Thromboelastographic assessment of the impact of mexiletine on coagulation abnormalities induced by air or normal saline intravenous injections in conscious rats

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Thromboelastography; Air embolism; Mexiletine; Coagulation; Animal model

Abstract
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Background: Thromboelastography (TEG) in venous air embolism (VAE) has been poorly studied. We induced coagulation abnormalities by VAE in a rat model, assessed by TEG with and without mexiletine, a lidocaine analogue local anesthetic.
Methods: Twenty-three Sprague Dawley rats instrumented under isoflurane anesthesia and allowed to recover five days prior to the experiments were randomized into three experimental groups: 1) VAE ($n = 6$); 2) VAE and mexiletine ($n = 9$); and 3) normal saline (NS) alone (control group, $n = 8$). Blood samples were collected at baseline, one hour (h) and 24 h in all groups and analyzed by TEG to record the R, K, angle α and MA parameters.
Results: In Group 1, VAE decreased significantly R at 1 h (31%), K at 1 h (59%) and 24 h (34%); α increased significantly at 1 h (30%) and 24 h (22%). While R returned to baseline values within 24 h, K, MA and α did not. In group-2 (Mexiletine + VAE), K and R decreased at 1 h (48% and 29%, respectively) and at 24 h the changes were non-significant. Angle α increased at 1 h (28%) and remained increased for 24 h (25%). In group 3 (NS), only R was temporarily affected. MA increased significantly at 24 h only in the VAE alone group.
Conclusion: As expected, VAE produced a consistent and significant hypercoagulable response diagnosed/confirmed by TEG. Mexiletine prevented the MA elevation seen with VAE and corrected R and K time at 24 h, whereas angle α remained unchanged. Mexiletine seemed to attenuate the hypercoagulability associated with VAE in this experiment. These results may have potential clinical applications and deserve further investigation.

Introduction

Venous air embolism (VAE) has been associated with coagulation abnormalities as air bubbles act as a foreign surface activating the coagulation cascade, contributing to platelet adhesion and aggregation to the vascular surface.[1,2] Air contacts the endothelium, which triggers complex interaction with blood products and fibrinogen, resulting in local fibrin deposition and endothelial expression of platelet activating factor. Leukocytes, fibrinogen, thrombin, and plasma proteins are activated, triggering the activation of the coagulation cascade. VAE may occur either from diving or iatrogenically, such as from central venous catheterization, semi-sitting craniotomy, penetrating and blunt chest trauma, high-pressure mechanical ventilation, thoracocentesis and several other invasive vascular procedures. Over time, the embolic obstruction may change to a thrombotic one that emphasizes the importance of early diagnosis. This hypercoagulable state has been documented after VAE using several isolated methods to establish the haemostatic profile (i.e., prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin and bleeding time).[3,4]

To our knowledge, no reports or studies have used thromboelastography (TEG) to assess the coagulopathy of VAE. TEG, a point-of-care testing for whole blood coagulation, has been used in other models and clinical monitoring as a sensitive method for detecting

hypercoagulable states.[6,7] Presently, TEG is widely used to assess global haemostatic function from a sample of whole blood, e,g., in trauma, obstetrics, cardiovascular surgery, early sepsis, liver transplantation and others.[2,7] Several studies have reported that TEG parameters are interrelated and reflect the initiation of the coagulation cascade from the initial platelet-fibrin interaction through platelet aggregation, clot strengthening, and fibrin cross linkage.[8,9]

There have been preclinical studies showing that various medications, e.g., lidocaine, ketamine, and magnesium sulphate can act as antiplatelet agents or affect coagulation.[10] For instance, lidocaine, a local anesthetic and intravenous antiarrhythmic agent, has been suggested to be of benefit in patients with decompression illness (DCI), mainly for cerebral arterial gas embolism (CAGE),[11–13] possibly by affecting membrane stability.[10] Mexiletine is a Class I-b local anesthetic and lidocaine analog.[14] It exerts similar antiarrhythmic properties and could have some beneficial effects during the treatment of VAE, possibly diminishing the hypercoagulable state.[10,11,13] In contrast to lidocaine, it can be ingested by mouth, increasing its clinical applicability.

