ORIGINAL ARTICLE

Sonoclot coagulation analysis of *in-vitro* haemodilution with resuscitation solutions

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SUMMARY

The colloid and crystalloid solutions used for resuscitation should preferably be free from effects on coagulation. In 10 volunteers, the effects of haemodilution with various concentrations of 0.9% sodium chloride and 4% succinylated gelatin were assessed by Sonoclot analysis, which describes the whole coagulation process.

Small and moderate haemodilution (up to 40%) with 0.9% sodium chloride promoted coagulation. Similar haemodilution with 4% succinylated gelatin impaired coagulation, and at 60% haemodilution coagulation was very poor.

These findings need to be confirmed *in vivo* and their clinical relevance determined.

INTRODUCTION

Exsanguination is second only to central nervous system injury as a cause of trauma death[1]. Haemostasis is therefore critical to the successful resuscitation of the injured patient: after adequate ventilation, control of haemorrhage and fluid resuscitation are a priority. At present in the UK various crystalloid and colloid solutions are available for resuscitation of hypovolaemic patients, and their effects on coagulation are clearly of interest. In injured patients, however, these are hard to assess because the stress of trauma or surgery can alter blood coagulation[2]. In addition, when blood is diluted with an intravenous fluid the haemodilution effect can itself alter haemostatic mechanisms[3]. As judged by routine coagulation screening tests, gelatin-based solutions are relatively safe, but such tests evaluate only part of the coagulation system. *In-vitro* studies of whole blood coagulation by thromboelastography indicate that these solutions may have an effect on clot stability.

In a controlled experiment we investigated the *in-vitro* effect on coagulation of dilution of blood with various concentrations of 0.9% sodium chloride and Gelofusine (4% succinylated gelatin).

METHODS

The coagulation profile was assessed by Sonoclot analysis, to give an overview of the coagulation process. The Sonoclot Coagulation & Platelet Function Analyser (Sonoclot Analyser, Sienco Inc, USA) measures the changing impedance to movement imposed by the developing blood clot on a small probe vibrating at an ultrasonic frequency in a coagulating blood sample[4]. The device consists of two parts—a cuvette containing the blood sample, and an open-ended plastic probe which vibrates vertically through it. The blood exerts a viscous drag on the probe, mechanically impeding its free vibration. The drag increases as the blood clots and fibrin strands form on the probe tip and between the probe and the wall of the cuvette, effectively increasing the mass of the probe. Increasing impedance to vibration of the probe, as the blood sample passes through the various stages of clotting, is detected by the electronic circuits driving the probe and converted to an output signal. Output signal data, transmitted to a computer every second, reflect the viscoelastic properties of the evolving clot. This record of the 'clot signal' versus time is called the Sonoclot signature (Figure 1) and describes the whole coagulation process *in vitro*. Several measurements can be taken from

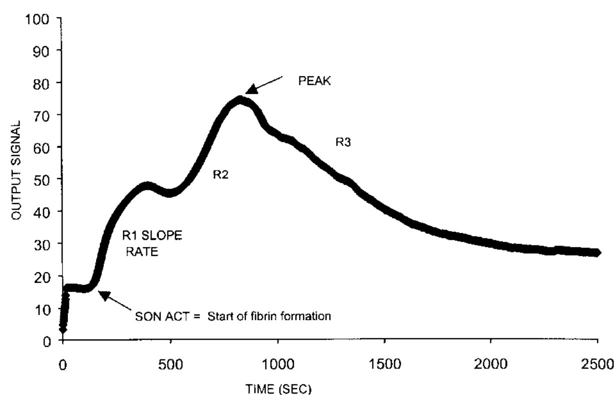

Figure 1 **The Sonoclot signature**

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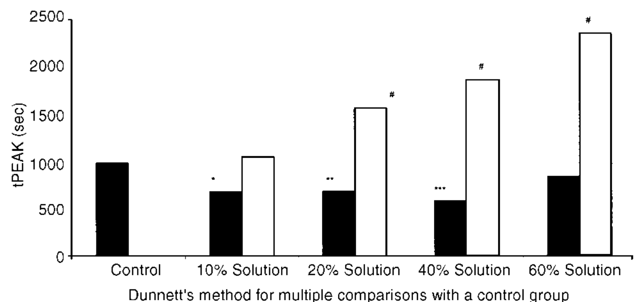

Figure 2 **tPEAK (time to reach maximum clot strength) in control and saline and Gelofusine haemodiluted blood.** ■0.9% sodium chloride; □ Gelofusine. *P=0.03; **P=0.003; ***P=0.001; #P=<0.0002

