Phospholipase Activity Provides a Simple Test for Systemic Inflammation in Acute and Chronic Disease

Published date: March 14, 2017

Technology description

The inflammatory response plays a role in illnesses from injury to infections to allergies. Initiation of inflammation involves the activation of immune cells that trigger a cascade of events leading to phospholipase A_2 (PLA_2)-involved inflammatory processes.

PLA_2s are enzymes that play a vital role in regulating the production of precursors to a number of pro-inflammatory lipid mediators, including prostaglandins, leukotrienes and platelet activating factor, which in turn exert a wide range of potent physiological effects. Excess production of PLA_2 is associated with diseases such as systemic inflammation, allergy, brain injury, cancer development and metastasis, and cardiovascular disorders. Studying the onset and severity of disorders associated with excess PLA_2 could provide useful diagnostic information as well as information on the efficacy of anti-inflammatory therapies. UW-Madison researchers have developed a fluorescence assay for measuring phospholipase activity, including secretory PLA_2 ($sPLA_2$) activity. This assay provides a simple, rapid and highly reproducible blood test that can be used to monitor systemic inflammation over an extended period and evaluate the effectiveness of anti-inflammatory therapies.

The assay uses a unique, fluorescently labeled liposome. When a sample containing a phospholipase is added to the liposome, the phospholipase hydrolyzes the phospholipid components of the liposome, causing a detectable change in fluorescence intensity. The degree of change indicates the activity of the phospholipase.

The sample can be compared to a control to determine if an individual has elevated PLA_2 activity, which may indicate a disorder associated with systemic inflammation, such as sepsis, heart disease, cystic fibrosis or chronic obstructive pulmonary disease. The assay also can be used to identify agents capable of altering phospholipase activity.

Because the activity of $sPLA_2$ in serum is a critical marker for monitoring the onset and severity of systemic inflammation in patients with sepsis or heart disease, this assay also could be used to create a portable, bedside device for early detection of hyper-inflammation. Such a device could provide

accurate, real-time monitoring of changes in fluorescence intensity resulting from changes in sPLA $_2$ activity and may enable early interventions for the prevention of multi-organ failure.
The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a simple, rapid and reproducible assay for systemic inflammation.

Additional Information

Tsao F.H., Shanmuganayagam D., Zachman D.K., Khosravi M., Folts J.D. and Meyer K.C. 2007. A Continuous Fluorescence Assay for the Determination of Calcium-Dependent Secretory Phospholipase A $_2$ Activity in Serum. Clin. Chim. Acta. 379, 119-126.

Tsao F.H., Shanmuganayagam D., Zachman D.K., Khosravi M., Folts J.D. and Meyer K.C. 2007. A Continuous Fluorescence Assay for the Determination of Calcium-Dependent Secretory Phospholipase A $_2$ Activity in Serum. Clin. Chim. Acta. 379, 119-126.

Figure 1.

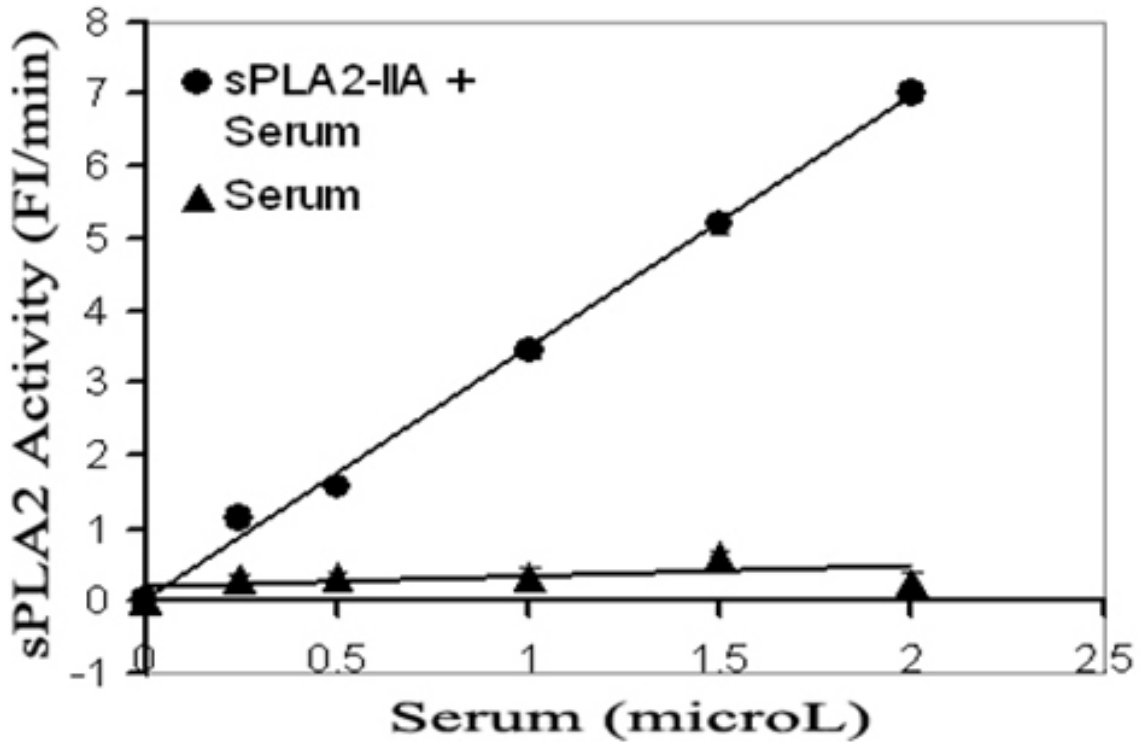

Figure 1 caption.

