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A-009

Early Identification of Cardiac Ischemia Patients in the Emergency Department

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Abstract

Background: **We demonstrated the rapid release of a potent 3 KDa *formyl peptide* chemotactic factor (Nourin) by reversible and irreversible ischemic myocardial tissues. Mass spectrometry analysis confirmed that Nourin is a formyl peptide. Using modified Boyden chambers, we found that Nourin was 3 fold higher in plasmas of ACS patients who presented to the Emergency Department (ED) within 1.5 - 3.5 hours after the onset of symptoms when compared to normal controls (P≤ 0.001). Additionally, 3 formyl peptide receptor competitive antagonists (soluble receptor fragment, t-Boc-FLFLF and Spinorphin) inhibited chemotactic activity detected in plasmas from heart attack patients by over 50%. We hypothesized that formyl peptides released by ischemic hearts can be used as early biomarkers for myocardial ischemia.**

Methods: **We developed an ELISA for the cardiac-derived formyl peptide Nourin using antibodies against its f-Met moiety, and determined its levels in serum samples collected from heart attack patients (n=10) with troponin levels below the clinical decision level and non-cardiac chest pain patients (n=10 - negative troponin). In a second study, the levels of cardiac Nourin were determined in frozen plasma samples (-70°C for 3 years) collected from 10 patients with heart attack and unstable angina and non-cardiac chest pain (n=5). Blood samples in both studies were collected within 8 hours of onset of chest pain.**

Results: **Figure 3 shows that samples from heart attack patients had significantly higher levels of the formyl peptide Nourin (p<0.0001) compared to non-cardiac patients. Similar results were obtained regardless of whether the blood samples were fresh or frozen (-70 °C for 1 month). In the second study using frozen plasma samples, we also demonstrated a significant difference between samples collected from patients with heart attack and unstable angina versus non-cardiac patients presenting to the ED with signs and symptoms suggestive of MI (p=0.012).**

Conclusion: **These data from a limited group of patients show a great promise for the use of cardiac Nourin ELISA for detection of myocardial ischemia/injury.**

Hypothesis

We hypothesize that formyl peptides released by ischemic hearts can be used as early biomarkers for myocardial ischemia.

Objectives

1. To determine a "proof of principle" that the formyl peptide Nourin is a clinically useful blood biomarker for early detection of patients with cardiac ischemia.

2. To generate polyclonal antibodies to Nourin and to develop a simple direct-ELISA assay to quantify serum levels of Nourin.

3. To determine the levels of Nourin using the Nourin ELISA in serum and plasma samples collected from AMI patients with low troponin values compared to samples collected from non-cardiac chest pain patients with negative troponin.

Nourin Stimulates the Release of High Levels of IL-8, IL-1ß & TNF-α by Human Peripheral Monocytes After 4 Hours of Incubation

Treatment	Nourin	Control Media
Interleukin-8	12,000 ng/ml	2,000 ng/ml
Interleukin-1ß	400 pg/ml	10 pg/ml
TNF-alpha	400 pg/ml	<10 pg/ml

Figure 1

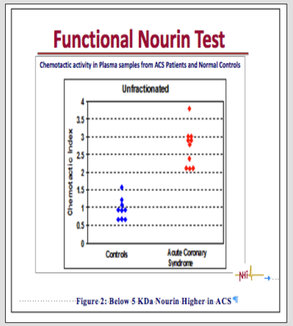

Figure 2

Results

- Using the Nourin ELISA assay, ***Figure 3*** shows serum samples from heart attack patients (n=10) had significantly higher levels of the formyl peptide Nourin (p<0.0001) compared to serum samples from non-cardiac chest pain patients (n=10). Similar results were obtained regardless if the patients' samples were tested fresh or frozen at -70 °C for 30 days.
- In the *second study* where cardiac and non-cardiac plasma samples were frozen at -70 °C for 3 years, we demonstrated a significant difference of p=0.012 between samples collected from patients with heart attack and unstable angina (n=10) versus non-cardiac patients (n=5) presenting to the ED with signs and symptoms suggestive of AMI.
- ***Figure 4*** shows that using the formyl peptide f-MLP ELISA and the Nourin ELISA we clearly demonstrated that serum samples collected from heart attack patients had significantly higher levels of f-MLP (p<0.0001) and Nourin (p<0.0001) antigens when compared to non-cardiac patients. The correlation of the ELISA data from the f-MLP vs. Nourin studies demonstrate that there was a correlation of R^2= 0.942 for non-cardiac samples and R^2= 0.621 for the cardiac AMI samples indicating that both assays can distinguish between cardiac and non-cardiac patients (***Figure 5***).

