Ethanol impairs coagulation and fibrinolysis in whole blood: a study performed with rotational thromboelastometry.

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Ethanol impairs coagulation and fibrinolysis in whole blood – A study performed with rotational thromboelastometry

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ABSTRACT

Objective: The objective of the study was to study the effects of ethanol on the coagulation and the fibrinolysis in whole blood. *Methods:* Blood samples from healthy volunteers were analysed before and after in vitro addition of ethanol in order to achieve ethanol concentrations of 0‰, 1‰, 2‰ and 4‰ respectively (0, 22, 44 and 88 mmol/l). Coagulation and fibrinolysis were then assessed using rotational thromboelastometry. *Results:* We found that increasing ethanol levels increasingly impaired coagulation as evaluated with rotational thromboelastometry with a maximum prolongation of Clot Formation Time of 118% at an ethanol level of 4‰ ($p<0.000001$). We also found a very strong impairment of fibrinolysis already at an ethanol level of 1‰. *Conclusions:* This is the first study assessing the effects of ethanol on coagulation and fibrinolysis in a whole blood model. The impairment of coagulation is similar in nature to the impairment found in patients suffering from hypothermia. The impairment is at a level that may be of clinical importance, e.g. in patients suffering from trauma. The inhibition of fibrinolysis is obvious already at an ethanol level of 1‰ and it may be a contributing factor to the increased amount of coronary and cerebrovascular ischemic events after binge drinking.

Keywords: alcohol, coagulation, ethanol, fibrinolysis, ROTEM, thromboelastometry,

INTRODUCTION

The use and abuse of alcoholic beverages are common features in trauma patients [1, 2]. More than 30% of injured drivers admitted to a trauma centre have detectable alcohol levels in their blood [3].

It is in our clinical experience common that patients suffering from traumatic brain injuries (TBI) have pronounced progression of their intracranial hemorrhages, e.g. contusions. It is also known that impairment of the coagulation of different origins, e.g. NSAID treatment or consumption coagulopathy, is associated with worse outcome in TBI patients[4-6].

It is in our clinical experience common that patients with high ethanol levels suffering from TBI have pronounced progression of their intracranial hemorrhagic complication. Effects on the coagulation and fibrinolytic systems by increased ethanol levels have been previously described. However, these studies have in general been performed on components of blood, rather than on whole blood, and the effects reported are not in agreement. Several studies have found an alcohol induced impairment of the primary hemostasis, whereas the humoral coagulation factors and the fibrinolytic system were unaffected [7-9]. El-Sayed et al [10] corroborated these results on the humoral coagulation factors and the fibrinolysis, but others have found an acute impairment of the fibrinolytic system by increased ethanol levels [11, 12]. One study also described an increase in fibrinolytic activity after alcohol withdrawal in chronic alcoholics[13]. Another study found somewhat contrasting results indicating an increase in platelet aggregation after binge drinking[14].

These observations have lead us to perform a study where we have evaluated the effects of increased blood ethanol levels on the hemostatic and fibrinolytic systems. We have used

rotational thromboelastometry (ROTEM) to perform the studies. ROTEM is a whole blood method assessing both hemostasis (humoral coagulation and platelet activity) and fibrinolysis in the same analysis. ROTEM is considered a better predictor of clinical bleeding tendency than other coagulation tests[15-17].

MATERIAL AND METHODS

The study was approved by the ethics committee of Lund University Hospital, Sweden.
Six healthy volunteers gave informed consent to participate in the study.

Blood sampling

An indwelling brachial venous catheter was used for blood sampling. Four blood samples were taken from each participant. Each blood sample consisted of 4 ml of blood collected in a siliconated test tube without additives. Before each sampling the initial blood drawn was discarded. One blood sample was analysed without addition of any ethanol. The other three were analysed after addition of 5, 10 and 20 μL of 96% pure ethanol resulting in ethanol concentrations of 1‰, 2‰ and 4‰ respectively.

Thromboelastometry

A rotating thromboelastometer (ROTEM) analyser (Pentapharm® GMBH, Munich, Germany) was used. All analyses were performed at 37°C and the analyses were performed on whole blood. Coagulation was activated with 20 μL of a tissue factor (TF) containing activator according to the EXTEG procedure described by the manufacturer. Four parameters were measured. The first parameter was Clotting Time (CT) measuring the time to initiation of clot formation and reflecting the initiation of the coagulation. Clot Formation Time (CFT) measuring the rate of increasing clot firmness once the clotting has started, reflecting platelet

activity and rate of fibrinogen to fibrin conversion, was also measured. Alpha Angle also reflects the rate of clot strengthening after clotting is initiated. Maximum Clot Firmness (MCF) describing the maximum strength of the clot is another parameter measured that is primarily affected by the platelet count. Lysis Index after 60 minutes (LI60), describing the fibrinolytic activity as the clot firmness 60 minutes after initiation of the analysis in percent of the MCF, was the final parameter measured (Figure 1).

