

Drug Therapy of Experimental Tuberculosis (TB): Improved Outcome by Combining SQ109, a New Diamine Antibiotic, with Existing TB Drugs[▽]

Boris V. Nikonenko,^{1,2*} Marina Protopopova,¹ Rowena Samala,¹ Leo Einck,¹ and Carol A. Nacy¹
Sequella, Inc., Rockville, Maryland 20850,¹ and Central Research Institute for Tuberculosis, Moscow 107564, Russia²

Received 24 October 2006/Returned for modification 27 November 2006/Accepted 13 January 2007

Substitution of the new diamine antibiotic SQ109 for ethambutol in a mouse model of chronic tuberculosis (TB) improved efficacy of combination drug therapy with first-line TB drugs rifampin and isoniazid, with or without pyrazinamide: at 8 weeks, lung bacteria were 1.5 log₁₀ lower in SQ109-containing regimens.

New antituberculosis drug regimens are clearly needed to reduce the therapy time interval required for a durable cure and to treat the expanding problem of drug- and multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains. Several new drug candidates in development show comparable or better performance than currently available antitubercular agents both in vitro and in animal models of tuberculosis (TB) and are effective against MDR mycobacterial strains (1, 5, 11, 16, 17). For the past several years, we have been characterizing the properties of a new diamine drug candidate with antitubercular activity, SQ109 (4, 7, 8, 10, 14). This new drug with a cell wall mode of action has excellent in vitro activity against both drug-sensitive, single-drug-resistant, and MDR *M. tuberculosis* strains (more than 50, including laboratory strains and clinical isolates), with a MIC range of 0.16 to 0.64 µg/ml against all *M. tuberculosis* isolates tested. Moreover, SQ109 by itself at 10 mg/kg of body weight was able to reduce the number of lung CFU by over 1.5 to 2 log₁₀ in a chronic mouse model of TB, activity that was similar to monotherapy with ethambutol (EMB) at 100 mg/kg (7, 14).

Recently we reported marked in vitro synergistic activity of SQ109 with isoniazid (INH) and rifampin (RIF) and additive activity with streptomycin (4). In preliminary studies to evaluate any synergistic activity with front-line TB drugs in vivo, we used a screening mouse model that followed weight loss as an indicator of TB severity (2, 12). Similar to in vitro observations of synergy, combination therapy with SQ109-containing regimens was also better at preventing TB-induced weight loss than the standard drug regimens (13).

In this report, we compared the efficacy of TB therapy with three- and four-drug combinations where SQ109 replaced EMB in the more standard chronic mouse model of TB.

Groups of C57BL/6 female mice were inoculated intravenously with *M. tuberculosis* H37Rv and treated each day for 5 days/week with saline (untreated controls; six mice/time point), individual drugs (six mice/time point), or drug combinations (six mice/time point) beginning 3 weeks after infection and continuing for 4 or 8 weeks. To assess efficacy of treatment, we

evaluated the average number of *M. tuberculosis* CFU in lungs of six mice/treatment each week during the course of therapy.

In the first set of experiments, we evaluated CFU in lungs of mice infected with 5×10^4 CFU *M. tuberculosis* H37Rv and treated with INH alone or with a combination of INH plus RIF, INH plus RIF plus EMB, or INH plus RIF plus SQ109 over 4 weeks (Table 1). INH was used at 25 mg/kg; RIF was used at 20 mg/kg; EMB was used at its minimal effective dose in mice, 100 mg/kg; SQ109 was used at its minimum effective dose, 10 mg/kg. The combination of INH plus RIF plus SQ109 was better than and statistically different from INH plus RIF plus EMB at weeks 2, 3, and 4. At 4 weeks, the average number of viable *M. tuberculosis* in lungs of untreated mice was 6.42 log₁₀ CFU; lungs of mice treated with INH contained an average 4.61 log₁₀ CFU, the INH plus RIF group had an average 4.27 log₁₀ CFU, the INH plus RIF plus EMB group had 3.86 log₁₀ CFU, and the INH plus RIF plus SQ109 group had 3.26 log₁₀ CFU, 0.6 log₁₀ lower than the standard drug combination (Table 1). In terms of CFU counts, that half-log decrease in CFU translated into a fourfold decrease in the number of viable bacteria: 7,330 in mice treated with INH plus RIF plus EMB versus 1,830 in mice treated with INH plus RIF plus SQ109. Moreover, the combination of INH plus RIF plus SQ109 resulted in the same CFU level in lungs at week 3 as in the INH plus RIF plus EMB group at week 4, a full week earlier. Two other 4-week experiments with the identical three-drug regimen demonstrated similar results: differences between log₁₀ CFU of mice treated with INH plus RIF plus EMB and INH plus RIF plus SQ109 were 0.44 and 0.50 (experiments 2 and 3, respectively), with the SQ109-containing regimen showing better elimination of bacteria from the lung.

