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

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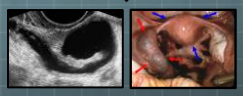
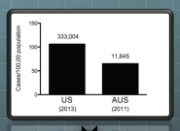
Gonorrhea – the many faces of a biological threat

- Disease burden
- Complications
- Antibiotic resistance
- Impact on HIV

Pelvic inflammatory disease (PID)



Infertility and chronic pelvic pain

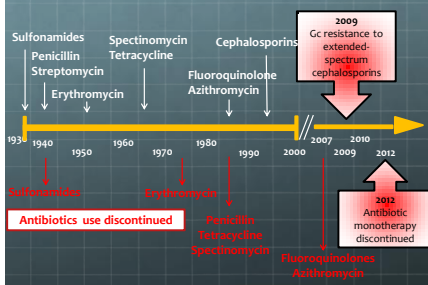


Estimated **worldwide** prevalence of gonorrhea – **106 million cases**

Ophthalmia neonatorum
Adverse pregnancy outcomes:
- premature delivery
- low birth weight
- failure to thrive

http://www.cdc.gov/std/stats13/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/4102.0Main+Features10Jun+2013#Bacterial

The threat of untreatable gonorrhea - antibiotic resistance in *Neisseria gonorrhoeae*



Recommended regimen for the treatment of gonorrhea (CDC)

250 mg IM Ceftriaxone
+
1 g Azithromycin

Single dose

Unemo and Shafer. 2014. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution and future. Clin Microb Rev 27:587-613. http://www.cdc.gov/std/tg2015/gonorrhea.htm;

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

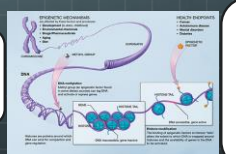
Study of factors that affect gene expression
Or
changes to the genome that do NOT affect its nucleotide sequence

Potential TARGET for novel therapies against *N. gonorrhoeae*

Modification of HISTONES

Proteins around which the DNA is compacted

Modification of HISTONES regulate gene expression



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

https://en.wikipedia.org/wiki/Epigenetics

Therapeutic potential of the HDACi sulforaphane (SFN)

Potent inducer of phase 2 detoxification enzymes and shown to induce apoptosis and prevent tumors

Induces expression of antimicrobial peptides (SLPI and beta-defensin-2)

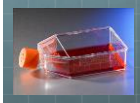
SFN
(natural isothiocyanate and HDACi first isolated from broccoli)

Anti-bacterial properties – directly bactericidal to certain bacterial pathogens (*Helicobacter pylori*)

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

Yedery and Jerse. 2015. Antibiotics 4:44; Choi et al. 2014. Korean J Physiol Pharma; Koo et al. 2013; Fahey et al. 2013. Biochem Biophys Res Commun 24:435; Greaney et al. 2015. J Leukoc Biol Aug 12; Reddy et al. Int Immunopharmacol 2015. 24(2):440; Geisel et al. J Immunol. 2014. 192(8):3530; Schwab et al. 2008. Immunology 125:241

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



Fresh media (+FBS)

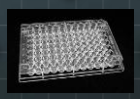
+SFN (24 hrs)

ME-180 (human endo-cervical cells)