Analyses

The blood was mixed with ethanol immediately after the blood was drawn. The ROTEM analysis was started exactly 90 seconds after the blood was drawn by adding 320 µL of blood to a cuvette already containing 20 µL of TF-containing activator according to standard ROTEM Procedure.

Statistics

Box plots show medians with the box representing 25^{th} to 75^{th} percentiles and the whiskers representing the range. All statistical analyses have been performed with non-parametric statistical methods. The Wilcoxon rank order test was used to study the effects on fibrinolysis. The Spearman rank order test was used for correlation analysis of the effects on the coagulation system. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

No participant withdrew from the study and the blood sampling was performed uneventfully. Increasing the ethanol concentrations progressively impaired the coagulation system.

The impairment was seen in CFT ($p<0.000001$, $r=0.91$) and alpha angle ($p=0.000001$, $r=0.88$) (Figure 2). The CFT was prolonged by 34% at an ethanol level of 1‰ and by 118% at an ethanol level of 4‰. There were no significant changes to CT and MCF.

The fibrinolysis was also significantly impaired with a virtually total elimination of fibrinolytic activity ($p=0.03$) already at an ethanol level of 1‰. Median LI60 was 90% when there was no ethanol added to the blood (Figure 3A). At an ethanol level of 1‰ the median LI60 was 97% (Figure 3B). At higher ethanol levels the LI60 was 98%.

DISCUSSION

ROTEM is gaining increasing popularity as a coagulation monitoring tool as the technique becomes more user-friendly and robust. It seems to be more sensitive and specific than routine coagulation tests in detecting defects of the coagulation system [16, 18-22].

It has also been used as a tool for detection of coagulation defects in patients undergoing cardiac and liver surgery [15, 23-26]. ROTEM measures the rate of clot formation and clot strengthening after coagulation. The result is displayed as a graph where the clot strength is found on the Y axis and time is displayed on the X axis (Figure 1).

In this study we have found that increased ethanol levels correlated to an impairment of the *in vitro* coagulation. The impairment was seen as a prolongation of CFT and a lowering of the alpha angle. These parameters primarily reflect the fibrinogen to fibrin conversion and the platelet activity, even though all parameters in some way is a reflection of thrombin generation which in turn leads to clot formation and stabilisation. There were no changes in the initiation phase or in the maximum strength of the clot. These findings may indicate that the reactions are still running, but at a lower rate. The coagulation would then be initiated at

the correct time but run slower and the clot strength would eventually reach the same strength but at a later point in time, explaining the absence of change in CT and MCF.

Previous studies investigating effects of acidosis and of hypothermia on the coagulation system have found impairments of the coagulation very similar in nature to the effects of increased ethanol levels [27, 28]. The effects are readily detectable already at an ethanol level of 1 ‰ and may be of importance in trauma patients. The link between impairment of the coagulation system and progression of intracranial hemorrhage in trauma patients has been well described. [29, 30]. A link between impairment of coagulation and worse outcome has also been found [5, 6]. The finding that thrombocytopenia at admission predicts progressive hemorrhage in TBI patients indicates a correlation between the coagulation system and progressive hemorrhage [31]. Altogether, many TBI patients have multiple reasons for developing delayed hemorrhagic complications and ethanol levels may be another factor necessary to consider during management of TBI patients. Ethanol has been found to decrease collagen induced platelet aggregation and this may be a problem viewed in the light of the finding that patients on NSAIDs have a higher mortality rate after TBI[4, 32]. In our study we also found a prolongation of CFT that may be interpreted as an impairment of the platelet activity.

Another aspect of the effects of ethanol on coagulation is the impairment of fibrinolysis. An impairment has been described previously, but not in whole blood. Several different studies have addressed this issue and found somewhat conflicting results. Most studies have found an increase in fibrinolytic activity after ethanol intake. Some studies have found an upregulation of tPA expression and a decrease in PAI-1 transcription, something that would explain the increased fibrinolytic activity[33-36]. However, there are also studies having found an acute

inhibitory effect or no effect at all of ethanol on fibrinolysis[9-11]. We found a decrease in fibrinolytic activity in this study, a finding supporting van de Wiel et al who found similar results in their study[11]. However, a weakness in our study is that we are not able detect interactions between the endothelium and components in blood that may be of importance in this process.

