Pharmacological manipulation of gastric juice: thrombelastographic assessment and implications for treatment of gastrointestinal haemorrhage

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Abstract

The impairment of formation and maintenance of a formed fibrin clot contributes to the prolonged bleeding and high incidence of rebleeding in upper gastrointestinal haemorrhage. To investigate the basis for the use of drug therapy in gastric bleeding, this study used thrombelastography to determine the effects of pharmacological manipulation of gastric juice on coagulation and fibrinolysis. The thrombelastograph is a mechanical device that provides a visual assessment of all stages of coagulation and fibrinolysis. The effects of fresh and pharmacologically changed gastric juice was assessed after its addition to fresh whole blood in the thrombelastograph cuvette. Pharmacological manipulation was achieved through alkalinisation or through addition of tranexamic acid, aprotinin, or sucralfate. Fresh gastric juice delayed clot formation, decreased maximum clot amplitude, and stimulated clot lysis. Alkalinisation inhibited the lytic effects of fresh gastric juice and improved the induced abnormalities in coagulation. Tranexamic acid partially inhibited gastric juice induced clot lysis but did not exhibit a beneficial effect on coagulation. Sucralfate, and to a lesser extent aprotinin significantly inhibited fibrinolysis but exacerbated the detrimental effect of gastric juice on the parameters of coagulation. Alkalinisation of gastric juice reduces the adverse effect on coagulation and fibrinolysis. Tranexamic acid, aprotinin, and sucralfate can all reduce or inhibit clot lysis, but the adverse effects on clot formation may outweigh any potential benefit in the treatment of gastrointestinal bleeding.

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bleeding although evidence that drugs are beneficial in arresting bleeding or preventing rebleeding is scant. Many agents have been advocated in the treatment of gastrointestinal bleeding. These include H₂ antagonists,² omeprazole,³ anti-proteolytics,⁴ and anti-fibrinolytics.⁵ Although controlled studies have failed to confirm the benefit of these agents in peptic ulcer bleeding, meta-analysis has suggested that anti-fibrinolytics⁶ and, possibly H₂ antagonists² may be beneficial. None the less, drug therapy for gastrointestinal bleeding remains an attractive prospect as treatments are universally acceptable, do not require special equipment, and are probably safe. In addition there is a sound physiological basis for their use in reducing bleeding and preventing rebleeding. The stomach and duodenum present a hostile environment to the processes of clot formation and clot lysis. Clot formation and platelet function are impaired in the presence of acid,⁷ and clot lysis is accelerated in the presence of gastric juice.⁸ The effect of currently available therapeutic agents on the adverse effects of gastric juice, however, have not been examined and thus it is unclear whether these agents are capable of ameliorating the gastric environment and thus optimising clot formation and stability. We have used thrombelastography, a sensitive technique for assessing the processes of coagulation and fibrinolysis to determine the effects of pharmacological manipulation of gastric juice on these haemostatic mechanisms.

Methods

THROMBELASTOGRAPHY

The thrombelastograph is a mechanically operated system that provides a continuous visual assessment of blood or plasma during all phases of coagulation and fibrinolysis. The graphic representation produced by the instrument is called a thrombelastogram (Fig 1). The instrument consists of two cylindrical cuvettes maintained at 37°C containing the blood or plasma to be analysed, which rotate back and forth about a vertical axis. A piston is lowered into each cuvette leaving a space of 1 mm for the sample between the piston and the cuvette. As long as the sample remains fluid the pistons remain motionless, but once fibrin strands begin to form the oscillations of the cuvette are transmitted to the piston and as fibrin formation and elasticity of the coagulum progress, the oscillations of the piston increase accordingly. The motion of the piston is

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The frequency with which upper gastrointestinal haemorrhage presents in modern clinical practice has resulted in great efforts to provide an effective treatment that will reduce the need for surgery and lower mortality. Endoscopic therapy has gained widespread acceptance for the treatment of ulcers that are actively bleeding or at high risk of rebleeding.¹ Pharmacological therapy is also widely used in the setting of upper gastrointestinal

Figure 1: Schematic representation of thrombelastogram and normal range of most commonly calculated variables.

transmitted electromechanically to a pen recording device resulting in the typical spindle shaped tracing.

