Nourexin-4: A Novel Anti-inflammatory Therapy for Influenza Flu

Program No. 52
Poster B1

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Abstract

Relatively little is known about the inflammatory mediators and mechanisms that drive the progression of influenza flu infection to cytokine storm, lung dysfunction, organ failure, and ultimately death. Vaccines and antiviral medications cannot control the excessive host inflammatory response. We demonstrated the rapid release of a potent inflammatory mediator, recently named Nourin, by local mammalian tissues in response to injury and infection. Nourin is a formyl peptide that acts through the formyl peptide receptor (FPR) on phagocytic leukocytes. As an "initial signal" in the "innate immunity", Nourin stimulates leukocyte chemotaxis, induce acute and chronic inflammation, and stimulates the release of cytokine storm mediators from monocytes and neutrophils. Nourin detected in plasma samples from patients with severe influenza infection was much higher compared to moderate influenza. We then tested the Nourin antagonist Nourexin-4, as specific competitive antagonist of formyl peptides on phagocytic leukocytes FPR. Nourexin-4 completely blocked neutrophil chemotaxis induced by the standard formyl peptide f-MLF and the host-derived Nourin released by **(1)** cultured epithelial cells infected with the H1N1 influenza virus (PR8) (6-24 hours), **(2)** Nourin detected in the serum of mouse model of H1N1 influenza (6 hrs), and **(3)** Nourin detected in severe and moderate influenza patients plasma samples. Nourexin-4 can be used to control virus-induced inflammation and protect patients.

Background

Nourin is a potent inflammatory mediator which is rapidly released (within 2 minutes) by local tissues (e.g., heart, vessels, stomach, corneas, conjunctiva, retina, brain, spinal cord, and urinary bladder, ect.) in response to injury induced by ischemia, chemical agents, physical trauma, as well as environmental and nutritional factors. Nourin also stimulates the release of cytokine storm mediators by human monocytes.

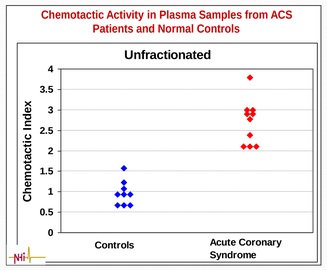


Figure 1

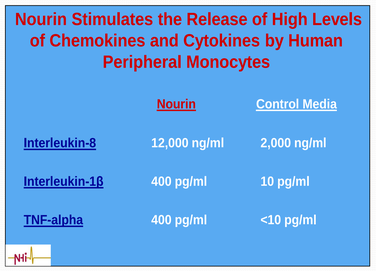


Figure 2

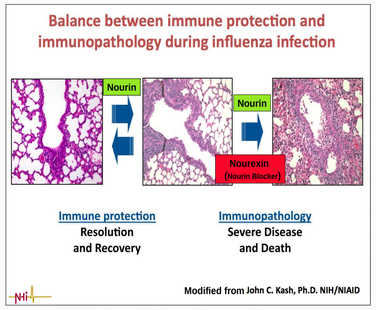


Figure 3

Hypothesis

Influenza virus infection of airway epithelial cells of patients triggers cell injury which results in the release of epithelial cell-derived pro-inflammatory mediators, including the formyl peptide Nourin and that the **Nourin antagonist Nourexin-4** will inhibit Nourin chemotactic activity.

Objectives

1. To determine the release of the formyl peptide Nourin by cultured **epithelial cells** (MDCK) infected with the H1N1 influenza virus (PR8) for 1-24 hours.
2. To determine the detection of the formyl peptide Nourin in serum samples of **mice** infected with the H1N1 influenza virus for only 6 hours.
3. To determine the detection of the formyl peptide Nourin in plasma samples of **patients** with severe and moderate influenza flu infection and whether there is a differential Nourin levels between severe and moderate influenza patients infected with the H1N1 influenza virus.
4. To determine whether the formyl peptide **antagonist Nourexin-4** inhibits chemotactic activity stimulated by the influenza-induced Nourin (cell culture, mice, and patients).
5. To **synthesize Nourexin-4** and to compared its activity to commercial Nourexin-4.

Methodology

Cultured Epithelial Cell Studies - Cultured epithelial MDCK cells were infected with H1N1 (PR8) influenza virus for 1, 3, 6, 12, and 24 hours. Each H1N1 infected cell supernatant was assayed both in the presence and absence of the formyl peptide specific antagonist Nourexin-4 ($5x10^{-6}$ M). Supernatant solutions were evaluated both undiluted (neat) and diluted 1/10 in hanks balance salt solution (HBSS).