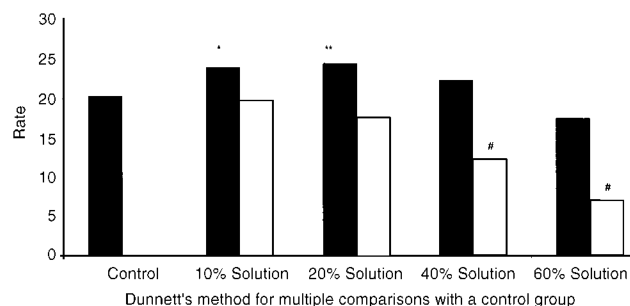

Figure 3 **RATE in control and saline and Gelofusine haemodiluted blood.** *P=0.04; **P=0.02; #P=<0.0001

the Sonoclot signature[4,5]. In this experiment the rate of fibrin formation (RATE), the time to reach maximum clot strength (tPEAK), and maximum amplitude at PEAK (Max Amp) were measured.

With local ethics committee approval 10 healthy volunteers were enrolled (8 men, 2 women, age range 30–38 years). Exclusion criteria were known coagulation disorder, oral anticoagulation, and aspirin or non-steroidal anti-inflammatory medication within the previous week. Blood samples were obtained from a free-flowing upper-limb vein, via an intravenous canula, into polypropylene plastic syringes. Blood was then added to an appropriate volume of each solution to make the required test sample. The test samples analysed were 10%, 20%, 40% and 60% dilutions with 0.9% sodium chloride and Gelofusine. A control sample of undiluted blood was also analysed. Specimens from each sample (0.360 mL) were then added to the cuvette of the Sonoclot analyser. Temperature was maintained at 37° C by making the dilutions with warmed solutions and by using the temperature plate and heat shield of the Sonoclot analyser during analysis. Results for control blood were compared with those for crystalloid and colloid diluted blood by analysis of variance (Dunett's method for multiple comparisons with a control group). The intrinsic effect on coagulation of the solution used to expand the plasma volume may be assessed by analysing the difference between the crystalloid diluted blood and the colloid diluted blood at similar haemodilutions. Wilcoxon's signed-rank test with 99% confidence interval was used to compare crystalloid and colloid diluted blood. The comparisons were made by use of the Arcus Quickstat software package (Addison Wesley Longman, Cambridge, UK).

RESULTS

The effects of dilution with 0.9% sodium chloride and Gelofusine on tPEAK, RATE and Max Amp are shown in Figures 2–4. Dilution with Gelofusine compromised blood coagulation with a progressive increase in tPEAK and a corresponding reduction in RATE compared with native blood. Haemodilution with 0.9% sodium chloride, however, induced different changes in the coagulation process: the time to peak was significantly shorter than control at 10%, 20% and 40% dilutions. At 60% dilution, tPEAK was returning toward the control value but rate of fibrin formation was now lower than that of control blood. Max Amp was affected only at 60% dilution with Gelofusine, which significantly reduced clot strength.

When values for 0.9% sodium chloride and Gelofusine were compared within each dilution (Table 1), the difference between the solutions was easily seen. In the direct comparison between solutions, haemodilution with Gelofusine resulted in significantly longer tPEAK values at all haemodilutions and reduced RATE values at 20%, 40%, and 60% dilutions. There was a significant difference in Max Amp between solutions at 60% dilution.

DISCUSSION

The effects of stress, tissue trauma, pain, endogenous catecholamine levels and anaesthesia *per se* on global coagulation status are poorly understood. Such factors can be confounding variables in any study of coagulation in relation to blood loss and fluid replacement. *In-vitro* and *in-vivo* haemodilution coagulation studies in normal individuals are therefore an important source of information regarding the effect on haemostasis of intravenous fluid resuscitation.