EVALUATION OF THE THROMBELASTOGRAM
Figure 1 shows a schematic representation of the thrombelastogram. As soon as the machine is switched on, a straight line begins to register, showing that no fibrin has formed and that movements of the cuvette have not yet been transmitted to the piston. The time from the starting point to the point at which the amplitude of the graph reaches 1 mm is called the reaction time (r time), and represents the rate of thromboplastin generation or the thromboplastin value.⁹ The coagulation time (k time) corresponds to the time taken from the end of the r time to a point where the amplitude of the tracing measures 20 mm. This is a measure of the rapidity of fibrin build up or the speed at which a clot of a certain solidity has been attained. The maximum amplitude of the coagulum (MA) reflects the firmness of the clot and is influenced by the dynamic properties of fibrin, calcium, other plasma factors and, to a significant degree, platelet function. Subsequently, there is often a gradual decline, which represents retraction or softening of the clot. If fibrinolysis is present, however, there is

a sustained regular decrease in maximum amplitude, which takes on a spindle type shape. Quantification of the degree of fibrinolysis is indicated by the fibrinolytic index (FI), which is the ratio of A₆₀ to MA where A₆₀ is the amplitude of the tracing one hour after the MA is attained.

COLLECTION AND MANIPULATION OF GASTRIC JUICE

After an overnight fast, gastric juice was obtained from patients referred for upper gastrointestinal endoscopy by aspiration through an air dried endoscope (Olympus Q10). Particular effort was made to avoid contamination with saliva or bile. The pH of the juice was measured and gastric juice was discarded if the pH was greater than 2.0 or if less than 5 ml could be aspirated. The juice was stored at 4°C and examined within a maximum of four days. Juice from patients with known pancreatic or biliary disease, or from patients receiving drugs known to change gastric juice was not utilised.

The effects of commonly used pharmacological agents on gastric juice was investigated through alkalisation or through the addition of sucralfate, aprotinin, or tranexamic acid. Drugs were added in concentrations that most closely resembled the dose used therapeutically but that did not change the pH of gastric juice: (tranexamic acid 20 mg/ml, aprotinin 5000 U/ml, sucralfate 12 mg/ml). To examine the effect of acid suppression, gastric juice was alkalised to a pH of 4 by the addition of 0.1 M sodium hydroxide (NaOH). About 50-200 µl of NaOH was required to increase the pH of 2 ml aliquots of gastric juice greater than 4. Hydrochloric acid (0.01 M) and 0.9 N isotonic saline were used as pH and volume controls respectively. After pharmacological manipulation, the juice was allowed to stand for 10 minutes and then centrifuged at 500 rpm for five minutes to remove particulate matter.

THROMBELASTOGRAPHIC TECHNIQUES
Fresh blood from a single donor was used in this study. For each study 50 µl of gastric juice or control was pipetted into the cuvette and the cuvette replaced into the instrument for five minutes to ensure that both the juice and cuvette were prewarmed to a temperature of 37°C. On each occasion fresh blood was obtained from the same donor by direct venepuncture using a 21 gauge needle but without the use of a tourniquet or syringe. The first five drops of blood obtained were discarded, and the blood that followed was allowed to drop directly into the cuvette. The piston was then immediately lowered and raised into and out of the cuvette three or four times to facilitate mixing of the blood and gastric juice. The piston was then lowered into the cuvette and the recording commenced. A thin layer of liquid paraffin was pipetted onto the surface of the sample to prevent drying of the sample during

Figure 2: Mean calculated reaction time in minutes. T acid=tranexamic acid.

Figure 3: Mean calculated coagulation time in minutes.

the recording. The time interval between the withdrawal of the blood sample and the start of the thrombelastographic recording was determined with a stopwatch and added to the *r* time to obtain the total recording value.

All readings were performed in duplicate and the mean of the calculated values from both readings obtained were utilised. Contamination of the specimen with particulate matter was readily apparent when it occurred. This was evidenced by irregular increases or decreases in amplitude. These recordings were always discarded and the measurements repeated. In each case thrombelastographic recordings were carried on for a minimum of two hours so that satisfactory assessment of the fibrinolytic index could be obtained.

Up to 15 samples of gastric juice were analysed and for each recording, the reaction time, coagulation time, maximum amplitude, and fibrinolytic index were calculated as previously described. The final results were expressed as a mean of these readings. Differences between groups were calculated using the Mann-Whitney U test and $p < 0.05$ considered significant.

Figure 4: Mean maximum amplitude in millimetres.