Mice Studies - Balb/C female, 5 weeks old mice were anesthetized with isofluorane and intranasally inoculated with 10 MLD50 of WSN (mouse adapted A/WSN/33 strain, H1N1) in 50 ul PBS (designated WSN-1 thru WSN-4). Four mice received sham treatment without H1N1 virus (designated Mock-1 thru Mock-4). **Six hrs** post virus or sham treatment, blood samples were collected from mice and the serum was stored at -70ºC until used for chemotaxis assay to determine chemotactic activity and the ability of Nourexin-4 ($5x10^{-6}$M) to inhibit that activity. Serum samples were diluted in HBSS at a dilution of 1/7.

Influenza Patients Studies - Plasma samples were obtained from patients with severe and moderate H1N1 influenza flu, as well as from patients with respiratory syncytial virus (RSV) infection. Severe influenza patients were admitted to the ICU with encephalopathy or respiratory failure, while moderate influenza patients and patients with RSV were admitted to the hospital with fever or wheezing (Ref. 1). We determined the presence and level of chemotactic activity in plasma samples (diluted 1/9 in HBSS) in the presence and absence of Nourexin-4 (Nxin-4) at 10^{-5}M.

Chemotaxis Assay – Samples were assayed for chemotactic activity using standard Neuroprobe chemotaxis system (Gaithersburg, Maryland) and human leukocytes as indicator cells.

Results

I. Release of Nourin by H1N1 Infected Cultured Epithelial Cells, Mice, and Patients – Inhibition by Nourexin-4

a. Cell Culture

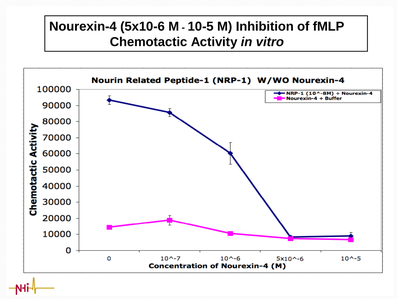
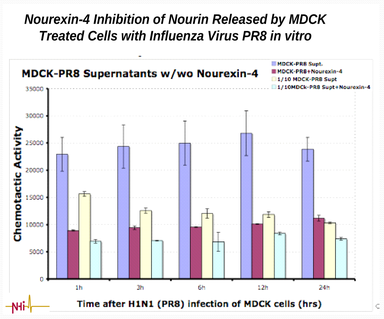