A possible explanation to the conflicting results regarding the effects of ethanol on fibrinolysis may be that ethanol acutely impairs fibrinolysis, but upregulates transcription of profibrinolytic genes, e.g. tPA genes and down regulates antifibrinolytic genes, e.g. PAI-1, leading to an increase in fibrinolytic activity in the longer perspective. This would also explain the increased risk for ischemic coronary or cerebrovascular disease after binge drinking, even though moderate intake of ethanol in the longer perspective may be protective against such diseases.

CONCLUSIONS

Ethanol impairs the coagulation system and seems to inhibit fibrinolysis in the short perspective. Intoxication by ethanol may be a risk factor for increased hemorrhage in patients suffering from trauma.

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FIGURE TEXTS

Figure 1A

An original ROTEM registration of blood explaining the different parameters measured. Clot strength is found on the Y axis and time on the X axis. The clot strength is arbitrary and measured in mm. Clotting Time (CT) is the time from initiation of clotting until the clot has gained a strength of 2 mm (thin black line).Clot Formation Time (CFT) is the time from clot strength 2 mm until clot strength 20 mm (black part of the curve). Alpha Angle is another measure of the rate of clot strengthening measuring the angle at which the clot strengthening takes place. Maximum Clot Firmness (MCF) is the maximum strength of the clot. Each square on the horizontal scale is equal to 10 minutes.

Figure 1B

An original ROTEM tracing showing Maximum Clot Firmness (MCF) and Lysis Index after 60 min (LI60). LI60 is the clot strength at 60 minutes as percentage of MCF.

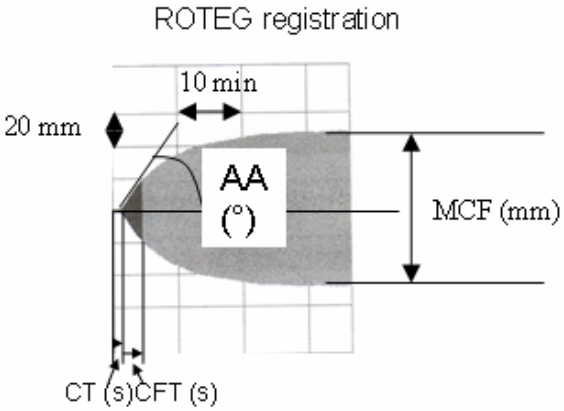

Figure 1A

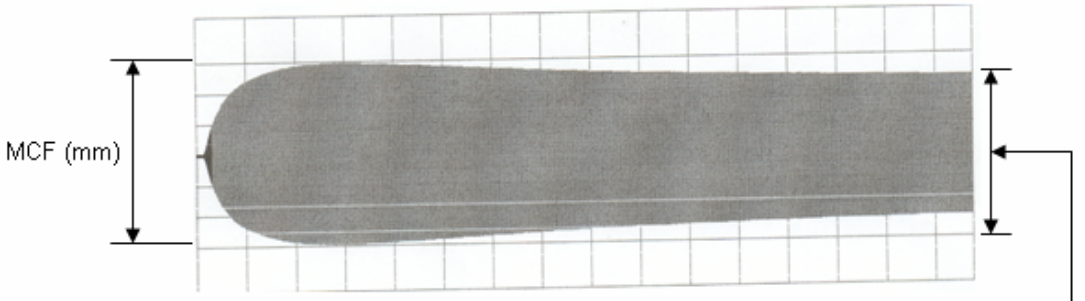

Figure 1B

Clot firmness at 60 min

Figure 2

The figure shows the correlation between ethanol level and the Clot Formation Time (CFT). The CFT is increasingly prolonged by increasing concentrations of ethanol ($p<0.000001$, $r=0.91$)