Results

REACTION TIME (Fig 2)

Acid alone (0.01 M hydrochloric acid), or pure gastric juice had no demonstrable effect on the reaction time of fresh blood when compared with volume control. Significant changes in reaction time were seen with pharmacologically changed gastric juice. The addition of sucralfate had the most noticeable effect prolonging the reaction time from 5.7 to 39.5 seconds ($p < 0.05$). The addition of both tranexamic acid and the non-specific protease inhibitor aprotinin to gastric juice also significantly though less profoundly prolonged the reaction time of whole blood when compared with the volume control.

COAGULATION TIME (Fig 3)

The addition of hydrochloric acid alone induced a significant prolongation of the coagulation time when compared with normal saline ($p < 0.05$). The addition of pure gastric juice to fresh blood caused a similar prolongation of the K value to 10.3 minutes, which was again significantly longer than seen for control ($p < 0.01$). This prolongation was found to be reversed by raising the pH of gastric juice. The addition of sucralfate to gastric juice again very noticeably changes the coagulation time in the absence of a change in pH (K value 61.9 (7.2)). The addition of aprotinin or tranexamic acid to gastric juice did not significantly change the K value when compared with that seen with gastric juice alone.

MAXIMUM AMPLITUDE (Fig 4)

The maximum amplitude, representing the maximum elasticity of the clot was significantly reduced by the addition of gastric juice when compared with normal saline (47.3 mm v 37.9 mm, $p < 0.01$). Alkalisiation of gastric juice reversed this effect. Sucralfate, however, once again had a very profound effect on the dynamics of haemostasis reducing the mean maximum amplitude of the clot to 19 mm. The adverse effect of gastric juice on clot formation was therefore exacerbated by the addition of sucralfate. Aprotinin in gastric juice also induced a significant reduction in clot elasticity though again less profoundly than with sucralfate. Tranexamic acid added to gastric juice also tended to reduce the maximum clot elasticity in comparison with gastric juice alone although this failed to reach statistical significance ($p = 0.07$).

FIBRINOLYTIC INDEX (Fig 5)

The mean fibrinolytic index of fresh blood when normal saline was added was 84%, which is within the published normal range. The addition of 0.01 M hydrochloric acid to blood did not cause any significant change to this value (84.3 v 88.8, $p = 0.08$). Pure gastric juice reduced the fibrinolytic index, however, from 84.6% in controls to 43.1% ($p < 0.001$). This shows lysis of a formed fibrin clot.

Figure 5: Mean fibrinolytic index.

Alkalisiation of gastric juice reverted the fibrinolytic index to greater than 80%. The addition of both tranexamic acid and aprotinin to the juice tended to reduce the observed lysis though both failed to inhibit lysis completely and return the fibrinolytic index to the normal range. The most profound effect on fibrinolysis seen with gastric juice was noted with the addition of sucralfate to the juice. Although the addition of 20 mg/ml of sucralfate had no effect on the pH of gastric juice, the lysis seen with gastric juice was completely abolished.

Discussion

There is a strong relation between the assessment of coagulation as measured with the thrombelastogram and the more common laboratory tests of coagulation. The thrombelastographic variables contain additional information on the haemostatic process that come from the measurement of interactions of various components of the clotting and lytic processes in whole blood.¹⁰ This additional information makes the thrombelastograph more sensitive to changes in the haemostatic balance of fibrinolysis^{11,12} and coagulation.¹³ Thus although the changes in haemostatic mechanisms detected by thrombelastography may not be clinically apparent in the setting of this study, changes in the thrombelastogram give important clues as to the effects of gastric juice on haemostasis. In addition, each of the thrombelastographic variables measured give independent information on various aspects of the haemostatic process.

The reaction time in this study was found not to be significantly changed by the addition of gastric juice or acid. Thus the rate of thromboplastin generation does not seem to be adversely affected by gastric proteases or an acid environment. Of interest, however, was the very noticeable effect of sucralfate on this variable. The mean reaction time of fresh blood was almost three times longer when gastric juice with sucralfate was added. Although sucralfate is of undoubted benefit in healing peptic ulcers,^{14,15} its role in active gastrointestinal bleeding has never been extensively studied.¹⁶ None the less, the prolonged thromboplastin generation time would be, at least theoretically, detrimental to haemostasis.

