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Short communication,

Endotoxin Potentiates the Procoagulant Effects of Tissue Factor

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Abstract:

Tissue Factor is the initiator of the extrinsic pathway of blood coagulation. It is generated in blood as a result of a variety of diseases and conditions and is, in part, responsible for the majority of morbidity and mortality in humans. In spite of this potential release during major trauma or diseases, there are few rapid clinical whole blood coagulation tests that can monitor the early generation of tissue factor. This study will evaluate whether endotoxin enhances the procoagulant effects of tissue factor on human blood and plasma.

Introduction:

Tissue factor (TF), also known as thromboplastin, plays a fundamental role in the initiation of the blood coagulation cascade. TF forms a complex with factor viia, inducing the activation of factor IX and factor X. These events are followed by the conversion of prothrombin to thrombin, which triggers fibrin formation, platelet activation, and finally the production of a blood clot [1]. A major complication of endotoxemia and sepsis, disseminated intravascular coagulation (DIC), is believed to be triggered mainly by the TF-dependent pathway of coagulation [2]. DIC can induce the formation of thrombi within blood vessels that may impede the delivery of blood to organs that is essential for survival. As a result, multisystem organ failure and subsequently mortality may occur [3].

During gram-negative septic shock, lipopolysaccharide (LPS, endotoxin) upregulates cellular TF expression [2]. Additionally, following exposure to endotoxin, proinflammatory cytokines are produced. In particular, tumor necrosis factor-α (TNF-α) and IL-1β are produced, ultimately leading to elevated levels of IL-6 and IL-8, both of which are associated with increased disease severity. TNF-α also promotes plasminogen activator inhibitor 1 (PAI-1) production, resulting in the inhibition of fibrinolysis. Consequently, there is a net deposition of fibrin within the microvasculature which can contribute to the development of DIC [4]. Recent studies have shown that bloods incubated with *E. coli* endotoxin for a period of two hours have an accelerated clotting time [5]. During briefer incubation periods, the effect of LPS on TF activity may not be observed due to the lapse in time between exposure to endotoxin and induction of TF expression. The purpose of this study is to demonstrate that, in addition to inducing monocytic and endothelial cell TF activity; endotoxin amplifies the procoagulant effects of TF shortly after exposure to endotoxin.

Methods:

Two-day-old samples of citrated whole blood (CWB) were obtained from the University Hospital's clinical labs according to IRB protocol. In the first experiment, 8 samples of CWB were each divided into two aliquots, one containing 500 µl of plasma isolated from CWB and the other containing 500 µl of CWB. Each aliquot was incubated at 37℃ for 10 minutes. Three hundred µl of each aliquot were added to cuvettes containing 32 µl 0.1 M $cacl_2$ to induce clot formation. The Sonoclot Coagulation Analyzer (Sienco, Wheat Ridge, CO, USA), which measures changes in viscosity due to fibrin strand formation, was then employed to determine clotting time. In the second experiment, 14 samples of CWB were divided into the following four aliquots. The first aliquot, comprised of 500 µl of CWB, served as a control. The second contained 495 µl of CWB with a final tissue factor concentration of 1%. The third consisted of 490 µl

of CWB with a final endotoxin concentration of 10 µg/ml. The fourth was composed of 485 µl of CWB with a final endotoxin concentration of 10 µg/ml and tissue factor concentration of 1%. These aliquots were incubated and clot formation was induced as in the first experiment. In the third experiment, 8 samples of plasma isolated from CWB were divided into four aliquots as in the second experiment. To compare clotting times; a statistical program was used to perform repeated measure analysis of variance (ANOVA) and paired t-tests. Significance was defined as test values with $p < 0.05$.

Results:

Experiment 1
Mean Plasma vs Blood Clotting Time (sec)

	Plasma	Blood
Mean ± SD	365 ± 41	283 ± 30

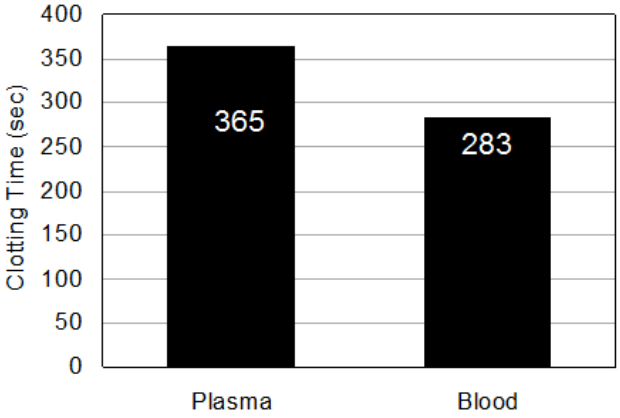

Table 1. Mean clotting time and standard deviation (seconds) of untreated plasma and blood are shown.

Figure 1. Mean clotting time (seconds) of untreated plasma and CWB.

Two-tailed paired t-tests revealed that CWB clots significantly faster than plasma ($p < 0.0005$). Additionally, in every trial performed, CWB clotted faster than plasma.