Figure 4



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b. Mice

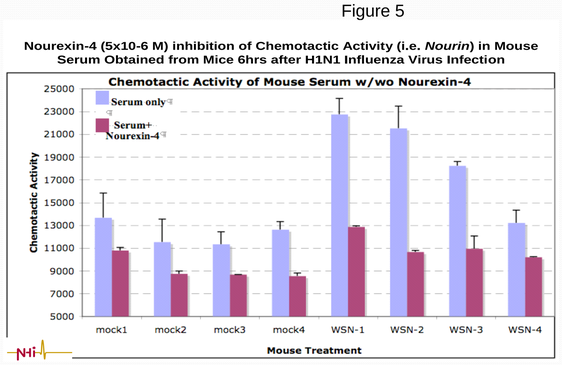

Figure 6

c. **Patients**

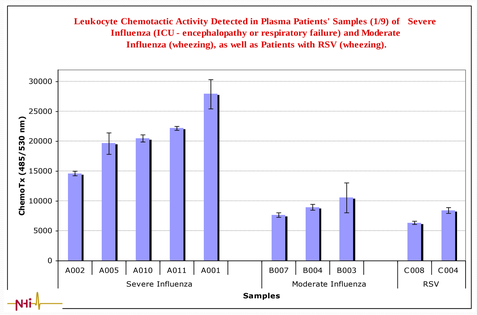

Figure 7

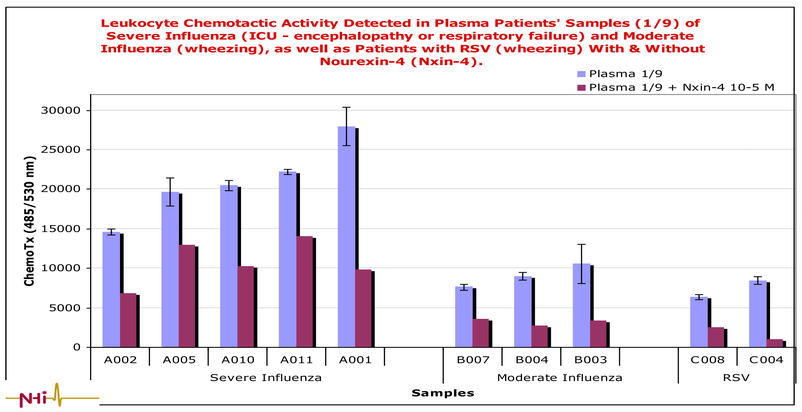

Figure 8

II. Synthesis of Active Nourexin-4

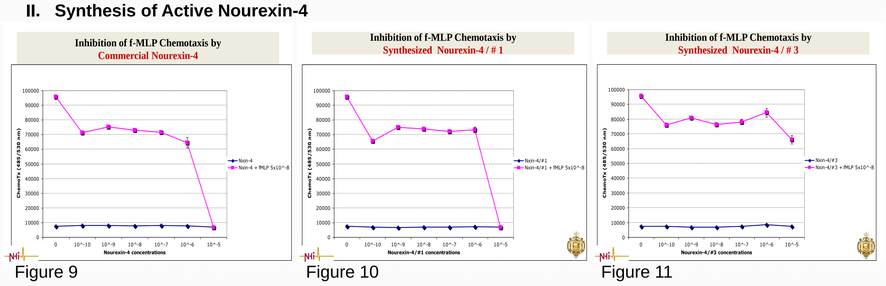

Figure 9 Figure 10 Figure 11

Conclusion

Rapid and sustained release of Nourin after H1N1 influenza flu infection. Differential levels of Nourin were detected in influenza patients' plasma samples where higher levels were observed in samples from severe influenza compared to patients with moderate influenza flu.

Nourexin-4 inhibits chemotactic activity induced by Nourin released after H1N1 influenza flu infection in cultured epithelial cell supernatant solutions, mouse sera, and plasma samples from patients with severe and moderate H1N1 influenza infection.

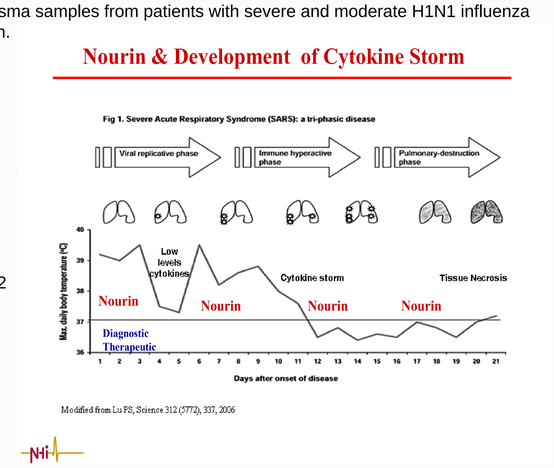

Figure 12

Diagnostic Application – The blood Nourin test can be used as a key inflammatory biomarker for "early" detection and monitoring of influenza flu patients proceeding to hyperactive inflammation and, thus, permitting "early" crucial anti-inflammatory therapy.

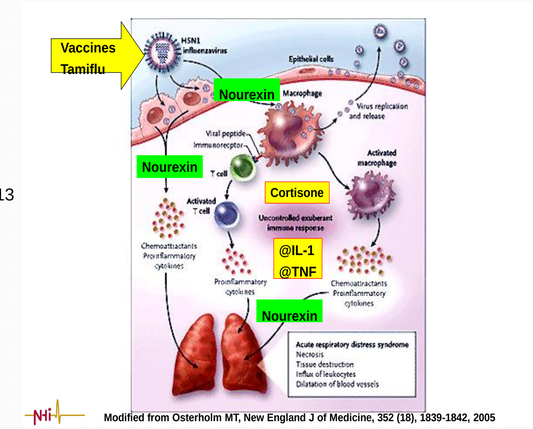

Figure 13

Therapeutic Application – Nourexin-4 will specifically block Nourin as a key stimulant of cytokine mediators and thus can control the "development" and "progression" of cytokine storm and organ inflammation which usually initiates 3-8 days post influenza.

Since Nourexin-4 does not target the virus, it will not develop drug resistance and will reduce the host uncontrolled inflammatory response induced by new strains of flu viruses and existing viruses with mutations.

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