In the second series of experiments, pyrazinamide (PZA; 150 mg/kg) was added to INH, RIF, EMB, or SQ109 (concentrations described above) in order to study the complete standard four-drug regimen used in the intensive phase of TB therapy in humans. Efficacy of the EMB-containing combination was compared to efficacy of the SQ109-containing combination. Figure 1 shows the changes in CFU in lungs of control mice and mice over 8 weeks of treatment with INH plus RIF plus EMB plus PZA or INH plus RIF plus SQ109 plus PZA. As above, treatment was initiated 3 weeks following infection of mice with 10⁵ CFU *M. tuberculosis* H37Rv, a time when the level of CFU in lungs reaches a plateau. Four-week therapy

* Corresponding author. Mailing address: Sequella, Inc., 9610 Medical Center Drive, Suite 200, Rockville, MD 20850. Phone: (301) 762-7776. Fax: (301) 762-7778. E-mail: borisnikonenko@sequella.com.

[▽] Published ahead of print on 22 January 2007.

TABLE 1. CFU in lungs of mice treated for 4 weeks with a three-drug regimen^a

Treatment duration and group (dose in mg/kg)	Log CFU/lung \pm SD
Time 0	
Untreated control.....	6.11 \pm 0.04
2 wks	
Untreated control.....	6.16 \pm 0.02
INH (24) + RIF (20) + EMB (100).....	4.64 \pm 0.23 ^b
INH (25) + RIF (20) + SQ109 (10).....	4.46 \pm 0.12 ^b
3 wks	
Untreated control.....	6.34 \pm 0.34
INH (24) + RIF (20) + EMB (100).....	4.38 \pm 0.05 ^c
INH (25) + RIF (20) + SQ109 (10).....	3.80 \pm 0.10 ^c
4 wks	
Untreated control.....	6.42 \pm 0.76
INH (25).....	4.61 \pm 0.15
INH (25) + RIF (20).....	4.27 \pm 0.15
INH (25) + RIF (20) + EMB (100).....	3.86 \pm 0.14 ^d
INH (25) + RIF (20) + SQ109 (10).....	3.26 \pm 0.12 ^d

^a Therapy with drug combinations was initiated 3 weeks after *M. tuberculosis* H37Rv infection.

^b Statistically significant difference (0.18), $P = 0.0011$.

^c Statistically significant difference (0.58), $P = 0.001$.

^d Statistically significant difference (0.60), $P = 0.008$.

with INH plus RIF plus EMB plus PZA reduced log₁₀ CFU in lungs to 4.11, while INH plus RIF plus SQ109 plus PZA reduced log₁₀ CFU to 3.48, a nearly 0.6 log₁₀ difference. In untreated mice at 4 weeks, the average log₁₀ CFU was 7.00. After 6 weeks of therapy with INH plus RIF plus EMB plus PZA, the average log₁₀ CFU in lungs was 3.42, and in mice treated with INH plus RIF plus SQ109 plus PZA, the average lung CFU count was 1.92 log₁₀ (over a 1.5-log₁₀ difference). Untreated mice at 6 weeks again had an average CFU count in lungs of 7.00 log₁₀. After 8 weeks of therapy, INH plus RIF plus SQ109 plus PZA almost eradicated infection (an average of 18 CFU on plates of undiluted whole lung tissue; bacilli were not detected in undiluted lung homogenate samples of two of the six mice), whereas INH plus RIF plus EMB plus PZA had an average of 580 CFU, a 32-fold higher number of bacteria than the SQ109-containing regimen. The average log₁₀ CFU of untreated mice was 7.08 at week 8 (Fig. 1). These results were reproduced in two other experiments with similar differences between SQ109- and EMB-containing regimens.

