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Fibrinolytic activity of endothelial cells from different venous beds

*Neil G. Kumar, MD, Amanda Clark, BS, Elisa Roztocil, BS, Xzabia Caliste, MD, David L. Gillespie, MD,[1] and John P. Cullen, PhD**

Division of Vascular Surgery, Department of Surgery, University of Rochester Medical Center, Rochester, New York

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ABSTRACT

Background: Little is known about the molecular biology of endothelial cells from different venous vascular beds. As a result, our treatment of deep vein thrombosis and pulmonary artery embolism remain identical. As an initial step in understanding venous thromboembolic disease in the trauma and surgical patients, this study sought to investigate the balance between coagulation and fibrinolysis in the pulmonary and deep venous vascular beds and how trauma might influence this balance.

Materials and methods: Confluent human iliac vein endothelial cells (HIVECs) and human pulmonary artery endothelial cells (HPAECs), were cultured in the absence or presence of tumor necrosis factor (TNFα; 10 ng/mL) for 24 h. The expression of mediators of coagulation and fibrinolysis were determined by Western blot analysis, and plasminogen activator activity was determined by a fibrin clot degradation assay.

Results: After TNFα stimulation, there was decreased expression of endothelial protein C receptor and thrombomodulin in both HIVECs and HPAECs. TNFα stimulation increased urokinase plasminogen activator expression in both HIVECs and HPAECs. There was an increase in the expression of tissue plasminogen activator and plasminogen activator inhibitor-1 in response to TNFα in HPAECs, but not in HIVECs. There was significantly greater clot degradation in the presence of both the conditioned media and cell extracts from HIVECs, when compared with HPAECs.

Conclusions: HPAECs and HIVECs react differently in terms of fibrinolytic potential when challenged with a cytokine associated with inflammation. These findings suggest that endothelial cells from distinct venous vascular beds may differentially regulate the fibrinolytic pathway.

1. Introduction

Major trauma is a hypercoagulable state often complicated by venous thromboembolism, with one study showing that up to 59% of major trauma patients developed venous thromboembolism without pharmacologic (anticoagulant) thromboprophylaxis within 14 d of injury [1]. Current recommendations are for all trauma patients with even a single risk factor to receive immediate thromboprophylaxis using graduated compression stockings and sequential compression devices, with consideration given to starting pharmacologic thromboprophylaxis with low molecular weight heparin as soon

* *Corresponding author*. Department of Surgery, University of Rochester Medical Center, 601 Elmwood Avenue, Box SURG, Rochester, NY 14642. Tel.: +1 5852735666; fax: +1 5857567819.
E-mail address: john_cullen@urmc.rochester.edu (J.P. Cullen).
[1] *Present address:* Division of Vascular Surgery, Heart and Vascular Center, Southcoast Healthcare System, Fall River/New Bedford, MA.
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as it is considered safe; usually within 72 h [2]. Despite this, thromboprophylaxis in trauma is variably performed for fear that pharmacologic anticoagulation could pathologically exacerbate hemorrhage [3]. Misperceptions of bleeding risk have led to a near exponential rate in the use of prophylactic retrievable inferior vena cava filters placed in trauma patients as a means of limiting the morbidity and mortality of venous thromboembolism instead of evidenced-based anticoagulation [4–6]. In addition to the bleeding risk that is felt to be inherent with pharmacologic thromboprophylaxis using anticoagulants, heparin-derived compounds also carry with them the immunologic risk of heparin-induced thrombocytopenia [7]. Heparin-induced thrombocytopenia can range from an asymptomatic drop in the platelet counts to a deadly syndrome involving bleeding from thrombocytopenia coupled with arterial and venous thrombosis.

