Diabetic Complications Consortium

Application Title: Arsenic Exposure, Endothelial Function and Platelet Activity in Type 2 Diabetes

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1. Project Accomplishments:

There are five major accomplishments with this project. First, we demonstrate that concentrations of inorganic arsenic (iAs) relevant to current exposure in the United States are associated with increased platelet activation and aggregation. Second, we show for the first time that a concentration of glucose common in type 2 diabetes (T2D) potentiates platelet activation induced by low-level inorganic arsenic. Third, we demonstrate that increased concentrations of arsenic and glucose acted synergistically to increase megakaryocyte adhesion. Fourth, we show that low-level arsenic substantially increased markers of endothelial adhesion in human aortic endothelial cell culture compared to no arsenic exposure. These findings suggest that alterations in platelet function may be a pathway through which exposure to environmental toxicants such as iAs increase cardiovascular (CVD) risk, particularly for patients with T2D. Finally, the investigations of this Pilot and Feasibility project were instrumental in supporting two grant submissions. The first is the PIs K23 application which received a competitive score (IF 20; NHLBI 2015 payline 31). The second is for the Trial to Assess Chelation Therapy 2 (NCCIH; UH2 mechanism) for which this P&F project provided novel data on the enhanced effects of environmental exposures among patients with T2D.

2. Specific Aims:

Specific Aim 1: To investigate whether increasing concentrations of glucose modify the effects of low to moderate concentrations of sodium arsenite on platelet function and aggregability.
Results: Healthy donor whole blood was prepared in a standard fashion and incubated with 0μM and 10μM sodium arsenite, a concentration clinically relevant to United States iAs exposure. iAs-induced platelet activation was assessed by unstimulated and thrombin-stimulated p-selectin expression and monocyte-platelet and leukocyte-platelet aggregation (MPA and LPA, respectively). All markers of platelet activation increased significantly with 10μM vs. 0μM iAs ($P<0.05$ for all; **Figure 1**). As expected, platelet activity increased with higher concentrations of D-glucose. After incubation of whole blood at hyperglycemic concentrations of D-glucose (15mM ≈270 mg/dl), platelet activity increased in response to subthreshold concentrations of iAs ($P<0.01$ for 1μM and 5μM iAs exposure). In contrast, platelet activity did not increase in response to subthreshold iAs after incubation with euglycemic concentrations D-glucose (5mM ≈90mg/dl; **Figure 2**). Next, we show that increasing iAs concentrations in both 5mM and 25mM concentrations of glucose significantly increase percent area of megakaryocyte adhesion ($P < 0.05$ for both) as illustrated in the representative photomicrographs shown in Figure 3. Ongoing experiments are comparing markers of megakaryocyte adhesion between conditions of 5mM and 25mM glucose with increasing sub-threshold iAs concentrations.

Figure 1.

