.1	22.2±2.6	13.4±2.3	47.6±5.5	21.2±2.2	11.8±1.5	50.0±4.9
10%	21.6±3.1	13.6±3.1	45.4±7.1	21.0±1.6	14.2±2.2	46.0±5.5	19.8±2.9	12.0±2.0	49.6±5.1
20%	22.2±3.3	15.6±2.7	41.4±6.2	20.6±2.6	14.5±2.6	44.6±5.9	18.2±3.1	11.2±1.9	49.2±5.8
50%	22.0±3.0	28.6±9.3	28.6±7.0	20.0±4.0	19.0±4.7	34.6±6.8	15.6±2.2	12.2±1.6	42.2±4.9

	6 HES®			6% Dextran 70			Saline		
	γ	k	ma	γ	k	ma	γ	k	ma
5%	20.3±2.9	11.6±2.3	50.8±6.6	19.3±1.2	11.8±3.8	53.3±4.1	21.2±1.7	12.5±2.6	47.2±5.9
10%	21.6±2.7	12.8±3.6	51.2±8.8	19.8±2.7	10.8±2.7	54.4±8.3	23.0±3.2	14.4±4.0	48.4±9.4
20%	20.8±1.9	17.8±2.1	42.5±3.7	20.5±1.7	17.0±1.4	42.5±2.7	19.8±2.6	17.0±1.8	43.3±5.0
50%	17.3±2.5	20.5±6.0	37.0±9.1	21.5±1.9	23.8±5.3	31.3±8.7	16.5±2.7	18.8±5.7	43.0±10.2

Comparison of γ, k, and ma values of each TEGm of 6% Hespander®, 3% Dextra 40, LRS, 6 HES®, 6% Dextra 70 Saline.

Fibrinolysis ratios (FLR) were calculated by the following formula:

$$FLR = \frac{ma - ma'}{ma} \quad (\%)$$

where ma′ is the amplitude value 60 min after ma.

The difference in r, k, ma, and FLR of between each plasma expander and the values of each control solution were compared. In this wasy, we could examine the exact effect on blood coagulability of 6% Hespander® 3% dextran-40, 6 HES® and 6% dextran-70.

Results

Table 2 shows the actual values of TEGm measured for each expander and control solvent solution at concentrations of 5, 10, 20, and 50% in blood.

The values of r, k, and ma of 6% Hespander® and 3% dextran-40 in LRS were measured and the control values of LRS were deducted from each value. The r, k, and ma values of 6 HES and 6 HES® and 6% dextran-70 in normal saline were also measured and the control values of normal saline were deducted from each value. Thus, a comparative study of each difference (Δr, Δk, Δma, and FLR) was made.

(1) The Δr values (reaction time) are shown in Fig. 2. The Δr value was prolonged when the concentration was increased in both 6% Hespander® and 3% dextran-40. There was no significant difference between these two low molecular weight plasma expanders, but the prolongation of the Δr value of 6% Hespander was less than that of 3% dextran-40. In other words, 3% dextran-40 showed a greater tendency to prolong thromboplastin formation time than 6% Hespander®.

In the high molecular weight plasma expanders at concentrations of 5 and 10% in blood, the Δr value was shortened in both 6 HES® and 6% dextran-70. Especially at

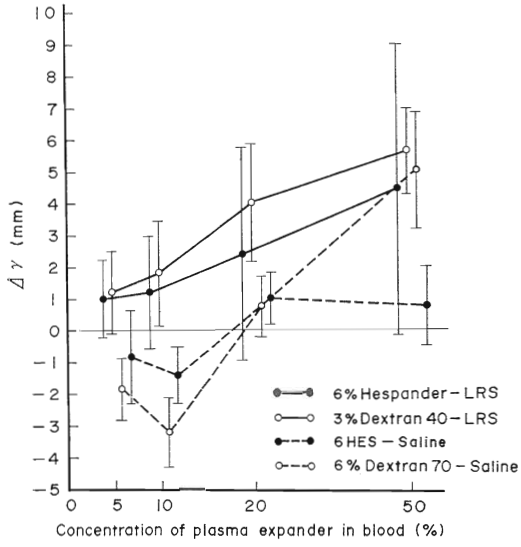

Fig. 2. Changes in $\Delta\gamma$ with the increase in concentration. In the low molecular weight plasma expanders the $\Delta\gamma$ value increased when the concentration was increased (solid lines). In the high molecular weight plasma expanders, a dual-phase pattern is seen (dotted lines).

