#B1301

EFFICACY OF SOLITHROMYCIN FOR TREATMENT OF EXPERIMENTAL SYPHILIS INFECTION

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Abstract

Background: Since 2004, macrolide resistance mutations have been identified in the 23S rDNA of *Treponema pallidum* strains from many geographical regions. Solithromycin (SOLI) is a fluoroketolide antibiotic that binds multiple sites on the bacterial 23S ribosome, which could overcome macrolide resistance conferred by a single basepair mutaton. We tested SOLI for efficacy against strains of *T. pallidum* with and without macrolide resistance mutations.

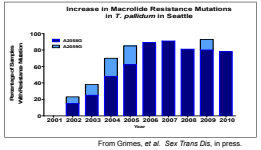
Methods: Groups of 3 rabbits were infected intradermally with *T. pallidum* Nichols (wild type) or a macrolide-resistance mutant: either Street 14 (mutation A2058G) or UW330 (mutation A2059G). After lesions developed, rabbits were treated with benzathine penicillin G (BPG; 200,000 MU IM single dose), Azithromycin (AZ; 15 mg/kg PO daily for 10 days), or SOLI (10 mg/kg/d or 15mg/kg/d IV for 10 days); controls were untreated. On days 0 (pre-treatment) and 3, 5, 7, 9, 11 after treatment initiation, lesion aspirates were examined for presence of *T. pallidum* by darkfield microscopy. Blood was collected weekly for quantitative Venereal Disease Research Laboratory (VDRL) testing. Popliteal lymph nodes (LN) of selected rabbits were transferred into naïve rabbits to detect latent infection.

Results: Following treatment, *T. pallidum* could not be detected in Nichols-infected rabbits treated with BPG, AZ or SOLI; VDRL titers were significantly lower in these groups than in untreated controls; and LN transfers were negative. Similar results were seen in Street 14- and UW330-infected rabbits treated with BPG. *T. pallidum* persisted in Street 14- and UW330B-infected rabbits treated with AZ, and VDRL titers were not significantly different from untreated controls. With SOLI at either dose, treponemes persisted in lesions after treatment, and VDRL titers were not significantly different from untreated controls.

Conclusions: SOLI was as effective as BPG and AZ in treating wild type *T. pallidum* infections in the rabbit model. BPG was effective for Street 14 and UW330 infections, while AZ and SOLI at the doses tested failed to cure *T. pallidum* infection by these strains harboring the A2058G or A2059G mutations.

Background

- The gold standard therapy for syphilis continues to be multiple painful injections of Benzathine Penicillin G.
- The advent of single dose oral treatment for syphilis, azithromycin (AZ), was welcomed by clinicians and patients, and made partner-carried treatment of sexual contacts possible.
- Recognition of azithromycin treatment failures in San Francisco and the Czech Republic led to demonstration of single base pair changes in the *23S rRNA* gene in *T. pallidum* from these patients.[a,b]
- Two *23S rRNA* gene mutations have been found in *T. pallidum* thus far:
  - The A2058G mutation is present in the historical Street 14 (which appears to show greater virulence in the rabbit model) and in currently circulating strains.
  - The A2059G mutation has been found in strains isolated from recent patient samples, such as UW330B.
- The prevalence of these mutations has increased rapidly in many cities since their first identification, shown below for Seattle, WA.



From Grimes, *et al. Sex Trans Dis*, in press.

- Solithromycin may provide an alternative oral treatment of syphilis in humans
  - Solithromycin has improved PK and bioavailability compared to Azithromycin

Mean Pharmacokinetic Parameters in Plasma Following Single Oral Dose (1200 mg)		
	Solithromycin[c] (n=8)	Azithromycin[d,e] (n=12)
C_{max} (µg/mL)	2.0	0.7
$t_{1/2}$ (h)	6.9	40
AUC_{0-24} (µg•h/mL)	26.8	6.8

	Solithromycin[c]	Azithromycin[d,e]
Bioavailability	67%	38%

References: [a]Lukehart *et.al., N Engl J Med* 2004, [b]Matějkov, *et.al., J Med Micro* 2009, [c]Still *et al.*, AAC 2011, [d]Zithromax prescribing information, [e]Foulds *et al.*, JAC 1990. [f] Presented at the 21st ECMIID Conference, 7 to 10 May 2011, Milan, Italy.