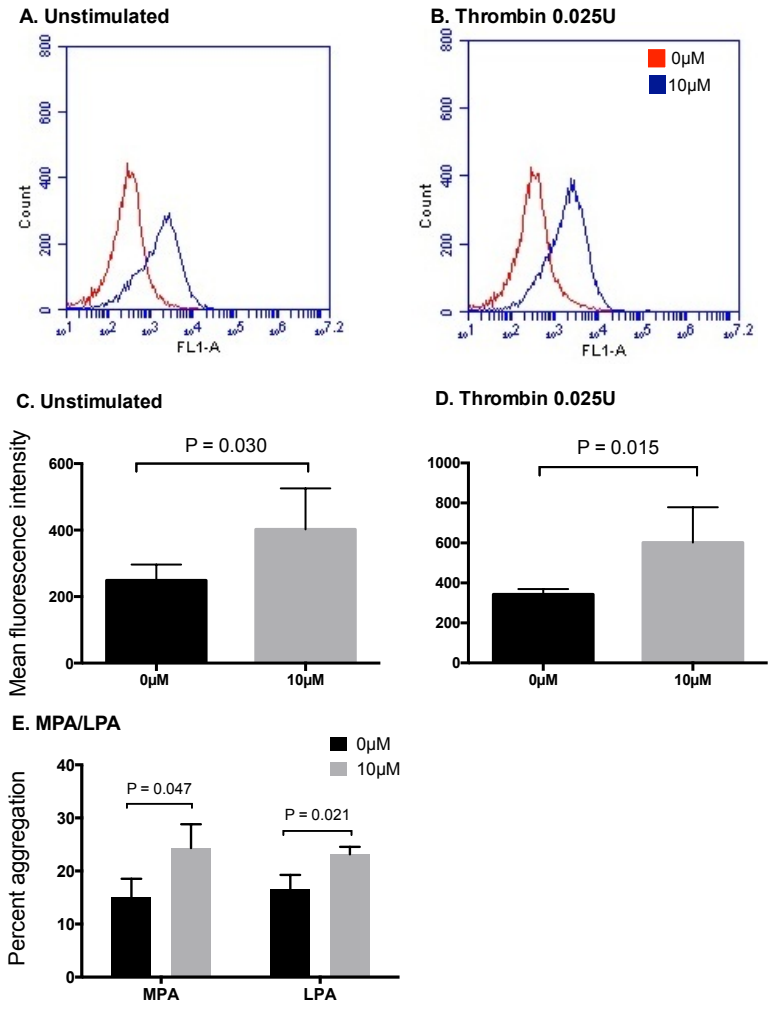


Figure 2.

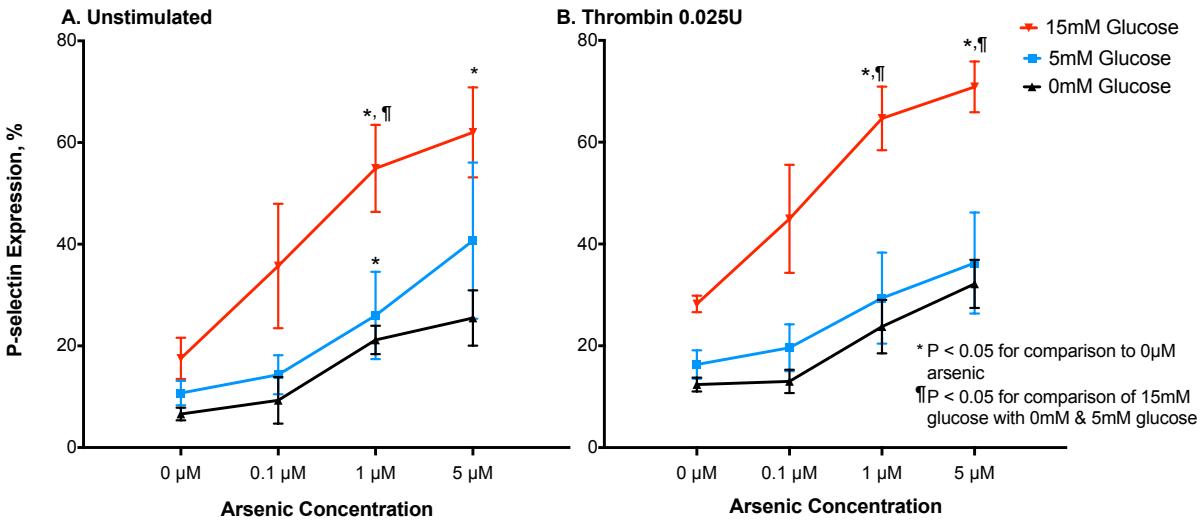
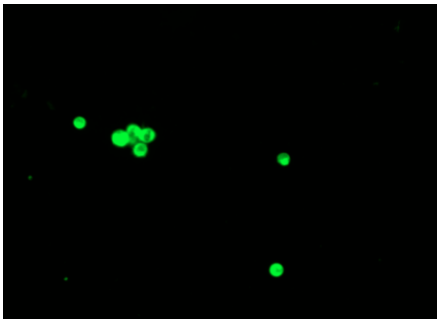


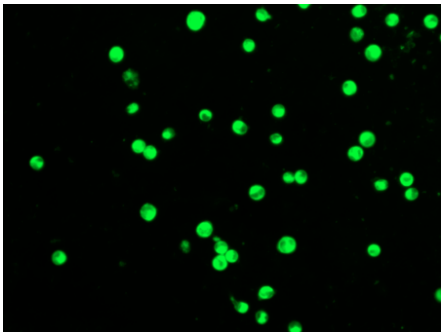
Figure 3.

