Poster 34

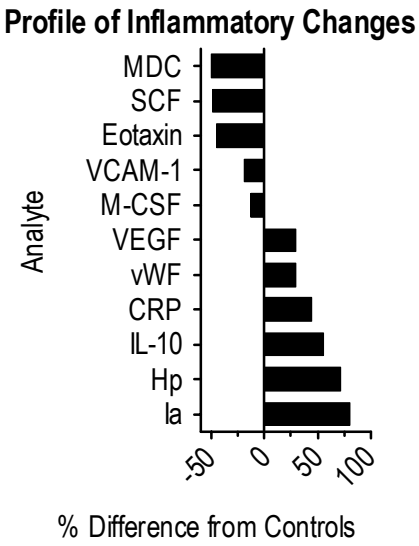
MULTI-ANALYTE IMMUNOASSAY PROFILES OF PLASMA BIOMARKERS OF INFLAMMATORY CHANGE IN A RAT MODEL OF EARLY MESENTERIC VASCULAR INJURY

O'Brien PJ, Chevalier S, Schenck E, Pawlowski V, Dagues N, Ledieu D.
Safety Sciences Europe, Pfizer, Sandwich UK and Amboise, France.

Introduction: Phosphodiesterase-inhibitors may induce mesenteric vascular injury in rats (1,2). We studied early plasma biomarkers of vascular injury using a novel, multi-analyte, immunoassay profile of 60 acute phase reactants, cytokines, chemokines, growth factors, and hormones using Luminex technology (Rules Based Medicine) that has recently become commercially available (Charles River Laboratories, Manston, Kent, UK). Although the immunoassays were developed for application in mice, they were stated by the vendor to have cross-reactivity for rats.

Material and Methods: Rats (25 Spraque Dawley males, aged 7 weeks) were treated with a single dose of 0, 160, or 320 mg / kg of a potent phosphodiesterase inhibitor administered by oral gavage. After 16 hours, plasma was analysed from blood collected into tubes with EDTA anticoagulant.

Results: Concentrations were below the lower limit of detection (mean ± 3 SD for blank) for 39 of 60 parameters. Of the measurable analytes, no treatment related effects were detected for 9: growth hormone, interleukin-11, insulin, leptin, aspartate aminotransferase, myoglobin, immunoglobulin A, and monocyte chemoattractant proteins 1 and 3. Treatment produced mild to moderate effects on 12 other measurable analytes. It increased fibrinogen (Ia), haptoglobin (Hp), interleukin-10 (IL-10), C-reactive protein (CRP), von Willebrand factor (vWF), and vascular epithelium growth factor (VEGF), by 80, 72, 56, 45, 30, and 30% respectively, and decreased macrophage-derived chemokine (MDC), stem cell factor (SCF), eotaxin, vascular cell adhesion molecule-1 (VCAM-1), and macrophage colony stimulating factor (M-CSF) by 50, 48, 45, 19, and 12% (1-way ANOVA; $p < 0.05$). There were differences (Student's unpaired, 2-tailed, t-test) between high and low groups for SCF, GCP-2 / IL-8, M-CSF, eotaxin, and vWF; SCF, eotaxin, M-CSF, and vWF were not affected at the low dose, whereas GCP-2 was increased at the low dose only. Granulocyte chemotactic protein-2 / interleukin 8 (GCP-2 / IL-8) concentrations were below the least detectable concentration for all controls, but above this limit for 6 low dose and 2 high dose rats.



Conclusion: Cross-species reactivity from mouse to rat occurred in only 35% of immunoassays in the mouse multi-analyte profile. However, the 21 assays of the profile that were able to measure analyte in rat plasma were effective in identifying a major effect of treatment with phosphodiesterase inhibitor. All treatment-related effects could be attributed to a mild, acute inflammatory response, characterized by increased release of acute phase proteins (Ia, Hp, CRP, vWF) and altered concentrations of cytokines and chemokines (eg IL-10, eotaxin, GCP-2, and MDC) that are modulatory of the inflammatory response. VEGF, an angiogenesis factor induced by inflammation was also slightly affected.

Reference:

1. Robertson DG, Reily MD, Albassam M, Dethloff LA. 2001. Metabonomic assessment of vascular injury in rats. Cardiovasc Toxicol. 1:7-19

2. Slim RM, Song Y, Albassam M, Dethloff LA. 2003. Apoptosis and nitrative stress associated with phosphodiesterase inhibitor-induced mesenteric vascular injury in rats. Toxicol Pathol. 31:638-45.