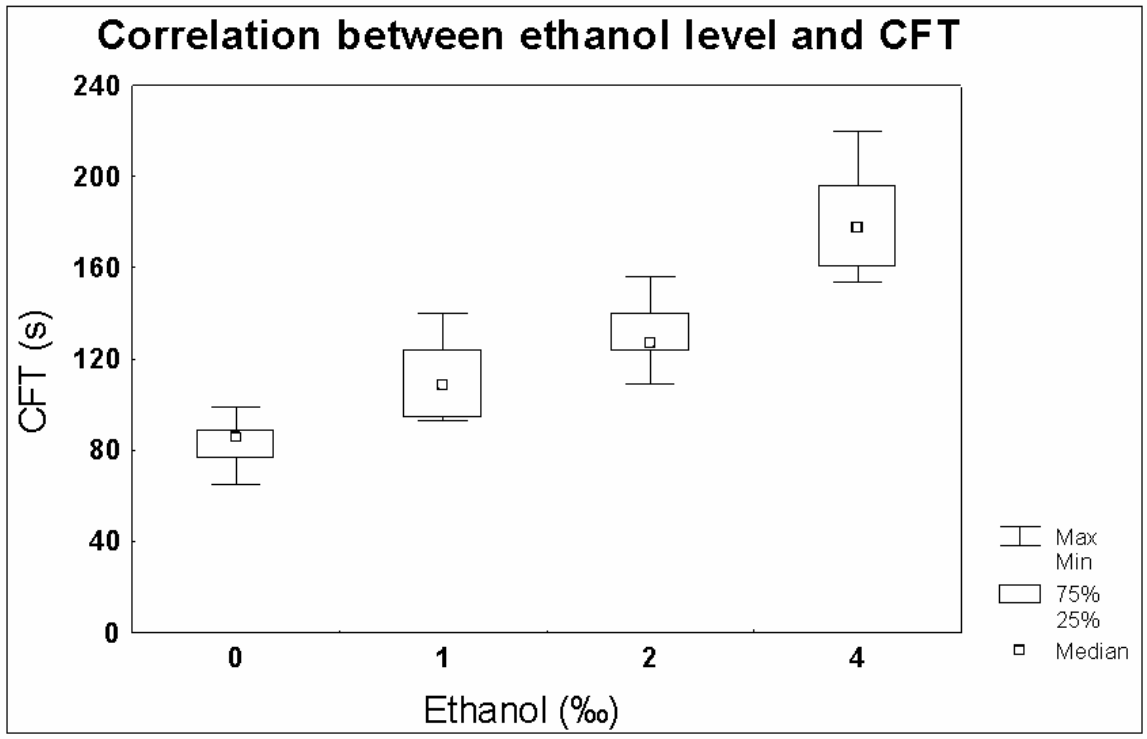

Figure 2

Figure 3A

Original ROTEM tracing at an ethanol level of 0 ‰. The fibrinolysis without ethanol. Lysis can be readily seen as a narrowing ROTEM tracing after MCF has been passed.

Figure 3B

Original ROTEM tracing at an ethanol level of 1 ‰. Fibrinolysis is impaired as compared to figure 3A.

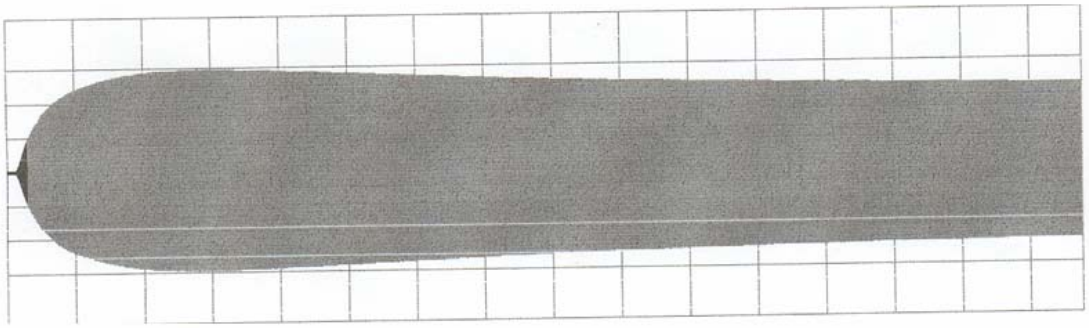

Figure 3A

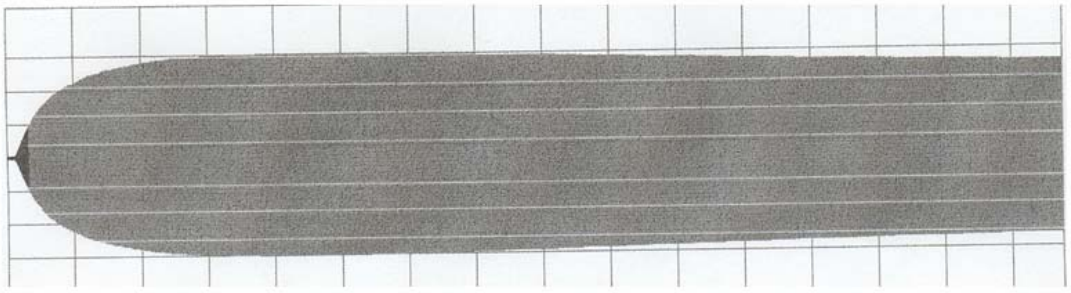

Figure 3B