SQ109 affects synthesis of *M. tuberculosis* cell walls (10, 14). Although the precise target(s) for SQ109 activity is not yet known, some of the genes switched on or off in *M. tuberculosis* by SQ109 are the same as EMB, but some are different (3). The importance of the differences between EMB-regulated and SQ109-regulated genes is underscored by the strong synergy between RIF/INH and SQ109 that is not present in the combination of RIF and EMB, either in vitro or in vivo. Moreover, the MIC range of SQ109 does not change for EMB^r strains or MDR strains compared to drug-sensitive strains of *M. tuberculosis* (3, 14), signifying a second important difference between SQ109 and cell wall-active EMB.

The combination of SQ109 with INH, RIF, and PZA in this study provided a new and very effective anti-TB intensive phase treatment regimen that achieved a better and faster rate

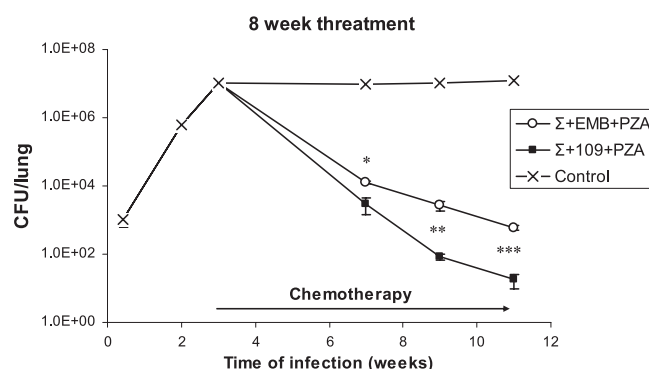


FIG. 1. Efficacy of four-drug combination treatment of mice infected with *M. tuberculosis* H37Rv. Differences between CFU recovered from lungs of mice treated with the EMB-containing regimen and the SQ109-containing regimen were significant (4-week treatment, $P = 0.001$; 6-week treatment, 1.5 log₁₀ CFU difference, $P = 0.022$; 8-week treatment, 1.5 log₁₀ CFU difference, $P < 0.001$). O, INH (25 mg/kg) plus RIF (20 mg/kg).

of mycobacterial kill than the therapeutic regimen of INH plus RIF plus EMB plus PZA. The long half-life of SQ109 in vivo (26 h in beagle dogs [6]) suggests that replacing RIF with longer-acting rifamycins that can be administered weekly in SQ109 combination therapies might also improve the timing of drug delivery for a TB therapy that includes SQ109 (9, 15). The half-life of SQ109 in humans is not yet known, but ongoing phase 1 safety studies of SQ109 in human volunteers will provide these data in the next several months.

This work was generously supported by the National Institutes of Health, NIAID challenge grant UC1 AI-049514-01 ("Second generation antibiotics from ethambutol"), SBIR phase I grant R43 AI060250-01 ("Development of dipiperidines as a new class of anti-TB drugs"), and corporate matching funds.