This study aimed to evaluate TEG changes in rats subjected to consistent and reproducible VAE, and to assess hypercoagulability changes in the presence and absence of mexiletine, an analogue of lidocaine. The null hypothesis tested was that mexiletine would not reduce the impact in the coagulation system produced by intravascular injection of air in rats. Whether TEG findings following VAE could be applied to humans in the diagnosis and treatment of VAE associated with DCI or surgical procedures is discussed. No similar study has been described in the literature.

Methods

After approval of the experimental protocol by the University of Texas Animal Welfare Committee, 23 Sprague-Dawley rats were included in the study. To avoid damage to the implanted catheters, animals were housed in individual cages in an air-conditioned (22 ± 1°C), light-controlled room (12-hrs light, 12-hrs dark) and were allowed to mobilize freely. Animals had free access to food and water. Their behaviour, posture and appearance were monitored daily.

INSTRUMENTATION

The rats were anesthetized with isoflurane 5%, intubated with a 16-gauge intravenous catheter and mechanically ventilated using a mixture of 30% oxygen, room air and 1.5 % isoflurane. Under sterile conditions, a Tygon® catheter was inserted into the femoral vein for drug administration and tunneled subcutaneously to the dorsum of the neck and secured in place after closure of the incisions. The animals were then allowed five days to recover prior to the start of the experiments and were individually housed and given free access to food and water.

EXPERIMENTAL DESIGN

To minimize the difference among groups, animals were randomized into three groups by a blinded investigator. Group 1 (n = 7, VAE); animals received air (0.5 ml) infused over two minutes (min) via the femoral vein catheter. The catheter was connected to a syringe driver (Medfusion, Medex, Inc; Duluth, GA) with PE tubing and used for drug administration. Group 2 (n = 9; Mexiletine and VAE). Mexiletine was administered in a dose of 10 mg·kg^{-1} IV over two min.[15] Thirty min following mexiletine administration air was infused as described in Group 1. Group 3 (n = 8); to test the adequacy of the TEG responses, animals in this group received NS only and were used as the control group. When the study was initiated, Group 1 included seven animals; Groups 2 and 3 had eight animals each. However, one animal died following surgery (during catheterization) for an unknown reason before the experiment began, resulting in six animals allocated to the VAE group. We added one animal to Group 2 due to the combination of treatments. To prevent artifacts from onset of coagulation to analysis, whole blood samples (with no activator) from all groups were drawn for immediate analysis using TEG (Haemoscope Corp., Skokie, IL) prior to VAE, mexiletine, saline and 1 hour (h) and 24 h thereafter, respectively.

THE SAMPLING PROCEDURES

TEG was performed with blood collected from the previously implanted femoral catheter. Citrate anticoagulation was achieved by collecting 900 µl of blood in 100 µl of 4% sodium citrate (1:10 dilution). Blood samples were gently inverted five times, and were placed on their sides for 30 minutes to allow adequate equilibration of the citrate throughout the sample. At this point, 340 µl of the blood was pipetted gently into a disposable plastic TEG cup containing 20 µl of 0.2M calcium chloride, being careful to avoid mixing. The cup was then transferred to a TEG 5000 thrombelastograph haemostasis analyzer (Haemoscope, Skokie, IL) for assay at 37°C.