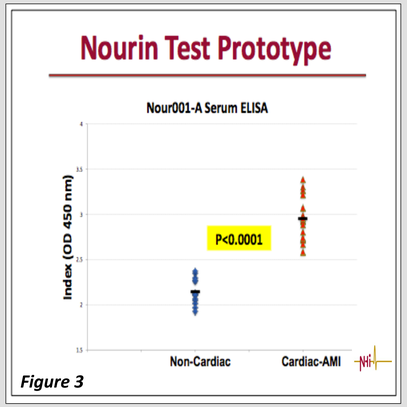

Figure 3

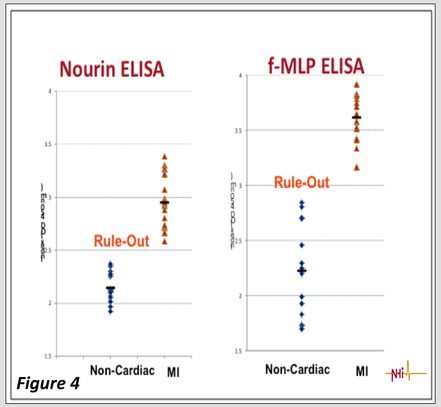

Figure 4

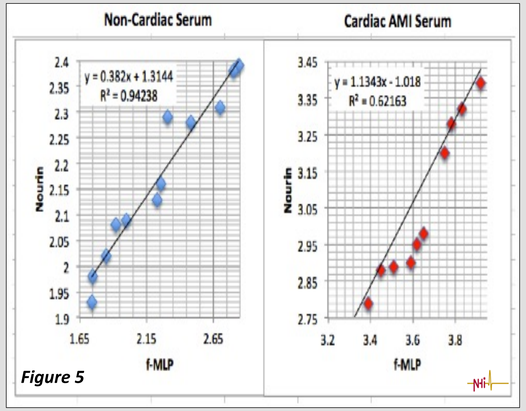

Figure 5

Background

Cardiac-Derived Nourin - We have previously demonstrated the rapid release of a potent leukocyte chemotactic factor (LCF) by myocardial tissues in response to both "*reversible*" and "*irreversible*" ischemic injury . We have designated this cardiac-derived LCF as ***Nourin***. The release of Nourin was associated with post ischemic myocardial inflammation. Nourin stimulates the release of chemokines and cytokines by peripheral monocytes (***Figure 1***) and stimulates the secretion of adhesion molecules by neutrophils (LECAM) and human aortic endothelial cells (ICAM-1 and ELAM-1).

Nourin is a formyl peptide – Using intact canine models of ischemia/reperfusion, we demonstrated the rapid release (5 minutes) of Nourin by ischemic myocardium in response to reversible and irreversible ischemic injury. In our earlier publications, we referred to Nourin as the cardiac-derived neutrophil chemotactic factor, while the name Nourin is used in our issued and pending patent applications. Nourin was purified from cardioplegic solutions collected from bypass patients who underwent cardiac arrest for coronary revascularization. Nourin is a 3 kilo Daltons (KDa) formyl peptide that acts on the formyl peptide receptor (FPR) of phagocytes. The amino acid sequence of Nourin was identified using mass spectrometry. Furthermore, 3 FPR competitive antagonists were used to determine their ability to inhibit chemotactic activity in AMI patient serum samples. The three FPR competitive antagonists Spinorphin, Soluble FPR fragment 17 aa loop peptide (RKAMGGHWPFGWFLCKF), and t-Boc-Phe-D.Leu-Phe-D.Leu-Phe inhibited chemotactic activity detected in plasmas from heart attack patients by over 50% .

Nourin Detected in plasmas of ACS patients - Using modified Boyden chamber chemotaxis assay (standard Neuroprobe chemotaxis system - Gaithersburg, Maryland) and human leukocytes as indicator cells, we found that Nourin was 3-fold higher in plasmas of ACS patients who presented to the ED within 1.5 to 3.5 hours after the onset of symptoms when compared to normal controls (P≤ 0.001). We next *fractionated* the ACS and control samples using size exclusion HPLC. As described in ***Figure 2***, the low molecular weight Nourin eluted in the less than 5 KDa fractions of the ACS samples showed chemotactic activity two to five fold higher than the fractionated control samples (P≤ 0.001). Furthermore, Nourin and other markers such as troponins were detected in AMI samples collected up to *30 hours* after onset of chest pain. For cardiac patients with unstable angina, while standard markers were not elevated, the below 5 KDa Nourin chemotactic activity was detected in patients' plasma samples .

Methodology

- We developed an ELISA for the cardiac-derived formyl peptide Nourin using antibodies against its N-formyl-Met moiety. Polyclonal antibodies were developed using the Tanaka et al method. Using the simple Nourin direct-ELISA assay we determined levels in serum samples collected from *confirmed* heart attack patients (n=10) with troponin levels ***below*** the clinical decision level and in serum samples from non-cardiac chest pain patients (n=10 - negative troponin). These patients' samples were tested both fresh and after being frozen at -70 °C for 30 days.
- In a *second study*, the levels of cardiac Nourin were determined in ***frozen*** plasma samples (-70°C for 3 years) collected from 10 patients with heart attack and unstable angina and 5 non-cardiac chest pain patients.
- Blood samples in both studies were collected within 8 hours of onset of chest pain. The cut-off level for classifying a sample as troponin positive was a troponin level of greater than 0.07 ng/ml (99th percentile) using the Dimension RxL Troponin I assay.
- Additionally, we developed a second ELISA assay for the ***formyl peptide Met Leu Phe (f-MLP)*** as a positive standard control for the presence of formyl peptides in AMI samples. The f-MLP ELISA assay was also used to determine the level of formyl peptides in the same above described serum samples collected from *confirmed* heart attack patients (n=10) with troponin levels ***below*** the clinical decision level, as well as from non-cardiac chest pain patients (n=10 - negative troponin).

Conclusions

- Samples collected from heart attack patients had significantly higher levels of ***formyl peptide antigens*** when compared to non-cardiac chest pain patients as detected by the formyl peptide Nourin (p<0.0001) and f-MLP (p<0.0001) assays.
- The present data support our hypothesis that formylated peptides are present in serum of AMI patients
- These data from a limited group of patients show a great promise for the use of cardiac Nourin ELISA for detection of myocardial ischemia/injury.

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