REFERENCES

- Andries, K., P. Verhasselt, J. Guillemont, W. H. Gohlmann, J.-M. Neefs, et al. 2005. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* **307**:223–227.
- Bogatcheva, E., C. Hanrahan, B. Nikonenko, R. Samala, G. Gearhart, F. Barbosa, L. Einck, C. A. Nacy, and M. Protopopova. 2006. Identification of new diamine scaffolds with activity against *Mycobacterium tuberculosis*. *J. Med. Chem.* **49**:3045–3048.
- Boshoff, H. I., T. G. Myers, B. R. Copp, M. R. McNeil, M. A. Wilson, and C. E. Barry III. 2004. The transcriptional responses of *Mycobacterium tuberculosis* to inhibitors of metabolism: novel insights into drug mechanisms of action. *J. Biol. Chem.* **279**:40174–40184.
- Chen, P., G. Gearhart, M. Protopopova, L. Einck, and C. A. Nacy. 2006. Synergic interaction of SQ109, a new ethylene diamine, with front-line antitubercular drugs in vitro. *J. Antimicrob. Chemother.* **58**:332–337.
- Ji, B., N. Lounis, C. Maslo, C. Truffot-Pernot, P. Bonnafous, and J. Grosset. 1998. In vitro and in vivo activities of moxifloxacin and ciprofloxacin against *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **42**:2066–2069.
- Jia, L., L. Coward, G. S. Gorman, P. E. Noker, and J. E. Tomaszewski. 2005. Pharmacoproteomic effects of isoniazid, ethambutol and SQ109 on *Mycobacterium tuberculosis* H37Rv. *J. Pharmacol. Exp. Ther.* **315**:905–911.
- Jia, L., J. E. Tomaszewski, C. Hanrahan, L. Coward, P. Noker, G. Gorman, B. Nikonenko, and M. Protopopova. 2005. Pharmacodynamics and pharmacokinetics of SQ109, a new diamine-based antitubercular drug. *Br. J. Pharmacol.* **144**:80–87.
- Jia, L., J. E. Tomaszewski, P. Noker, G. S. Gorman, E. Glaze, and M. Protopopova. 2005. Simultaneous estimation of pharmacokinetic properties in mice of three anti-tubercular ethambutol analogs obtained from combinatorial lead optimization. *J. Pharm. Biomed. Anal.* **37**:793–799.
- Kelly, B. P., S. K. Furney, M. T. Jessen, and I. M. Orme. 1996. Low-dose aerosol infection model for testing drugs for efficacy against *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **40**:2809–2812.
- Lee, R., M. Protopopova, E. Crooks, R. A. Slayden, M. Terrot, and C. E.

- Barry III. 2003. Combinatorial lead optimization of [1,2]-diamines based on EMB as potential anti-tuberculosis preclinical candidates. *J. Comb. Chem.* **5**:172–187.
11. Lenaerts, A. J., V. Gruppo, K. S. Marietta, C. M. Johnson, D. K. Driscoll, N. M. Tompkins, J. D. Rose, R. C. Reynold, and I. M. Orme. 2005. Preclinical testing of the nitroimidazopyran P-824 for activity against *Mycobacterium tuberculosis* in a series of in vitro and in vivo models. *Antimicrob. Agents Chemother.* **49**:2294–2301.
12. Nikonenko, B. V., R. Samala, L. Einck, and C. A. Nacy. 2004. Rapid, simple in vivo screen for new drugs active against *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **48**:4550–4555.
13. Nikonenko, B. V., M. Protopopova, R. Samala, E. Bogatcheva, L. Einck, and C. A. Nacy. Drug therapy of experimental TB: combination of new diamine SQ109, and piperidine SQ609 with existing TB drugs improves efficacy of the treatment. *Probl. Tubercul., in press.* (In Russian.)
14. Protopopova, M., C. Hanrahan, B. Nikonenko, R. Samala, P. Chen, J. Gearhart, L. Einck, and C. A. Nacy. 2005. Identification of a new antitubercular drug candidate SQ109 from a combinatorial library of 1,2-ethylenediamines. *J. Antimicrob. Chemother.* **56**:968–974.
15. Shoen, C. M., S. E. Chase, M. S. DeStefano, S. Haspster, A. J. Chmielewski, and M. H. Cynamon. 2000. Evaluation of rifalazil in long-term treatment for tuberculosis in mice. *Antimicrob. Agents Chemother.* **44**:1458–1462.
16. Tygai, S., E. Nuermberger, T. Yoshimatsu, K. Williams, I. Rosenthal, N. Lounis, W. Bishai, and J. Grosset. 2005. Bactericidal activity of the nitroimidazopyran P-824 in a murine model of tuberculosis. *Antimicrob. Agents Chemother.* **49**:2289–2293.
17. Yoshimatsu, T., E. Nuermberger, S. Tyagi, R. Chaisson, W. Bishai, and J. Grosset. 2002. Bactericidal activity of increasing daily and weekly doses of moxifloxacin in murine tuberculosis. *Antimicrob. Agents Chemother.* **46**:1875–1879.