Experiment 2
Mean Blood Clotting Time (sec)

	Control	Tissue Factor	Endotoxin	Endotoxin + Tissue Factor
Mean ± SD	285 ± 60	220 ± 62	285 ± 64	174 ± 47

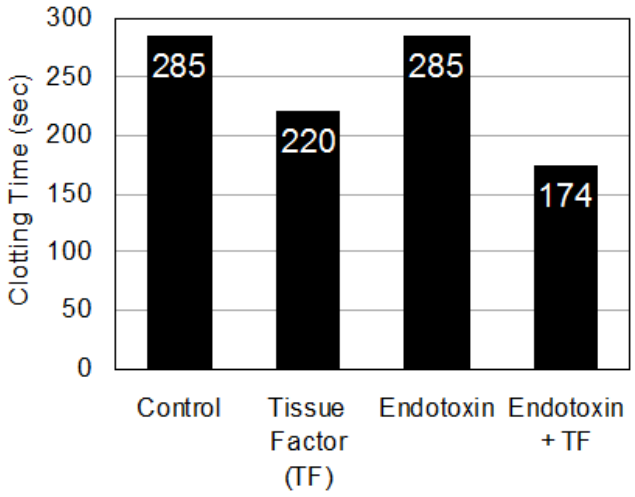

Table 2. Mean clotting time and standard deviation (seconds) of CWB treated with tissue factor alone, endotoxin alone, or both tissue factor and endotoxin are shown.

Figure 2. Mean clotting time (seconds) of CWB incubated with tissue factor alone, endotoxin alone, or both tissue factor and endotoxin.

Two-tailed paired t-tests indicated that, compared to the control, tissue factor alone significantly shortened blood clotting time ($p < 0.001$), but endotoxin alone did not significantly reduce blood clotting time ($p = 0.99$). Endotoxin and tissue factor, when combined, significantly decreased blood clotting time, not only when compared to the control ($p < 0.0001$), but also when compared to tissue factor alone ($p < 0.001$). Furthermore, in every trial performed, blood clotting time was shorter with endotoxin and tissue factor combined than with tissue factor alone.

Experiment 3
Mean Plasma Clotting Time (sec)

	Control	Tissue Factor	Endotoxin	Endotoxin + Tissue Factor
Mean ± SD	399 ± 48	242 ± 34	403 ± 43	176 ± 53

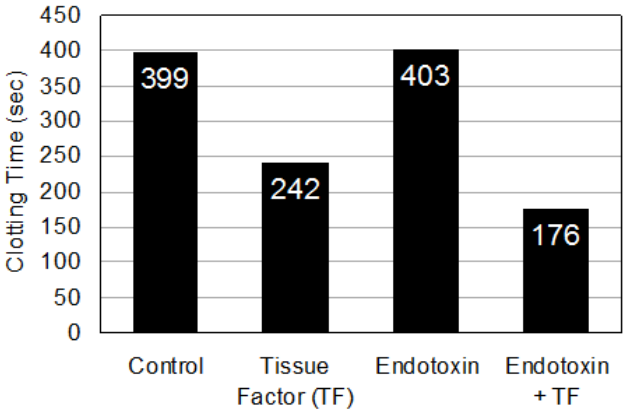

Table 3. Mean clotting time and standard deviation (seconds) of plasma treated with tissue factor alone, endotoxin alone, or both tissue factor and endotoxin are shown.

Figure 3. Mean clotting time (seconds) of plasma incubated with tissue factor alone, endotoxin alone, or both tissue factor and endotoxin.

Two-tailed paired t-tests demonstrated that tissue factor alone significantly lowered plasma clotting time relative to the control ($p < 0.0001$), while endotoxin alone did not ($p = 0.83$). Endotoxin and tissue factor combined significantly lessened plasma clotting time relative to both the control ($p < 0.0001$) and tissue factor alone ($p < 0.005$). Moreover, in every trial performed, plasma clotting time with endotoxin and tissue factor combined was shorter than with tissue factor alone.

Discussion:

The assay that demonstrated the difference in clotting time between CWB and plasma highlights the limitations of assays such as prothrombin time (PT) and activated partial thromboplastin time (aptt), which require the addition of the reagents, tissue factor and phospholipid (partial thromboplastin), respectively. In particular, due to the addition of reagents that reduce clotting time to a range that is in seconds, PT and aptt may fail to detect a hypercoagulable state. The significant reduction in clotting time when both endotoxin and TF are present, versus when only TF is present, indicate that endotoxin potentiates the effects of TF to promote clot formation. The lack of alteration in clotting time relative to the control in the presence of endotoxin but absence of TF confirms that the synergistic action of endotoxin and tissue factor is responsible for reducing clotting time. Additionally, the complementary results obtained with plasma in the place of CWB suggest that the mechanism by which endotoxin enhances the effects of TF involves a constituent of plasma. These results suggest that hypercoagulable conditions increase susceptibility to DIC and its deleterious complications following exposure to endotoxin. Future studies should be conducted to investigate the mechanism by which endotoxin intensifies the effects of TF.

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