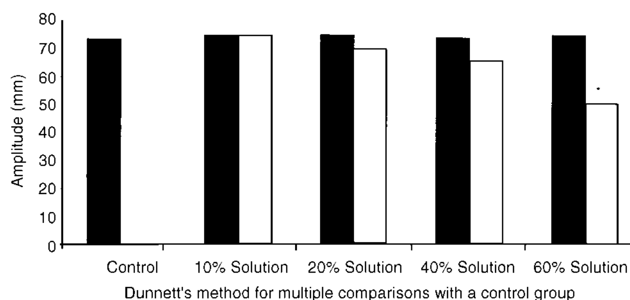

Figure 4 **Maximum amplitude at PEAK in control and saline and Gelofusine haemodiluted blood.** ■ 0.9% sodium chloride; □ Gelofusine *P=0.0003

Table 1 **Sonoclot values for 0.9% sodium chloride and Gelofusine diluted blood**

		10% solution		20% solution		40% solution		60% solution	
	Control	CR	COL	CR	COL	CR	COL	CR	COL
PEAK (seconds)	982	679	1052*	686	1568*	586	1870*	847	2368*
RATE	20.3	24	19.7	24.5	17.6†	22.4	12.3	17.6	7.27†
Max Amp	73.5	74.9	75	75.3	70.1	74.4	66.5	75.6	51.3*

*$P<0.008$; †$P=0.002$ Wilcoxon signed rank test
CR=0.9% sodium chloride; COL=Gelofusine

As early as 1951 it was noted that dilution of blood with 0.85% sodium chloride accelerated the blood clotting process[3]. Recent studies, though few in number, have confirmed that haemodilution with isotonic crystalloid solutions increases blood coagulation. Using the Thromboelastogram (TEG), investigators have demonstrated that, *in vitro*, saline haemodilution at 20% and 30% dilutions promotes coagulation[6–8]. Our study confirmed this effect, demonstrated by increased RATE and reduced tPEAK. Maximum amplitude, however, was unaltered. The mechanism by which saline haemodilution promotes coagulation is still poorly understood.

Until recently the gelatin solutions Gelofusine and Haemaccel (polygeline) have been thought to have no important effect on the clotting mechanism, but this may not be so. In an *in-vitro* study, Mardel reported reduced quality of clot formation with gelatin-based plasma substitutes[9]. He found a significant decrease in clot strength in blood diluted with Gelofusine or Haemaccel compared with saline diluted blood and he postulated that the gelatin-based products might become incorporated into the developing clot, thus interfering with polymerization of fibrin. Ruttmann, however, found that 20% haemodilution with Hamaccel or saline caused an increase in coagulation as measured by thromboelastography[6]. In a further *in-vitro* study, Egli reported that haemodilution with 4% succinylated gelatin (Physiogel) at both 30% and 60% significantly compromised coagulation[8]. Mortier found, however, that even with 50% Gelofusine haemodilution, coagulation parameters were unaltered as measured by TEG[10]. In our study, haemodilution with Gelofusine impaired coagulation, reduced RATE, prolonged tPEAK and reduced maximum amplitude. It appears therefore that the coagulation system can compensate for dilution with Gelofusine in small amounts (10%–20%) *in vitro* but that clotting is impaired at higher dilutions. In normal individuals a similar haemodilution could be achieved *in vivo* by administration of between one and two litres of intravenous fluid. These volumes of fluid are often administered to moderately injured and bleeding patients.

In-vivo studies have been performed to help elucidate the effect of colloid and crystalloid on coagulation. Some have been limited, however, by the relatively small volumes of fluid administered. De Jonge found that a 1000 mL infusion of Gelofusine resulted in a 1.7-fold increase in bleeding time, and ristocetin-induced platelet aggregation was impaired[11]. In clinical studies Haemaccel fluid resuscitation in traumatized patients was associated with an increased bleeding time and pronounced blood loss[12]. Van Wyk, however, found no difference in coagulation indices in 20 patients undergoing surgery when patients received either 500 mL Haemaccel or 1500 mL Ringer's lactate[13]

In trauma patients it is tempting to assume that promotion of coagulation is clinically of benefit whereas reduced coagulation is not. Injury, however, is a process associated not only with haemorrhage but also with thrombosis. Thrombosis is less clinically evident and less commonly appreciated until late in a downward course toward end-organ failure, when gross disseminated intravascular coagulopathy (DIC) supervenes.

Fluid resuscitation with colloid solutions in the early phase of trauma resuscitation may adversely affect outcome by decreasing blood coagulation and impairing surgical haemostasis. Once the bleeding process has been controlled, however, intravenous saline may promote coagulation and lead to a thrombotic state. Perhaps the promotion of a DIC-like effect by saline could be just as hazardous to the trauma patient as the impairment of haemostasis by colloid. Clearly, therefore, the choice of initial resuscitative fluid in a trauma patient may be influenced by many factors, both short-term and long-term. Further understanding of coagulation following injury may enable us to adjust different parts of the haemostatic system during different phases of resuscitation in attempts to maintain normal coagulation.

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