The cytokine response to trauma is complex, but has been found to include increased levels of tumor necrosis factor α (TNFα) [8–11]. Interestingly, elevated levels of TNFα may be associated with venous thrombosis [12]. Undoubtedly, the trauma patient's increased risk for venous thromboembolism is multifactorial, but when the previously described observations regarding TNFα are taken together, it is possible that TNFα in part, may contribute to this increased susceptibility to venous thromboembolism. We hypothesize that TNFα is a systemic mediator that is responsible for the endothelial response to trauma. Intriguingly, Velmahos *et al.* [13] found that only seven of forty-six trauma patients who experienced pulmonary embolism (PE) had any evidence of preexisting deep vein thrombosis (DVT). This study suggests that in trauma, *de novo* pulmonary thrombosis (as opposed to PE) may play a larger role in trauma-related venous thromboembolism than originally hypothesized. These findings are further supported by a previous study that demonstrated that only 30% of patients with fatal PE at autopsy were found to have previous DVT [14]. In addition, a study of predictors of early *versus* late timing of PE after traumatic injury suggests that early PE after trauma may occur as a result of unknown biochemical processes and may not even originate in peripheral veins [15]. However, the question arises if the endothelial response to trauma in the pulmonary artery is inherently different from that in the endothelial cells in the iliac veins [16]. In the present study, we seek to answer this question. Specifically, we investigated the differential response of the endothelial cells of the deep venous system of the lower extremities and the endothelial cells of the pulmonary artery in an *in vitro* cell culture model.

2. Materials and methods

2.1. Cell culture

Human pulmonary artery endothelial cells (HPAEC; catalog ID WC00107) and human iliac vein endothelial cells (HIVEC; catalog ID WC00110) were purchased from the Coriell Institute for Medical Research (Camden, NY). Cells were grown to confluence in Medium 199 (Corning Cellgro, Manassas, VA) supplemented with 10% heat-inactivated fetal calf serum (Fisher Scientific, Pittsburgh, PA), unfractionated heparin (Sagent Pharmaceuticals, Schaumburg, IL), endothelial cell growth supplement (BD Biosciences, Bedford, MA), penicillin-streptomycin (Invitrogen, Carlsbad, CA), and amphotericin B (Invitrogen). All cells were cultured at 37°C with 5% carbon dioxide. Additionally, the cells were grown on sterile culture plates, which were pretreated with 0.1% gelatin as an attachment factor. Cells were assessed for endothelial cell phenotype by morphology and expression of platelet endothelial cell adhesion molecule (PECAM-1, Fig. 1). Endothelial cells between passages three and six were used for all experiments.

2.2. TNFα treatment

A stock concentration of 10 ng/μL of TNFα (BD Pharmingen, San Jose, CA) in phosphate buffered saline (PBS) was made fresh and diluted in culture medium for a final concentration of TNFα

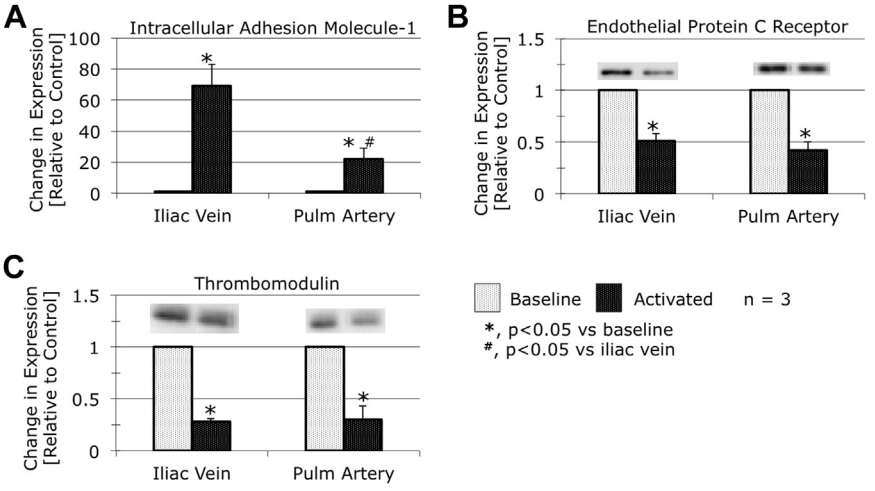

Fig. 1 – Effect of TNFα on HIVEC and HPAEC expression of ICAM-1, EPCR, and TM. Endothelial cells were incubated with TNFα (10 ng/mL) for 24 h and (A) surface ICAM-1 expression was determined by FACS analysis, or (B) and (C) EPCR and TM expression were determined by Western blot analysis. Representative Western blot and cumulative protein data are shown. *P < 0.05 *versus* control, #P < 0.05 *versus* iliac vein (*n* = 3).