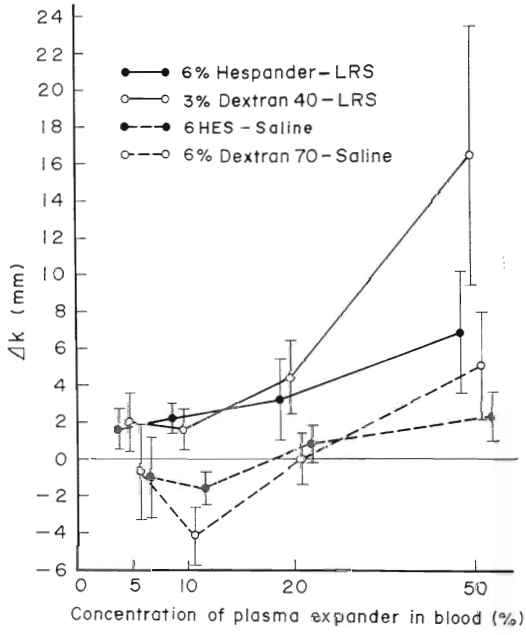

Fig. 3. Changes in Δk with the increase in concentration. In low molecular weight plasma expanders the Δk value increased when the concentration was increased to 20–50% (solid lines). In the high molecular weight plasma expanders the Δk value showed a dual-phase pattern (dotted lines).

10% in blood, the Δr value in 6% defftran-70 was significantly shorter than that of 6 HES® ($p<0.05$). At 20% in blood, prolongation of the Δr value was slight in both 6 HES® and 6% dextran-70. At 50% in blood, prolongation of the Δr value became significant in 6% dextran-70.

(2) The Δk values (coagulation speed) are shown in Fig. 3. In the low molecular weight plasma expanders, the Δk value was prolonged when the concentration was increased to 20% and 50%. This means that coagulation speed became slower at higher concentrations and consequently thrombin formation time became slower.

In the high molecular weight plasma expanders at concentrations of 5 and 10% in blood, the Δk value was shortened in both 6 HES® and 6% dextran-70, and especially at 10% the value in 6% dextran-70 was significantly shorter than that in 6 HES® ($p<$ 0.05). However, at a concentration of 50% in blood, the Δk value was prolonged in both 6 HES® and 6% dextran-70, but the degree of prolongation was less than that in the low molecular weight plasma expanders.

(3) The Δma values (maximum ampliture) are shown in Fig. 4. In the low molecular weight plasma expanders, the Δma value decreased with the increase in concentration. At 50% in blood, the Δma value of 3% dextran-40 was significantly lower than that of 6% Hespander®. This means that 3% dextran-40 significantly decreased the blood clot elasticity as compared with 6% Hespander®.

In the high molecular weight plasma expanders, at concentrations of less than 10% in blood, the Δma value increased in both 6 HES® and 6% dextran-70. Especially the

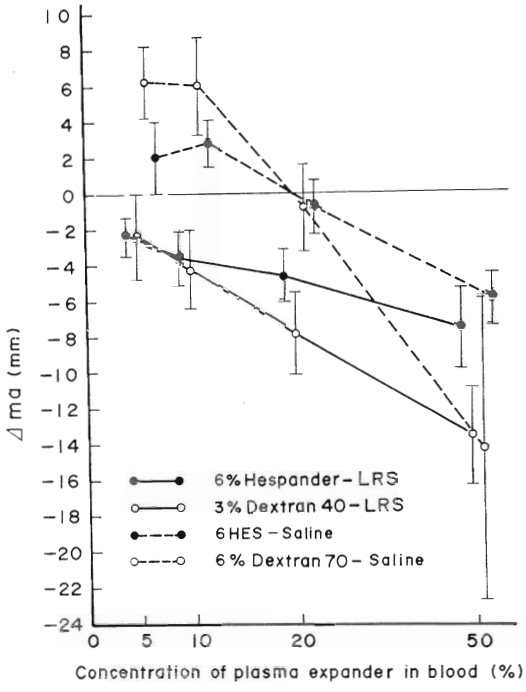

Fig. 4. Changes in Δma with the increase in concentration. In the low molecular weight plasma expanders the Δma value showed a dual-phase pattern (dotted lines).