MEASUREMENTS

All TEG parameters were recorded from standard tracings: split point (SP, min), reaction time (R, min), coagulation time (K, min), angle (α, degrees), maximum amplitude (MA, mm), clot strength (G, dynes·cm^{-2}), and lysis at 30 min (LY30, %).[7] The SP is a measure of the time to initial clot formation, interpreted from the earliest resistance detected by the TEG analyzer causing the tracing to split; this is the terminus of all other platelet-poor plasma clotting assays (e.g., PT and aPTT). The R value, the time elapsed from start of the test until the developing clot provides enough resistance to produce a 2 mm amplitude reading on the TEG tracing, represents the initiation phase of enzymatic clotting factors. K measures the time from clotting factor initiation (R) until clot formation reaches amplitude of 20 mm. The

angle (α) is formed by the slope of a tangent line traced from the R to the K time measured in degrees. K time and angle (α) denote the rate at which the clot strengthens and is most representative of thrombin cleavage of fibrinogen into fibrin. The MA indicates the point at which clot strength reaches its maximum amplitude in millimeters on the TEG tracing, and reflects the end result of the platelet-fibrin interaction via the GPIIb-IIIa receptors. G is a calculated measure of total clot strength derived from amplitude (A, mm) G ¼ (5000 3 A)/ (100 3 A). The process of clot dissolution, or fibrinolysis, leads to a decrease in clot strength. The LY30 measures the degree of fibrinolysis 30 minutes after MA is reached. A hypercoagulable state was defined by having at least two of the following four TEG parameters: a short R time, a short K time, an increased α angle, and an increased MA.[9]

STATISTICAL ANALYSIS

Data were analyzed by a one-way analysis of variance (ANOVA) to assess overall significance among groups. When differences were significant, multiple pairwise-comparisons were performed using the post hoc Dunnett's *t* test. When changes were significant, the magnitude of change in each experimental condition was compared using an unpaired *t*-test, $P < 0.05$ was considered significant. The target sample size of six to nine animals in each group was chosen to provide an 80% power analysis and to detect the size of the effect at 1.33 between control and treatments. Data are expressed as mean ± standard deviation.

Results

The results are presented in Table 1 and Figures 1 A–D. VAE alone (Group 1) affected all TEG parameters. Air infusion decreased significantly R at 1 h (54%), K at 1 h and 24 h (59% and 34%, respectively); angle α increased significantly at 1 h and 24 h (30% and 22%, respectively). MA rose steadily to achieve a significant increase at 24 h.

In contrast, in Group 2 (VGE + mexiletine) fewer TEG parameters were significantly affected. K, R and MA had no significant changes at 1 h and 24 h. However, angle α increased significantly at 1 h and 24 h by 28% and 25%, respectively.

In Group 3 (control group), saline induced a significant decrease in R at 1 h by 48% but the R-values returned to normal at 24 h. K, (α), and MA were not significantly affected.

Mostly, R returned to baseline in all groups. K did not return to baseline following VAE (Group 1) but returned to baseline in the presence of mexiletine (Group 2). Angle α did not return to baseline at 24 h following VAE either alone (Group 1) or despite mexiletine (Group 2). MA increased significantly following VAE administration in Group 1 at 24 h.

Discussion

In our study, VAE produced a hypercoagulable state with a significantly shortened R and K time and increased α angle occurring at 1 h and 24 h after air was injected intravenously. These findings are in agreement with previous studies using other methods (i.e., PT, aPTT, thrombin and bleeding time) to assess the haemostatic process.[3,16,17] Mexiletine appeared to ameliorate the changes at 1 h and correct the hypercoagulability at 24 h, thus rejecting the null hypothesis.

TEG identified the viscoelastic changes associated with the coagulation abnormalities induced by the exposure to air with

