Nano-mupirocin Characterization as a Potential Candidate for MDR Gonorrhea Treatment

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Background

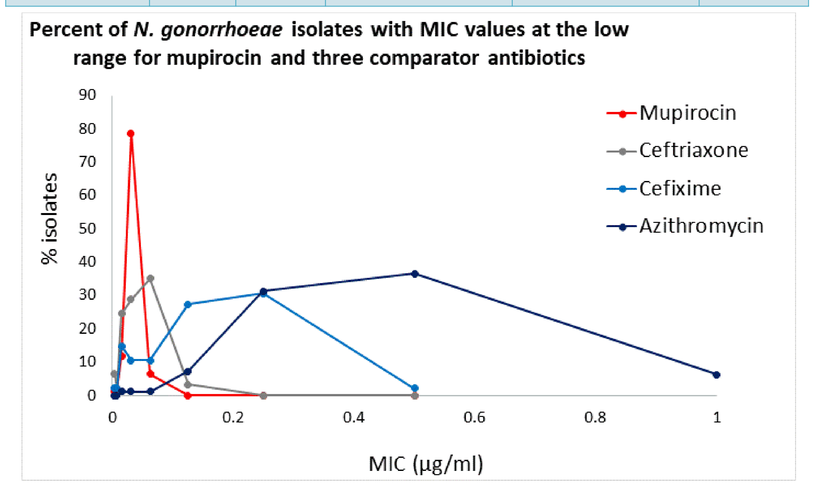
Mupirocin is an antibiotic having a unique mode of action used for the treatment of staphylococci skin infections. It has high protein binding and it is rapidly eliminated from the circulation, limiting its use to topical settings. Loading mupirocin into PEGylated nano-liposomes to form Nano-mupirocin protects the drug, allowing its parental use against a wider range of infections. Mupirocin is highly active against *N. gonorrhea* for which resistance for all marketed antibiotics is emerging. In order to test the suitability of Nano-mupirocin for gonorrhea treatment, in vitro susceptibility of *N. gonorrhea* strains to mupirocin and in vitro resistance studies were performed. Additionally, the distribution of Nano-mupirocin to the vaginal mucus was studied in mice.

Mupirocin MIC against *N. gonorrhea* strains

(Cern A, Connolly KL, Jerse AE, Barenholz Y. 2018. Antimicrob Agents Chemother)

MIC was determined by the agar dilution method.
No cross-resistance with mupirocin was shown for isolates resistant to other antibiotics.
A study performed at Uniformed Services University of the Health Sciences (USUHS) additionally showed that an isolate with resistant to cefixime and ceftriaxone was sensitive to mupirocin (H041).

Compound	MIC_{90} (µg/ml)	MIC_{50} (µg/ml)	EUCAST breakpoint (R>) (µg/ml)[b]	No. of isolates with MIC above the breakpoint	Number of isolates tested
Mupirocin	0.031	0.031	NA	NA	94
Azithromycin	8	0.5	0.5	21	96
Cefixime	0.25	0.125	0.125	31	95
Ceftriaxone	0.063	0.031	0.125	0	94
Ciprofloxacin	16	16	0.06	71	96
Penicillin	2	1	1	43	95
Tetracycline	2	2	1	55	96



Fluorescent microscopy observation of vaginal smears

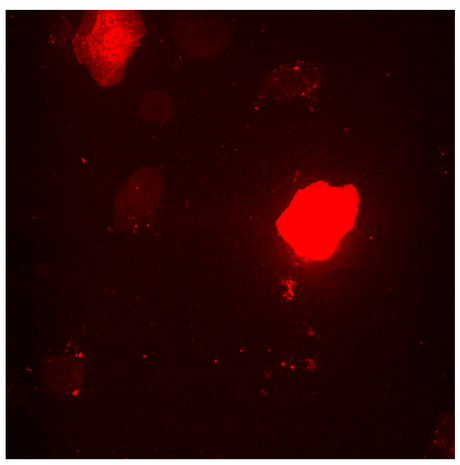
Two female BALB/c mice were injected IP with Lissamine-Rhodamine (LR) labelled Nano-mupirocin (69 mg/kg mupirocin, the label is on the liposomal membrane). 3.5 h after injection, vaginal swabs were taken. An additional swab was taken from an untreated mouse. Smears of these swabs were observed under a spinning disk confocal microscope. The images showed substantial fluorescence in the smears of LR-Nano-mupirocin treated mice. The intense fluorescence signal was found inside cells and on bacteria. These observations show that the liposomes themselves reached the mucus and interacted with bacteria and cells.

