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Thrombelastography indicates limitations of animal models of trauma-induced coagulopathy

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ABSTRACT

Background: Thrombelastography (TEG) has been used to characterize the coagulation changes associated with injury and shock. Animal models developed to investigate trauma-induced coagulopathy (TIC) have failed to produce excessive bleeding. We hypothesize that a native TEG will demonstrate marked differences in humans compared with these experimental models, which explains the difficulties in reproducing a clinically relevant coagulopathy in animal models.

Methods: Whole blood was collected from 138 healthy human volunteers, 25 swine and 66 Sprague–Dawley rats before experimentation. Citrated native TEGs were conducted on each whole blood sample within 2 h of collection. The clot initiation (R-time, minutes), angle (degrees), maximum amplitude (MA; millimeter), and lysis 30 min after MA (LY30; percentage) were analyzed and contrasted between species with data represented as the median and 25th to 75th quartile range. Difference between species was conducted with a Kruskal–Wallis test with alpha adjusted with a Bonferroni correction for multiple comparisons (alpha = 0.016).

Results: Median R-time (clot initiation) was 14.65 min (IQR: 13.2-16.3 min) for humans, 5.7 min (4.9-8.8) for pigs, and 5.2 min (4.4-6) for rodents. Humans had longer R-times than both pigs ($P < 0.0001$) and rats ($P < 0.0001$); pigs were not different from rats ($P = 0.4439$). Angle (fibrin cross-linking) was 42.3° (interquartile range [IQR]: 37.5-50.2) for humans, 71.7° (64.3-75.6) for pigs, and 61.8° (56.8-66.7) for rats. Humans had reduced angle compared with both pigs ($P < 0.0001$) and rats ($P < 0.0001$); pigs were not different from rats ($P = 0.6052$). MA (clot strength) was 55.5 mm (IQR: 52.0-59.5) for humans, 72.5 mm (70.4-75.5) for pigs, and 66.5 mm (56.5-68.6) for rats. Humans had reduced MA compared with both pigs ($P < 0.0001$) and rats ($P < 0.0001$); pigs were not different from rats ($P = 0.0161$). LY30 (fibrinolysis) was 1.5% (IQR: 0.975-2.5) for humans, 3.3% (1.9-4.3) for pigs, and 0.5% (0.1-1.2) for rats. Humans

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had a lesser LY30 than pigs ($P = 0.0062$) and a greater LY30 than rats ($P < 0.0001$), and pigs had a greater LY30 than rats ($P < 0.0001$).

Conclusions: Humans, swine, and rodents have distinctly different coagulation systems, when evaluated by citrated native TEG. Animals are hypercoagulable with rapid clotting times and clots strengths nearly 50% stronger than humans. These coagulation differences indicate the limitations of previous models of trauma-induced coagulopathy in producing coagulation abnormalities associated with increased bleeding. The inherent hypercoagulable baseline tendencies of these animals may result in subclinical biochemical changes that are not detected by conventional TEG and should be taken into consideration when extrapolated to clinical medicine.

Introduction

Thrombelastography (TEG)-based resuscitation compared with conventional laboratory coagulation assays reduces mortality in trauma patients undergoing massive transfusion.[1] The implementation of TEG into clinical practice has been because of the ability to characterize the coagulation changes associated with injury and hemorrhagic shock.[2,3] However, it has been challenging to develop animals that replicate the excessive bleeding observed in seriously injured patients.[4] This is likely because of inherent differences between human and animal coagulation systems. Previous work has emphasized differences in various coagulation components in animal models in an attempt to define the ideal model that mimics the coagulation profile of humans.[5] Furthermore, rodents are resistant to shock-induced fibrinolysis, and the amount of exogenous tissue plasminogen activator (t-PA) to elicit fibrinolysis in whole blood in a rat is 10-fold higher than a human.[6]

Viscoelastic assays provide a comprehensive assessment of clot formation and clot remodeling and degradation. There is a paucity of data that contrasts TEG measurements between animal models to human subjects. Therefore, the purpose of this study was to explain the difficulties in reproducing a clinically relevant coagulopathy in animal models through discrepancies between animal and human TEG indices. We hypothesize that TEGs demonstrate substantial differences in humans compared with animals used in these experimental models, which underscores the challenges in reproducing a clinically relevant coagulopathy.

Materials and methods

Materials

Whole blood was collected from 138 healthy human volunteers under the Colorado Multiple Institutional Review Board protocol number 14-0366. Whole blood was also collected from 25 outbred pigs (Colorado State University farm, Fort Collins, CO) and 66 Sprague–Dawley rats (Envigo, Indianapolis, IN) before experimentation, through a protocol approved by the University of Colorado Institutional Animal Care and Use Committee (Protocol 90814(11)1D, 90814(12)1D, and 90811(11)1D). Human data were compiled from a database evaluating the normal baseline viscoelastic parameters of healthy volunteers. Pig and rat data were compiled from a database of TEGs collected on previously conducted animal experiments. There were no additional animals used for these experiments. The primary outcome was differences in baseline viscoelastic parameters.

Blood collection

Human blood samples were collected from healthy volunteers after informed consent was obtained. Blood was collected in citrate (3.2%) tubes (Vacutainer; Becton-Dickinson, Franklin Lakes, NJ) after venipuncture of the antecubital fossa. Rat blood was collected after femoral artery cannulation. The process of rat blood collection was done as previously described.[7] Pig blood was similarly collected after femoral artery cut down and cannulation. Rat and pig blood was collected in 3.2% citrate similar to that of human blood. For humans and pigs, blood was collected in a single 3.2% citrate tube. For rats, 900 μL of blood was collected from the femoral artery cannula in 100 μL of sodium citrate in a standardize method as previously reported.[7] All animal studies and procedures were carried out in accordance with University of Colorado University of Colorado Institutional Animal Care and Use Committee guidelines and in accordance with the National Institute of Health guide for care and use of laboratory animals.

Thrombelastography

Citrated whole blood samples were analyzed at 37°C using a Model 5000 Thrombelastograph Haemostasis Analyzer (Haemonetics, Boston, MA) per the manufacturer's instructions. Native TEG was used because standard activators, such as tissue factor or kaolin, can mask subtle changes in coagulation.[7] The following parameters were recorded from the temporal impedance tracings of the TEG: R-time (minutes), angle (α, degrees), maximum amplitude (MA; millimeter), and lysis 30 min after MA (LY30; percentage). These values were contrasted between species with data represented as the median and 25th-75th quartile range.

Statistics

Statistics were done using GraphPad Prism version 7.0a (GraphPad Software, Inc, La Jolla, CA) and Excel version 12.2.5 (Microsoft Corporation, Redmond, WA). Citrated native TEG