Host-directed therapeutics as adjunctive therapy for antibiotic-resistant Neisseria gonorrhoeae

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Gonorrhea – the many faces of a biological threat

Disease burden
Complications

Antibiotic resistance
Impact on HIV

Pelvic inflammatory disease (PID)
Infertility and chronic pelvic pain

Ophthalmia neonatorum

Estimated worldwide prevalence of gonorrhea – 106 million cases

http://www.cdc.gov/std/tg2015/gonorrhea.htm;
http://apps.who.int/iris/bitstream/10665/70603/1/WHO_RHR_11.14_eng.pdf;
http://www.cdc.gov/amd/project-summaries/treating-gonorrhea-threat.html;
http://www.abs.gov.au/AUSSTATS/abs@.nsf/Lookup/4102.0MainFeatures10Jun2012#

With the possibility that untreatable gonorrhea exists in the near future, there is an URGENT need to develop novel or alternate therapies for treating gonorrhea

Novel/alternate therapies could be used alone or in combination with current treatments:
- decrease the amount of antibiotic used
- diminish the development of antibiotic resistance

Epigenetics
Study of factors that affect gene expression
Or changes to the genome that do NOT affect its nucleotide sequence
Potential TARGET for novel therapies against N. gonorrhoeae

Histone deacetylases (HDAC) are enzymes that remove an acetyl group from lysines in histones, allowing the histone to wrap DNA more tightly
HDAC inhibitors (HDACi)
- block the action of HDACs
- cause a state of hyperacetylation
- change global gene expression

Modification of HISTONES
Proteins around which the DNA is compacted

Modification of HISTONES regulate gene expression
Therapeutic potential of the HDACi sulforaphane (SFN)

SFN (natural isothiocyanate and HDACi first isolated from broccoli)

Induces expression of antimicrobial peptides (SLPI and beta-defensin-

Antibacterial properties - directly bactericidal to certain bacterial pathogens (Helicobacter pylori)

Anti-inflammatory properties - SFN inhibits inflammasomes and LPS-stimulated inflammatory responses

Assay for measuring the bactericidal activity of supernatants from SFN-treated cervical cells

SFN induces CATIONIC soluble factors in human cervical tissue culture cells that KILL N. gonorrhoeae

Depletion of cationic peptides

SFN significantly reduces the recovery of N. gonorrhoeae from mice

Can SFN induce host factors that kill recently isolated multiple drug resistant (MDR) N. gonorrhoeae strains?
Supernatants treated with SFN kill laboratory and antibiotic-resistant N. gonorrhoeae

Do supernatants from ME-180 cells treated with SFN enhance killing of N. gonorrhoeae in the presence of antibiotics?

Is there SYNERGY between soluble factors released during SFN treatment and antibiotics against N. gonorrhoeae?

The combination of SFN-treated supernatants with antibiotics decreases the MICs of N. gonorrhoeae strains

The checkerboard is the accepted method to measure SYNERGY between two antibiotics

Antibiotic dilutions on one side of plate, and supernatants from ME-180 cells treated with different concentrations of SFN on the other

Conclusions

Supernatants from SFN-treated cervical tissue culture cells kills both sensitive and multiple-antibiotic resistant N. gonorrhoeae

The soluble factors responsible for this activity are cationic

Cationic antimicrobial peptides were found to be expressed in genital tissues of mice treated with SFN

N. gonorrhoeae recovery was reduced in SFN-treated mice

Preliminary results indicate that treatment of cervical cells with SFN in combination with antibiotic therapy may reduce the amount of antibiotic necessary to kill N. gonorrhoeae, including MDR strains.
**Future studies**

Using the CHECKERBOARD method, define conditions in which the combination of SNF-Tx supernatants and antibiotics reduce the MICs of laboratory and antibiotic-resistant Ng.

In vivo (mouse) experiments – can SFN treatment reduce the dose of antibiotic needed to clear infection?

Subject SFN-Tx supernatants for mass spectrometry analysis to identify potential effector(s) of SFN treatment on ME-180 on growth of N. gonorrhoeae.

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**MICs**

(Agar dilution method)

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**Antibiotic-sensitive strains**

**Antibiotic-resistant strains**

CLSI – CRO + CEF, <0.25 µg/ml = sensitive; CIP, >1 µg/ml = resistant