SO release from leukocytes via NADPH oxidase activation contributes to oxidative stress under various diseases, such as ischemia/reperfusion (IR) injury and vascular complications in diabetes. NADPH oxidase has seven isoforms with NOX2 being the predominant isoform of NADPH oxidase in polymorphonuclear leukocytes (PMNs). Activation of NOX2 requires the assembly of cytosolic subunits (p47phox, p67phox, Rac) to membrane subunits (gp91phox and p22phox) (1). NADPH oxidase is activated during IR injury via cytochrome oxidase or chemotactic factor (N-formyl-L-methionyl-L-phenylalanine (MLP, MW=438 g/mol) and utilizes molecular oxygen to produce SO (2) (fig1). Gp91ds-tat is a peptide which selectively inhibits NADPH oxidase assembly by blocking p47phox and p22phox leukocytes (PMNs).

We have previously shown that myristic acid conjugated caveolin-1 and protein kinase C (PKC) beta II and zeta peptide inhibitors significantly attenuated (MLP-induced SO release compared to their native counterparts). However, it is not known if differences exist in the effectiveness of myristic acid versus tat conjugated gp91ds-tat peptides to their native counterparts or untreated controls.

**Methods**

**Isolation of PMNs**

Male Sprague-Dawley rats (350–400 g, Charles River), used as PMN donors, were anesthetized with 2.5% isoformure and given a 16 ml intraperitoneal injection of 0.5% glycogen (Sigma Chemical) dissolved in PBS. Rats were reanesthetized with isoflurane 16–18 h later, and the PMNs were harvested by peritoneal lavage in 30 ml PBS in the presence or absence of myristic acid conjugated caveolin-1 and protein kinase C (PKC) beta II and zeta peptide inhibitors.

**Measurement of SO Release From Rat PMNs**

The SO release from PMNs was measured spectrophotometrically (model 260 Gilford, Nova Biotech; El Cajon, CA) by the reduction of ferricytochrome c (2,5). The PMNs (5×10⁶) were resuspended in 450 µl PBS and incubated with ferricytochrome c (100 µM, Sigma Chemical) in a total volume of 900 µl PBS in the presence or absence of myristic acid conjugated (2 to 10 µM), tat conjugated (80 µM) or native gp91ds (80 µM) for 15 min at 37°C in spectrophotometric cells. The PMNs were stimulated with 1 µM MLP (calbiochem) in a final reaction volume of 1.0 ml. Absorbance at 550 nm was measured every 30 sec for up to 120 sec for MLP and the change in absorbance (SO release) from PMNs was determined relative to time 0.

**Cell Viability**

Cell viability was determined by counting 0.5 ml of the samples from spectrophotometric analysis using trypan blue exclusion (0.3%). Then, 20 µl of the combined sample was placed on to a hemocytometer, and 100-150 cells were subsequently counted using microscopic analysis.

**Statistical Analysis**

All data in the text and figures are presented as means ± S.E.M. The data were analyzed by analysis of variance using post hoc analysis with the Fisher’s test. Probability values of <0.05 are considered to be statistically significant.

**Results**

We hypothesized that myr-gp91ds (2–10 µM) would dose-dependently attenuate MLP-induced PMN SO release at lower concentrations compared to tat conjugated or tat conjugated gp91ds-tat peptides. Moreover, we also predict that both myristic acid and tat conjugated gp91ds-tat peptides would significantly attenuate MLP-induced leukocyte SO release compared to native or untreated controls without affecting cell viability.

**Hypothesis**

Unconjugated native sequence did not inhibit the MLP-induced SO response at the highest dose tested (80 µM). Myr-pep-gp91ds NADPH oxidase peptide inhibitor significantly attenuated leukocyte SO release dose dependently compared to untreated or native sequence (myr-pep-gp91ds; 5–10 µM). The tat conjugated gp91ds inhibitors (both 80 µM) significantly attenuated MLP-induced leukocyte SO release, but to a lesser extent than the myr-pep linked inhibitor and were not different from native gp91ds. These results suggest that myr-pep-gp91ds is more cell permeable and therefore can inhibit MLP-induced SO release from leukocytes at lower doses compared to tat gp91ds.