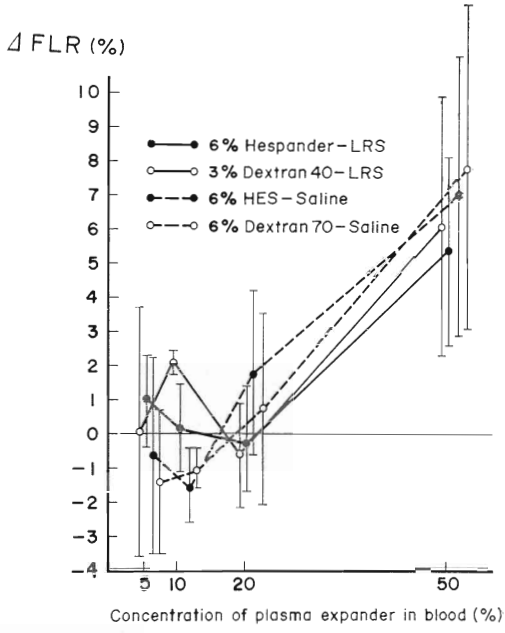

Fig. 5. Changes in the ΔFLR with the increase concentration. In the low molecular weight plasma expanders of the ΔFLR increased with the increase in concentration (solid lines). In the high molecular weight plasma expander the ΔFLR showed a dual-phase pattern (dotted lines).

Δma value of 6% dextran-70 increased more than that of 6 HES® at 5% and 10% in blood, with a significance of $p<0.05$. However, when the concentration were more than 20% in blood, the Δma value returned to normal at 20% and decreased significantly at 50%.

(4) The ΔFLRs (fibrinolysis rates) are shown in Fig. 5. In the low molecular weight plasma expanders, the ΔFLR increased with the increase in concentration. At a 10% concentration, the ΔFLR of 3% dextran-40 increased most slightly. The ΔFLR became minimum at 20% of both 6% Hespander® and 3% dextran-40, but increased when the concentration was increased. In the high molecular weight plasma expanders at concentrations less than 10%, the ΔFLR decreased a little in both 6 HES® and 6% dextran-70. At 20% or more, the ΔFLR increased when the concentration was increased. A dual-phase pattern was also seen in the high molecular weight expanders.

All these experiential results are summarized in Table 3 and the changes in Δr, Δk, and Δma values are shown by arrows.

Discussion

Up to this time, research on the effect of plasma expanders on blood coagulability has mainly been focussed on the bleeding time, and individual blood coagulation factors.[2] However, in order to evaluate the blood coagulability 4 aspects of blood coagulation have to be explored; blood vessel function, platelets and related factors, each blood coagulation factor, and fibrinolysis factors. Overall exploration of the above 4 factors can be done by the thromboelastography in

Table 3. Summary of changes in $\Delta\gamma$, Δk, and Δm values

	6 % Hespander®–LRS			3 % Dextran 40–LRS			6 HES®–Saline			6 % Dextran 70–Saline		
	$\Delta\gamma$	Δk	Δma	$\Delta\gamma$	Δk	Δma	$\Delta\gamma$	Δk	Δma	$\Delta\gamma$	Δk	Δma
5%	—	—	—	—	—	—	—	—	—	—	—	—
10%	↗	—	↘	—	—	↘	↘	↘	↗	↘	↓	—
20%	↑	↗	↓	↗	↗	↓	↗	↗	↓	↑↑	↗	↓↓
50%	↑↑↑	↑↑	↓↓↓	↑	↑↑	↓↓	↗	↑	↓↓	↑↑↑	↑	↓↓↓

Comparison of $\Delta\gamma$, Δk, and Δma values of each TEGm of 6% Hespander®–LRS, 3% Dextran 40–LRS, 6 HES®–Saline, and 6% Dextran 70–Saline

— : no significance
↗, ↘ : tendency toward increase or decrease, but no significance
↑ , ↓ : statistically significant increase of decrease ($p > 0.05$)
↑↑, ↓↓ : ($p < 0.01$)
↑↑↑, ↓↓↓ : ($p < 0.001$)