Spin + freeze media at -20 C

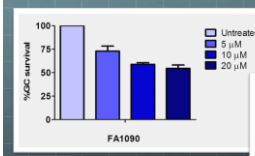
150 µl supernatant per well

Gc = 500 CFU

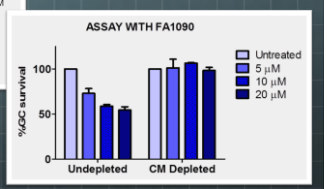


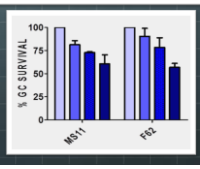
Quantitation at 4 and 20 hrs

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



Depletion of cationic peptides

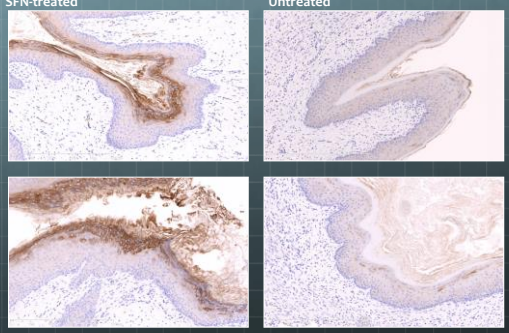




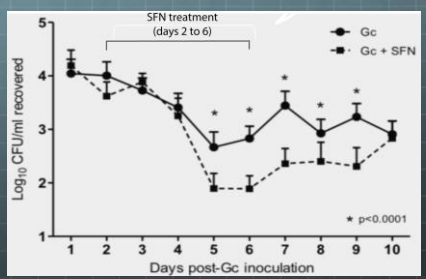
SFN increases CATIONIC antimicrobial peptide expression in female mice

SFN-treated

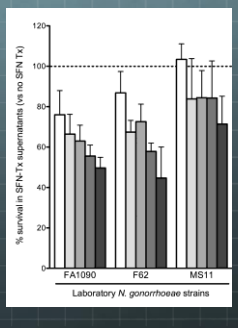
Untreated



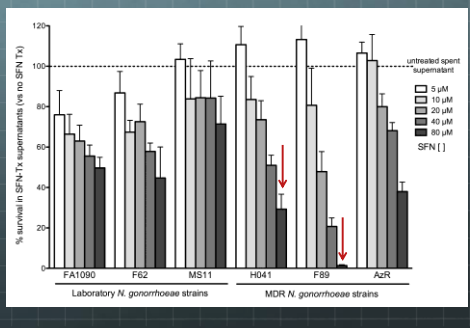
SFN significantly reduces the recovery of *N. gonorrhoeae* from mice



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*?

Testing antimicrobial combinations – the checkerboard

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

FIC index= $(MIC_{A+B}/MIC_A) + (MIC_{B+A}/MIC_B)$
FIC = fractional inhibitory concentration

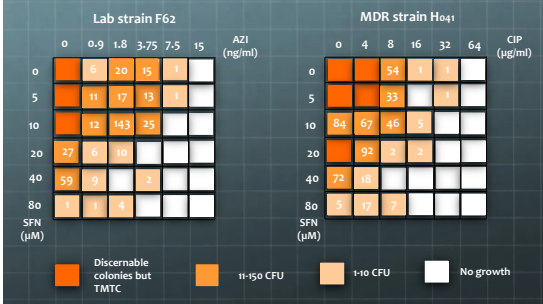
Synergy	FICI < 0.5
Indifference	FICI 0.5-4
Antagonism	FICI >4

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

MIC's are defined as the lowest concentration of antimicrobial with NO bacterial growth after 16-24 hrs

Orhan et al. 2005 J Clin Microbiol 43:140; Pillai, Moellering and Eliopoulous. 2005. Antimicrobial combinations, pp. 365-440.

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



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*

Acknowledgements

- Ann E. Jerse
- Members of the Jerse laboratory
  - Kristie Connolly
  - Carolina Gomez
  - Michelle Pilligua
  - Leah Vincent
  - Afrin Begum
  - Riley Sennett
  - Claire Costenoble-Caherty
  - Nazia Rahman
- Funding sources
  - NIH – NIAID, RO1-AI42053

MICs

(Agar dilution method)

	FA1090	F62	MS11	H041	F89	AzR
Ciprofloxacin	<0.075	<0.075	<0.075	32-64	8-16	<2
Ceftriaxone	<0.075	<0.075	<0.075	2	1-2	0.0075
Cefixime	<0.015	<0.015	<0.015	4	2	0.031
Azithromycin	0.015	0.015	0.062	0.125	0.25	4

Antibiotic-sensitive strains | Antibiotic-resistant strains

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