Overlay of DIC and Fluorescence Light

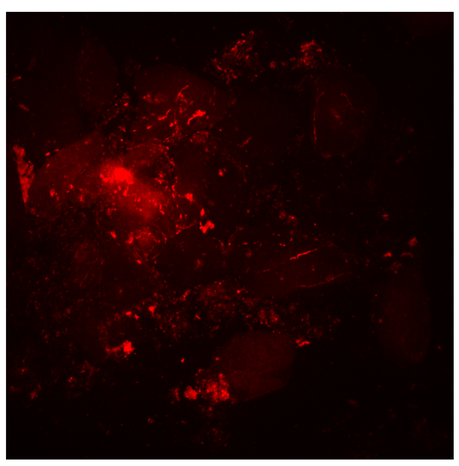
Control untreated mouse

LR Nano-mupirocin treated mouse

Fluorescence Light



LR Nano-mupirocin treated mouse



LR Nano-mupirocin treated mouse

Resistance assays

(Performed at IHMA)

Serial passage

MIC determinations were repeated daily using the growth at 0.5 × MIC. These passages were run for a total of 15 days.

Results: **The MICs for mupirocin and Nano-mupirocin were stable over time against the three *N. gonorrhea* isolates**. Only eight isolates had increased MIC for either mupirocin or Nano-mupirocin and only one isolate was confirmed as mutant, although the increase in MIC was low.

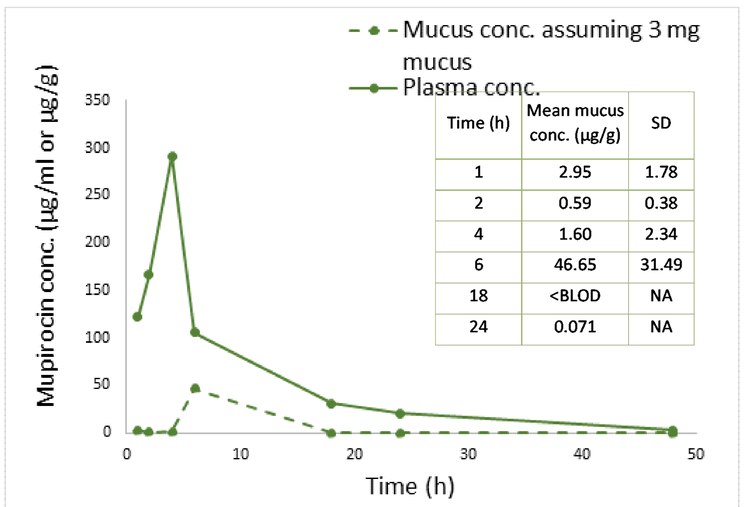
Spontaneous mutation frequency (SMF)

High bacterial inocula (10^8 - 10^{10}) were spread onto agar plate containing the test compounds at 4 ×, 8 × and 16 × MIC. The plates were incubated for 24 h, to allow the growth of resistant mutants. SMF was determined as the proportion of resistant mutants in the total population.

Results: No mutant was obtained.

Plasma profile and distribution to vaginal mucus of Nano-mupirocin following IP administration

Nano-mupirocin (50 mg/kg) was administrated IP to BALB/C female mice aged 7-8 weeks. N=3 mice per time point. At each time point, blood samples were taken and vagina was swabbed. Extracted swabs were analyzed by LCMS/MS method.



Time (h)	Mean mucus conc. (µg/g)	SD
1	2.95	1.78
2	0.59	0.38
4	1.60	2.34
6	46.65	31.49
18	<BLOD	NA
24	0.071	NA

Conclusions

The in vitro activity of Nano-mupirocin against MDR isolates, as well as its distribution to vaginal mucus strongly support the development of Nano-mupirocin for the treatment of MDR gonorrhea.

Acknowledgement

The studies were sponsored by Rebiotics Rx, Israel (**http://www.rebioticsrx.com)**.