Methods

- ***T. pallidum* Infection**
  - •Intradermal infection of rabbits
  - •3 rabbits per treatment and control group; 4 strains
    - •Wild Type (Nichols)
    - •Strain Street 14 (Mutant A2058G)
    - •Strain UW330B (Mutant A2059G)
    - •Strain UW249 (Mutant A2058G)
- **Antibiotic Treatment**
  - • Four treatment groups and controls per strain
  - • BPG (Penicillin) 200,000U IM once only
  - • AZ (Azithromycin) 15mg/kg orally daily
  - • SOLI (Solithromycin) 10 or 15mg/kg IV daily
  - • Untreated
- **Microscopic Analysis**
  - • Examination for *T. pallidum*
  - • Aspirate 2 lesions per day
  - • View aspirate by darkfield microscopy for presence of *T. pallidum*
  - • Results in **Tables 1, 2, & 3**
- **Serological Analysis**
  - • Antibody Titers
  - • Blood collection weekly
  - • Venereal Disease Research Laboratory titers
  - • Titer development in **Figures 1, 2, 3, & 4**
- **Rabbit Infectivity Test**
  - • Detection of persistent infection post-treatment
  - • Transfer of lymph node tissue to susceptible recipient rabbit
  - • Detect persistent infection by microscopic analysis and/or serum antibody titer
  - • Results shown in **Table 5**

Results

Detection of *T. pallidum* in Lesion Aspirates Over the Course of Treatment

All antibiotics tested were effective against wild type Nichols strain

Table 1. Wild type (Nichols) Infection

	Days after initiation of treatment						
Treatment	Pre	D3	D5	D7	D9	Post	*T.p.* after Rx?
BPG (200,000 U IM)	6/6	1*/6	0/6	0/6	0/6	ND	NO
AZ (15mg/kg/d PO)	6/6	0/6	0/6	0/6	0/6	ND	NO
SOLI (10mg/kg/d IV)	6/6	0/6	0/6	0/6	0/6	ND	NO
Untreated	6/6	6/6	6/6	6/6	6/6	6/6	YES

Table 1. DF microscopic examination of aspirates of 2 lesions per rabbits.
*=monmotile. ND = Not Determined

Only BPG was effective against the A2058G mutant Street 14 strain

Table 2. A2058G mutation (Street 14) Infection

	Days after initiation of treatment						
Treatment	Pre	D3	D5	D7	D9	Post	*T.p.* after Rx?
BPG (200,000 U IM)	6/6	0/6	0/6	0/6	ND	ND	NO
AZ (15mg/kg/d PO)	6/6	6/6	6/6	5/6	6/6	4/6	YES
SOLI (10mg/kg/d IV)	6/6	6/6	5/6	5/6	6/6	6/6	YES
SOLI (15mg/kg/d IV)	6/6	6/6	3/6	5/6	4*/6	4/6	YES
Untreated	6/6	6/6	6/6	6/6	6/6	6/6	YES

Table 2. DF microscopic examination of aspirates of 2 lesions per rabbits.
*=monmotile. ND = Not Determined

Only BPG was effective against the A2059G mutant UW330B strain

Table 3. A2059G mutation (UW330B) Infection

	Days after initiation of treatment						
Treatment	Pre	D3	D5	D7	D9	Post	*T.p.* after Rx?
BPG[a] (200,000 U IM)	7/8	0/6	ND	0/6	0/6	ND	NO
AZ[a] (15mg/kg/d PO)	6/6	6/6	ND	4/6	5/6	5/6	YES
SOLI[a] (10mg/kg/d IV)	6/6	4/6	ND	5/6	6/6	6/6	YES
SOLI[a] (15mg/kg/d IV)	6/6	6/6	4/6	4/6	4/6	4/6	YES
Untreated[a,b]	10/12	10/12	4/6	9/12	10/12	11/12	YES

Table 3. DF microscopic examination of aspirates of 2 lesions per rabbits.
[a,b] Separate experiments, duplicate controls. ND = Not Determined

Rabbits infected with the A2058G mutant UW249 strain failed to develop lesions that were consistently DF positive for *T. pallidum*.

Antibiotic treatment efficacy was limited to serological evaluation (see Fig 4).