sPLA$_2$-IIA spiked in serum. The sPLA$_2$-IIA activity was determined in human serum spiked with recombinant human sPLA$_2$-IIA. The sPLA$_2$-IIA concentration in the serum was 0.5 ng sPLA$_2$-IIA/1 µl serum. The serum pool was used as control. R^2 for sPLA$_2$-IIA + serum linearity was 0.9975. The conditions of serum described in this figure can be used for sPLA2 standard preparation. Standard sPLA2 in serum stored at -70°C is stable for more than 2 years.

Figure 2.

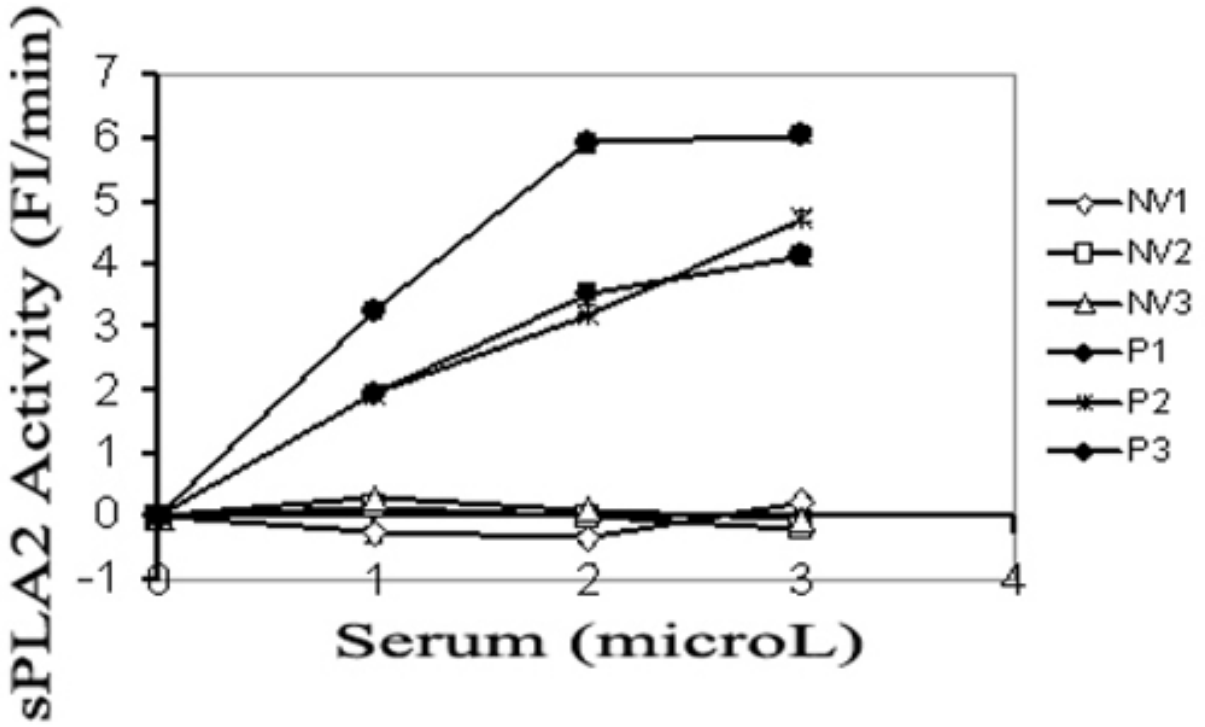

Figure 2 caption.

$sPLA_2$ activity in serum from normal healthy volunteers (NV1, NV2, and NV3) and from patients with sepsis and organ failure (P1, P2, and P3).

The assays were conducted in 96-well microplates at 30ºC. The $sPLA_2$-IIA activity was determined in Tris buffer containing 10 mM Ca^{2+} and then subtracting from this any background FI changes in the presence of 10 mM Ca^{2+} and 20 mM EGTA. The data is expressed as mean ± SEM (n=3).

Application area

Diagnosis and analysis of sepsis and other disorders
Evaluation of anti-inflammatory therapies, including dietary interventions
Identification of phospholipase modulators
Creation of a portable, bedside device for early detection of hyper-inflammation in patients with sepsis for the prevention of multi-organ failure

Advantages

Capable of determining the level of systemic inflammation response in micro-liters of serum
Substrate and assay standards are stable for months at -20°C.
Simple, rapid and highly reproducible
Capable of monitoring the inflammatory process over a time course of five to six days, rather than three to four hours as seen with traditional inflammatory markers
Phospholipase activity can be determined based on data collected at a single time point or recorded on a continuous basis.

Institution

Wisconsin Alumni Research Foundation

Inventors

Keith Meyer
Francis Tsao