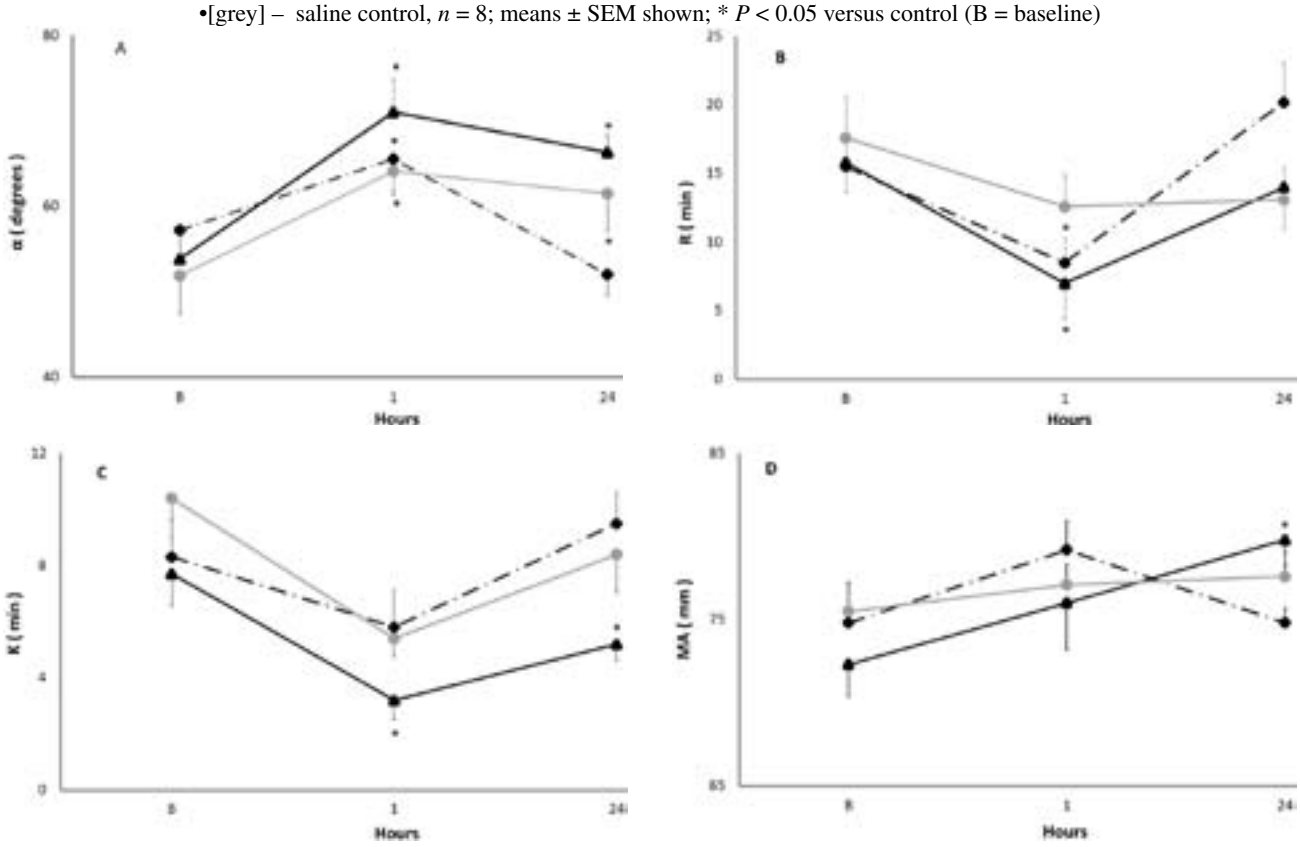
Table 1

Changes in four of the measured components of elastography (see text for details) in the three experimental groups: venous air embolism (VAE); VAE + mexiletine pretreatment; saline control (means and standard deviation shown); * $P < 0.05$; ** $P < 0.01$