Table 4. Significance of γ, k, and ma values of the TEGm and their relationship with coagulation components

γ : REATION TIME Time for thromboplastin formaton	Platelet factors (except the material components) Thrombin Coagulation factors which influence prothrombin time Red blood cells
k : COAGLATION SPEED Time for thrombin formation and speed of blood clood bild.	Coagulation factors which influence the γ value Fibrinogen
ma : MAXIMUM AMPLITUDE Maximum elasticity of blood clot	Platelets (material components) Fibrinogen Red blood cells Fibrin stabilizing factors

a single procedure.

The TEGm shows the whole process of blood coagulation, from the beginning of endogenous coagulation to fibrinolysis, as a series of coagulation changes, including the platelets and other coagulation factor functions, all judged objectively. In addition, the TEGm will detect the state of hypercoagulability, hypocoagulability, and hyperfibrinolysis.[3,4]

The r value represents thromboplastin formation in the whole process of blood coagulation, which is effected by platelets, thrombin, and prothrombin-producing factors, and red blood cells (RBC) (Table 4). The k value represents thrombin formation and the speed of blood clot formation, which is effected by fibrinogen and the same factors which influence the r value. The ma value represents blood clot elasticity, which is

affected by platelets, fibrinogen, RBCs, and fibrin-stabilizing factors.

Blood coagulation dysfunction will occur after massive infusion of plasma expander, as other authors have warned.[1] In our experiments also, as can be seen from Table 2, at the high concentration of 50% the value of k is nearly double the normal value and that of ma is decreased to nearly one-half the normal value. Even taking into account the effect of diluting blood with the control solutions (LRS and normal saline), it is evident that coagulability has been affected. Suppression of coagulability after massive infusion of plasma expander has been attributed to many different causes by various authors. Bloom *et al.*[5] and Ballinger *et al.*[6] attributed its cause mainly to changes in blood vascular function, Harada[7] and Thompson *et al.*[8] mainly to platelet system changes, and Ricketts[9] and Sampel[10] to a series of blood coagulation factor dysfunctions. Langdell *et al.*[11] and Lewis[12] said that the causes were combination of these factors.

Contrary to these reports, there were some reports which suggested that a slight hypercoagulability occurred after hydroxyethyl-starch infusion. In conclusion, the effect of plasma expander on blood coagulability is still debatable.

Table 3 summarizes the results of our study. In the low molecular weight plasma expanders, Δr and Δk values were prolonged and Δma value decreased when the concentration was increased. 6% Hespander® was milder than 3% dextran-40 in this tendency, and was similar to the control solution (LRS). These results resemble the reports of Lewis *et al.*[12] and Russel *et al.*[16] which stated that the effect of hydroxyethyl-starch on blood coagulability was milder than that of dextran.

Thompson *et al.*[8] attributed the cause of coagulability dysfunction after plasma expander infusion to weakness of the platelets, suppression of fibrinogen production, and dilution of blood coagulation factors. Hydroxyethyl-starch, however, had a milder influence on these factors than dextran.

In the high molecular weight plasma expanders, at concentrations less than 20% in blood, Δr and Δk values were reduced but Δma value increased a little, indicating increased coagulability. At concentrations of more than 20% in blood, Δr and Δk values were prolonged and Δma value was reduced, indicating reduced coagulability.

In other words, the high molecular weight plasma expanders seem to have a dual-phase effect on coagulability according to the concentration; at low concentrations coagulability increases a little and at high concentrations coagulability decreases. 6 HES® seems to have a milder effect on these tendencies than 6% dextran-70 and resembles the control solution (normal saline) in regard to blood coagulability.

In general clinical use the volume of plasma expander is less than 20% of the circulating blood volume. Therefore, examination of blood coagulability changes at clinical concentrations of plasma expander in blood is desirable and important. In our experiments, a dual-phase effect on coagulability was seen in the high molecular weight plasma expanders; at low concentrations they increased coagulability a little and at high concentrations they decreased coagulability. In other words, at the high concentration of 50% blood coagulability is clearly affected, but, at a clinical concentration of less than 20% of the circulating blood volume, the increase and decrease in coagulability are minimum and have no clinical significance.

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