5mM Glucose

A. 0µM iAs

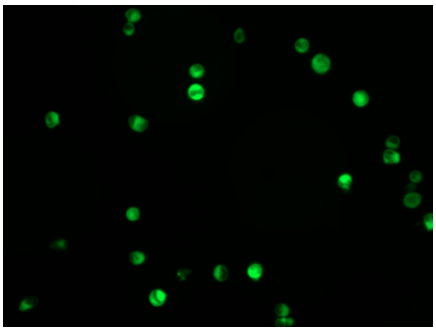


B. 10µM iAs

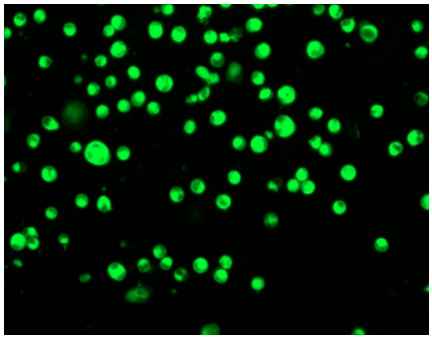


25mM Glucose

C. 0µM iAs

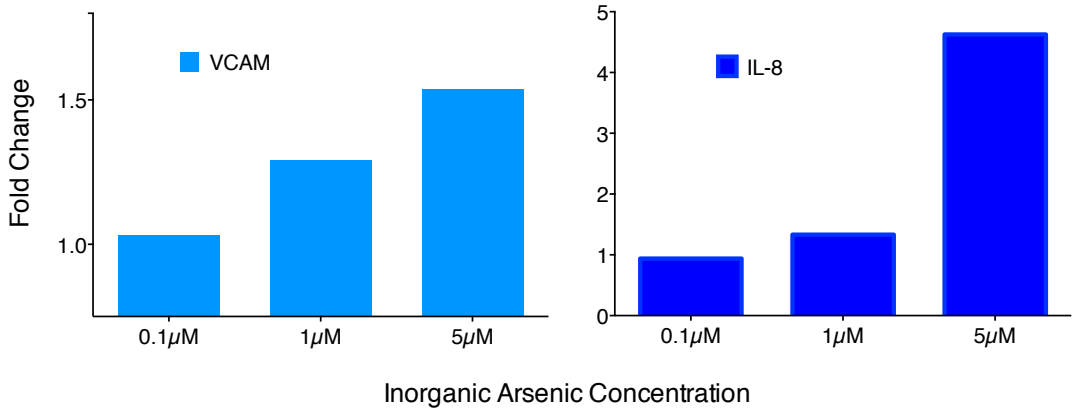


D. 10µM iAs



Specific Aim 2: To investigate whether increasing concentrations of glucose modify the effects of low to moderate concentrations of sodium arsenite on markers of human aortic and coronary endothelial cell dysfunction.
Results: We developed endothelial cell cultures of second to third passage human aortic endothelial cells (HAEC) from two healthy non-smoking, non-diabetic human donors. These HAECs were incubated for 24 hours with 0.1µM, 1µM and 5µM sodium arsenite. RNA from cell cultures was extracted using a Trizol isolation for gene expression and cDNA was synthesized. Conventional reverse- transcriptase (RT)-PCR quantification was then performed for endothelial markers including vascular cell adhesion molecule-1 (VCAM) and interleukin-8. As demonstrated in **Figure 4**, increasing concentrations of sodium arsenite at levels relevant to current US exposure resulted in a nearly 5-fold increase in IL-8 expression, and a greater than 50% increase in expression of VCAM. We are in the process of optimizing cell conditions to model clinically relevant concentrations of glucose exposure (5mM, 10mM and 15mM) with a fixed concentration of insulin at 100nM as a physiologic stimulus for T2D. Exploratory analyses with L-glucose as an osmotic stimulus for endothelial cells are being planned.

Figure 4. Fold Change in HAEC Gene Expression From Arsenic Exposure



3. Publications

The data above is being prepared for submission to the Journal of Translational Medicine.