Serum Antibody Titer Over the Course of Infection and Treatment

Figure 1. WILD TYPE NICHOLS STRAIN

- Titers were significantly lower in all treated groups at 6 weeks, compared to untreated controls; p<0.05 (ANOVA).
- VDRL titer was different between AZ- and Soli-treated groups only at week 3; p<0.05 (ANOVA)

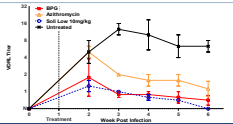


Figure 2. A2058G MUTANT STREET 14 STRAIN

- Titer in BPG group at week 8 was significantly lower than untreated controls; p<0.05 (ANOVA)
- AZ- and SOLI-treated groups had titers that were not significantly different from untreated controls at 8 weeks.
- Although not significantly different from untreated controls at week 8, rabbits treated with 15mg/kg/day SOLI trended toward a lower antibody titer (N.S.) compared to rabbits treated with 10mg/kg/day, perhaps consistent with a dose-response.

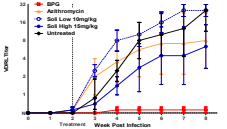


Figure 3. A2059G MUTANT UW330B STRAIN

- Titer in BPG group at week 6 was significantly lower than untreated controls; p<0.05 (ANOVA).
- AZ- and SOLI-treated groups had 6 week titers that were not significantly different from untreated controls.

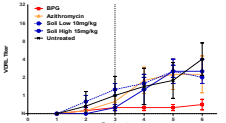
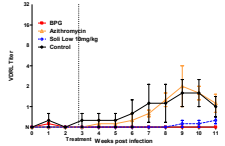


Figure 4. A2058G MUTANT UW249 STRAIN

- Titers in all groups were not significantly different from each other (p>0.05, ANOVA) at weeks 9-11.



Rabbit Infectivity Test for Persistent Infection Post-therapy

Table 5. Persistence of *T. pallidum* after Treatment as Determined by the Rabbit Infectivity Test

Treatment	Strain					
	Nichols (WT)		Street 14 (A2058G)		UW330 (A2059G)	
	Results	Persistent Infection?	Results	Persistent Infection?	Results	Persistent Infection?
BPG (200,000 U IM)	0/3	NO	0/3	NO	0/3	NO
AZ 15mg/kg/d PO)	0/3	NO	ND*	YES	ND*	YES
SOLI (10mg/kg/d IV)	0/3	NO	ND*	YES	ND*	YES
SOLI (15mg/kg/d IV)	Not tested**		ND*	YES	3/3	YES
Untreated	2/2	YES	2/2	YES	4/4	YES

*ND: Not Determined due to microscopic evidence of *T. pallidum* after treatment, showing persistent infection.
**Not tested: The low dose of Solithromycin was sufficient to determine sensitivity of the Nichols strain.

- **Benzathine Penicillin G is effective against all strains, with no persistent infection.**
- **Both Azithromycin and Solithromycin are effective only against the wild type strain, with persistent infection in rabbits infected with mutant strains.**
- **Untreated rabbits have persistent infection for all strains.**

Conclusions

- Benzathine penicillin G is effective in treatment of infection by wild type, A2058G mutant, and A2059G mutant strains of *T. pallidum* in the rabbit model.
- Azithromycin at 15mg/kg/day is effective against wild type *T. pallidum*, but not strains with the A2058G or A2059G mutations.
- Solithromycin at 10 mg/kg/day for 10 days is as effective as benzathine penicillin in treatment of wild type *T. pallidum* infection in the rabbit model.
- Solithromycin at 10 mg/kg/day or 15 mg/kg/day for 10 days is not effective in treating infection with macrolide-resistant *T. pallidum* strains Street 14 and UW330B.
  - However, solithromycin at 15mg/kg/day showed a trend toward lower antibody titer compared to 10mg/kg/day for the Street 14 strain, suggesting a possible dose-response.
  - Although testing doses higher than 15/mg/ml is not feasible in rabbits, treatment of the more robust macrolide-resistant Street 14 strain *may* be effective with higher doses of solithromycin.
- With improved plasma exposure (AUC) and a shorter half-life ($t_{1/2}$), solithromycin may be less likely to select for resistance than azithromycin.

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