A similar, though less noticeable effect was seen with the non-specific protease inhibitor aprotinin, and again would suggest that this agent has some properties that would make it less than ideal in upper gastrointestinal haemorrhage. This agent has previously been shown to significantly prolong the partial thromboplastin time in vitro.¹⁷

The coagulation time was, however, significantly prolonged by the addition of fresh gastric juice. The similar effect seen with hydrochloric acid, and the reversal of the prolonged coagulation time by alkalinised gastric juice would suggest that this effect is related to a change in pH and not a property of gastric juice in itself. The adverse effect of acid on both the more conventionally measured coagulation pathways and on platelet function have previously been described.⁷ It is probable that this effect is represented here by a rise in the K value. Although tranexamic acid had no significant effect on the coagulation time, sucralfate, and to a lesser extent aprotinin, both have profound effects on the coagulation time. This finding in conjunction with prolonged thromboplastin generation would seem to confirm that these agents do indeed delay clot formation. However, not only does sucralfate delay the formation of a formed clot but it also reduces the elasticity or firmness of the clot. This is represented by the very significant reduction in the mean maximum amplitude when compared with control. Interestingly this effect was not seen with aprotinin. Pure gastric juice but not hydrochloric acid or normal saline significantly reduces the maximum amplitude and this effect is almost completely reversed by alkalisiation. This suggests that an acid dependant factor in gastric juice such as a gastric protease is responsible rather than acid alone. Tranexamic acid does not play any part in reversing the effect of gastric juice in terms of the maximum amplitude. Again aprotinin exaggerates the detrimental effect of gastric juice on clot formation.

This study shows that with regard to haemostasis, acid alone plays only a minor part in impairing clot formation. Pure gastric juice interferes with haemostasis both by delaying the rate of clot formation and by reducing the quality of the final clot formed. This effect is not purely acid related as the effect of hydrochloric acid alone has shown. Aprotinin, and more particularly sucralfate, are likely to have profound detrimental effects on haemostasis as both impair both the rate of clot formation and the maximum elasticity of the final clot formed.

Lysis of a formed fibrin clot is also likely to be important in the clinical setting of upper gastrointestinal haemorrhage. Gastric juice has been shown to possess significant fibrinolytic activity,⁸ and levels of fibrinogen degradation products have been shown to correlate with survival in acute upper gastrointestinal bleeding.¹⁸ Measurement of the fibrinolytic index in this study shows that acidic gastric juice but not 0.01 M hydrochloric acid causes significant fibrinolysis. In terms of pharmacological

inhibition of fibrinolysis, all three agents tested possessed some inhibitory effects. Tranexamic acid is a potent antifibrinolytic agent, which acts by blocking the lysine binding sites of the plasminogen molecule that are essential for its binding to fibrin. This prevents its activation by a plasminogen activator and thus reduces its capacity to degrade fibrin.^{19 20} Thus although it may be capable of inhibiting the plasmin mediated pathway, it has no activity against non-specific protease mediated clot lysis. Aprotinin, a polypeptide derived from bovine lung tissue, has a greater inhibitory effect on clot lysis, and this may be explained by its ability to inactivate gastric proteases.²¹ Of particular interest, however, is the profound effect of sucralfate on the thrombelastographic measurements of clot lysis. Very little lysis was seen in any of the recordings made when sucralfate was added to gastric juice. The mechanism by which sucralfate inhibits fibrinolysis is not clear. Its ability to bind pepsin in addition to other proteins such as fibrinogen and albumin²²⁻²⁴ suggests that it may be capable of inactivating many of the proteases in the proteolytic pathway.

In summary, thrombelastography can provide useful information on the effects of gastric juice on dynamics of clot formation and clot lysis. We have shown that gastric juice causes both significant impairment of clot formation and accelerates lysis of a formed fibrin clot. Both these aspects are likely to be of clinical relevance in a patient with an upper gastrointestinal bleed. Acid neutralisation is clearly of benefit both in optimising clot formation and in preventing fibrinolysis. This supports the hypothesis that profound acid suppression such as that obtained with agents such as omeprazole may have a part to play in the setting of peptic ulcer bleeding. Agents such as sucralfate, aprotinin, and tranexamic acid would seem to be less appropriate on the basis of this work. Aprotinin and tranexamic acid both reduce clot lysis but their adverse effects on the dynamics of clot formation show that their use in active bleeding is not ideal. Sucralfate, which is effective in healing peptic ulcers also reduces clot lysis by gastric juice. The adverse effect that sucralfate clearly has on clot formation at least in vitro, however, would suggest that caution should be exerted before

this agent is recommended in patients with an actively bleeding ulcer.

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