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Gene deletion of protein tyrosine phosphatase 1B limits cardiovascular dysfunction and mortality during endotoxic shock
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Sepsis-associated cardiovascular dysfunction is one of the main causes of mortality in critically ill patients. PT1B1 is a negative regulator of insulin signaling and endothelial Nitric Oxide (NO) production, two mechanisms likely involved in the pathogenesis of sepsis. We recently showed that gene deletion or pharmacological inhibition of PT1B1 improves endothelial dysfunction and reduces the severity of heart failure. The aim of the present study was to assess the effect of PT1B1 gene deletion on lipopolysaccharide (LPS)-induced cardiovascular dysfunction and mortality in mice. PT1B1-/- or wild-type (WT) mice received LPS (15mg/kg) or vehicle followed by subcutaneous saline resuscitation (30ml/kg). LPS significantly enhanced cardiac and arterial PT1B1 mRNA expression. Compared with WT, PT1B1-/- mice displayed markedly reduced mortality (Survival 4 days after LPS: WT: 0%; PT1B1-/-: 70%, p<0.01), associated with a higher locomotor activity.

Evaluation of vascular function 8h after LPS in isolated mesenteric resistance arteries (arteriograph system) showed that PT1B1-/-mice had improved contractile response to phenylephrine, and restored flow mediated, NO dependent-dilation (% dilation to 200μm: WT 6.3±3; PT1B1-/- 16.5±2, p<0.05) associated with significant increases in phosphorylation of Akt and eNOS, and reduced mRNA expression of VCAM-1, ICAM-1, COX-2, IL-1, iNOS and Gp91phox (PCR).

Evaluation of left ventricular (LV) function by echocardiography, and pressure-volume curves 8h after LPS revealed reduced cardiac dysfunction in PT1B1-/- mice as shown by an increased LV fractional shortening (WT 17.3±1; PT1B1-/- 24.1±1, p<0.05) and LV end systolic pressure-volume relation (WT 16±2; PT1B1-/- 20±2, p<0.05) together with reduced LV end diastolic pressure-volume relation (WT 4.7±1.7; PT1B1-/- 2.4±0.9 mmHg/RVU, p<0.05). Improved cardiac contractility was also observed ex-vivo in hearts isolated and perfused 8h after LPS (LV Developed Pressure: WT 26±5; PT1B1-/- 50±5 mmHg, p<0.05). Improved cardiac function was associated with significant decreases in myocardial markers of inflammation and oxidative stress (VCAM-1, ICAM-1, CD45, IL-10, TNFα and Gp91phox), reduced P38 and ERK1/2 phosphorylation and limited decrease of phospholamban phosphorylation (WT-42%; PT1B1-/- -22.8, p<0.05).

Thus, we demonstrate for the first time that PT1B1 gene deletion protects against lipopolysaccharide-induced cardiovascular dysfunction and mortality. These results suggest that PT1B1 is an attractive target for the treatment of sepsis.

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High glucose-induced increased expression of endothelin-1 in human endothelial cells is mediated by activated CCAAT/enhancer-binding proteins
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Purpose: High glucose-induced endothelial dysfunction is partially mediated by the down-stream pathophysiological effects triggered by increased expression of Endothelin-1 (ET-1). The molecular control mechanisms of ET-1 synthesis are yet to be discovered. Members of the CCAAT/enhancer-Binding Proteins (C/EBP) family are important regulators of key metabolic processes, cellular differentiation and proinflammatory genes. In this study, we aimed at elucidating the role of C/EBP in mediating the high glucose effect on ET-1 expression in human endothelial cells.

Methods and results: Human umbilical vein cells (EhAhy520) and primary cultures of human aortic Endothelial Cells (EC) were exposed to high levels of glucose (16.5-25 mM). Real-time PCR, Enzyme-Linked Immunosorbent Assay (ELISA), Western blot, ET-1 promoter-luciferase reporter analysis, and chromatin immunoprecipitation assays were employed to investigate ET-1 regulation. The expression of various C/EBP isoforms was selectively downregulated by siRNA-mediated gene silencing. High glucose dose-dependently induced activation and transcription of C/EBPβ, C/EBPδ and C/EBPβ, δ-induced significantly the high glucose-induced upregulation of ET-1 mRNA, pro ET-1, and ET-1 secretion. Chemical inhibition of JNK, p38MAPK and ERK1/2 diminished significantly the high glucose-induced nuclear translocation of C/EBP and ET-1 expression. In silico analysis indicated the existence of typical C/EBP binding sites in human ET-1 gene promoter. Transient overexpression of C/EBPβ, C/EBPδ or C/EBPβ, δ upregulated the luciferase level controlled by the ET-1 gene promoter. The direct interaction of C/EBPβ, C/EBPδ or C/EBPβ, δ proteins with the ET-1 promoter in high glucose-exposed EC was confirmed by chromatin immunoprecipitation assay.

Conclusions: High glucose-induced ET-1 expression is mediated via multiple mechanisms. We present evidence that members of the C/EBP proinflammatory transcription factors are important regulators of ET-1 in high glucose-exposed human endothelial cells. High glucose-induced activation of C/EBP-related signaling pathways may induce excessive ET-1 synthesis, thus promoting dysfunction of the vascular wall cells in diabetes.


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Pioglitazone prevents endothelial dysfunction induced by ischemia and reperfusion via up-regulating anti-oxidative effects: a human study
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Background: Animal studies have demonstrated that pioglitazone can limit myocardial damage induced by ischemia. No study has investigated whether pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, protects against ischemia and reperfusion (IR)-induced endothelial dysfunction in humans.

Methods: In a placebo-controlled, crossover design, 22 volunteers (25 to 44 years old) were randomized to one week administration of oral pioglitazone (30 mg) or placebo. Endothelium-dependent, flow-mediated dilation (FMD) of the brachial artery was measured before and after IR (15 min of upper arm ischemia followed by 15 min of reperfusion). In a separate protocol, 5 volunteers received the cyclooxygenase (COX)-2 inhibitor meloxicam (10 mg) for one week in with pioglitazone. On day 7, subjects were underwent the same protocol. Gene expressions in human umbilical vein endothelial cells after 24 hours of pioglitazone treatment (10μM) were evaluated by DNA microarray.

Results: Pre-IR brachial artery diameter and FMD were similar between groups. After placebo administration, IR significantly blunted FMD (pre: 9.9±2.1%; post: 3.8±0.7%, p<0.01). Pioglitazone limited this impairment in FMD (pre: 10.2±2.5%; post: 7.5±2.5%, p<0.05). Meloxicam did not affect FMD post-IR, suggesting no involvement of COX-2 pathway. Pioglitazone significantly increased serum levels of extracellular superoxide dismutase and adiponectin and decreased high sensitive CRP but not affect ADMA level. Finally DNA microarray revealed pioglitazone significantly increased anti-oxidative stress genes such as superoxide dismutase and peroxiredoxin-1 in endothelial cells.

Conclusions: In humans, pioglitazone induces potent protection against IR-induced endothelial dysfunction through activation of anti-oxidative stress effect.