Group	Baseline	1 hour	24 hours
α (degrees)			
VAE	53.8 ± 7.7	71.0 ± 9.4**	66.3 ± 4.9*
Saline	51.9 ± 11.1	64.1 ± 7.0*	61.5 ± 11.1*
VAE + mexilitine	57.2 ± 7.6	65.5 ± 9.1*	52.0 ± 5.9*
R (min)			
VAE	15.8 ± 5.4	7.0 ± 6.3**	14.0 ± 7.5
Saline	17.6 ± 7.4	12.6 ± 5.6	13.1 ± 5.7
VAE + mexilitine	15.5 ± 4.6	8.5 ± 4.4*	20.2 ± 6.9
K (min)			
VAE	7.7 ± 2.8	3.2 ± 1.7**	5.2 ± 1.5*
SALINE	10.4 ± 3.4	5.4 ± 1.6**	8.4 ± 8.3
VAE + MEXIL	8.3 ± 3.2	5.8 ± 3.3	9.5 ± 2.7
MA (mm)			
VAE	72.3 ± 4.7	76.0 ± 7	79.8 ± 3.9*
Saline	75.5 ± 4.2	77.1 ± 3	77.6 ± 3.5
VAE + mexilitine	74.8 ± 2.4	79.2 ± 4.2	74.8 ± 2.1

Figure 1

Effects of venous air embolism (0.5 ml air over 2 min) on elastographic components (see text for details) with or without IV mexiletine pretreatment in rats; A – angle (α), B – R, C – K, D – MA (see text for details); ▲ – VAE, $n = 6$; •[black] – VAE + mexiletine, $n = 9$; •[grey] – saline control, $n = 8$; means ± SEM shown; * $P < 0.05$ versus control (B = baseline)



and without mexiletine or NS in the animals' blood samples. Our results parallel previous studies showing that the presence of an air-blood interface activates the coagulation cascade, adhesion and aggregation of platelets.[1,2,17–19] It has also been shown that bubbles induce platelet aggregation regardless of the type of gas in the bubbles (e.g., He, N_2, or O_2-CO_2-N_2 mixture).[19] Furthermore, the contact of the gas with rat's blood could bring coagulation events, activating the complement system and fibrinolytic cascade.[20]

The results of this experiment could be explained by the inherent properties of mexiletine. Numerous studies have shown that local anesthetics block membrane ion channels, stabilize the platelet membrane, inhibit alpha granule release, prevent thrombin and ADP-induced platelet aggregation.[21–24] TEG has been shown to detect the *in vitro* coagulation and fibrinolysis alterations produced by lidocaine.[21] A recent study also showed that substances used during anesthesia, like lidocaine and ketamine, have antiplatelet effects.[25]

Haemodilution has also been associated with coagulopathies; a hypercoagulable state has been described following dilution with normal saline. Some researchers have shown the value of *in vitro* haemodilution with normal saline and the correlation of their results with the incidence of deep venous thrombosis in patients following abdominal surgery.[26] However, the authors determined that to reach a hypercoagulable state, it was necessary to dilute the blood between 75 to 85%. In another study, normal saline induced a significant decrease in R and MA as well as a significant increase in the alpha angle in humans but only after reaching 30% haemodilution.[27] The small volume (0.5 ml) of normal saline in our experiment was not enough to reach that degree of haemodilution (in a 250–300 gm rat the blood volume is about 16 to 21 ml). Therefore, although we observed a significant R decrease, it was temporary and not accompanied by any other changes necessary to reach a true hypercoagulable state (requiring two or more TEG parameters affected).

LIMITATIONS OF THE STUDY

Despite this study being a controlled animal laboratory experiment, there are several limitations to consider. First, after an extensive literature review, this seems to be the only study to date addressing whether mexiletine had any effect on the coagulation abnormalities induced by air embolism and the ability to measure the effects with TEG; this lack of studies limits our ability to compare our results with others. Secondly, the small size and, therefore, small blood volume of the animals impeded the ability to perform a more comprehensive haematological study. Finally, there is a need for confirmatory studies in similar or comparable settings.

Conclusions

VAE in rats produced a persistent hypercoagulable state that was attenuated by the lidocaine analogue, mexiletine, as assessed by TEG at 1 h and at 24 h. These results suggest that mexiletine may have a potential role in the clinical management of VGE. Given the lack of comparable studies, more laboratory and clinical studies are needed to confirm our findings.

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Conflict of interest

The authors do not have any significant conflict to declare associated with this study.

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