

## Thiosemicarbazole (Thiacetazone-Like) Compound with Activity against *Mycobacterium avium* in Mice

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Received 10 March 2003/Returned for modification 3 April 2003/Accepted 14 May 2003

**In vitro screening of thiacetazone derivatives indicated that two derivatives, SRI-286 and SRI-224, inhibited a panel of 25 *Mycobacterium avium* complex (MAC) isolates at concentrations of 2 µg/ml or lower. In mice, SRI-224 and thiacetazone had no significant activity against the MAC in livers and spleens, but treatment with SRI-286 resulted in significant reduction of bacterial loads in livers and spleens. A combination of SRI-286 and moxifloxacin was significantly more active than single drug regimens in liver and spleen.**

*Mycobacterium avium* is an environmental organism that can cause opportunistic infection in immunosuppressed individuals, such as patients with AIDS or with specific abnormalities in the immune system (9, 12). In addition, disease-causing organisms of the *M. avium* complex (MAC) are isolated from patients with chronic lung diseases and individuals without predisposing conditions (14–16). One of the characteristics of *M. avium* is its resistance to several first-line antimicrobials used in the treatment of tuberculosis (11).

The treatment and prophylaxis of *M. avium* infection were remarkably improved with the introduction of the new macrolides, clarithromycin (5), azithromycin (17), and roxithromycin, although for the latter no clinical trials have been reported (3). However, once resistance to macrolides develops, the options for treatment of *M. avium* infection are limited.

Thiacetazone is a thiosemicarbazole antimicrobial that has been widely used for treatment of tuberculosis in Africa and South America (6). The drug is bacteriostatic against *Mycobacterium tuberculosis* but has been found to be quite useful in combination with isoniazid (8). Because thiacetazone is associated with dermatologic side effects and Stevens-Johnson syndrome in AIDS patients, it is not available in the United States (8). Thiacetazone has been demonstrated to have anti-*M. avium* activity in vitro, with MICs ranging from 0.02 to 0.15 µg/ml (7), but evidence for in vivo activity is lacking.

Recently, a number of analogs have been synthesized, with some showing promising results in vitro against *M. tuberculosis* (unpublished data). In this report we show the results obtained in vitro and in vivo with two thiacetazone analogs.

**Mycobacteria.** MAC 101 is a well-characterized human isolate that has been used in many previous in vivo studies (4). MAC strains 100, 108, 109, and 116 were also isolated from the blood of AIDS patients. MAC organisms were cultured on Middlebrook 7H11 agar (Difco) supplemented with oleic acid-

albumin-dextrose-catalase for 10 days at 37°C. For animal experiments, transparent colony morphotypes were harvested and suspended in Hanks' buffered salt solution at a concentration of  $3 \times 10^8$  CFU/ml for infection of mice. The size of the inoculum was confirmed by colony plating onto 7H11 agar.

**Antibiotics.** Antimicrobial preparations for therapy were made by suspending the agent with 2.5% gum arabic (Sigma) in 0.1% Tween 80 (Sigma). SRI-224, SRI-286, and thiacetazone were synthesized at Southern Research Institute (Fig. 1). Moxifloxacin was kindly provided by Bayer Corp.

**In vitro susceptibility testing.** MICs were determined by a radiometric broth macrodilution method and the T100 method of data analysis (10). The inoculum was prepared as previously reported (2). It was placed into 7H9 broth and frozen at –70°C. The concentration was adjusted to approximately  $5 \times 10^4$  CFU/ml as previously described (2). Controls included the inoculum undiluted without added drug, the inoculum diluted 1:100 (99% control), and the inoculum diluted 1:1,000 (99.9% control). The period of observation and the end point were determined by daily monitoring of the control and test cultures. Seven days was sufficient for most isolates.

**In vivo testing.** C57BL/6J-Lyst<sup>bg-J</sup>/Lyst<sup>bg-J</sup> female mice, aged 8 to 10 weeks, were obtained from Jackson Laboratories for challenge studies. Mice were infected through the caudal veins with  $3 \times 10^7$  CFU of MAC 101 in a volume of 0.1 ml. After 7 days, treatment was initiated with SRI-224, SRI-286, or thiacetazone at 40 mg/kg of body weight/day as oral monotherapy.

TABLE 1. MICs of thiacetazone and thiacetazone analogs for *M. avium* strains

| Strain <sup>a</sup> | MIC (µg/ml)  |         |         |
|---------------------|--------------|---------|---------|
|                     | Thiacetazone | SRI-224 | SRI-286 |
| MAC 100             | 16           | 0.125   | 0.5     |
| MAC 101             | 0.25         | 0.125   | 0.5     |
| MAC 108             | 32           | 0.125   | 1       |
| MAC 109             | 0.25         | 0.125   | 0.5     |
| MAC 116             | 32           | 16      | 16      |

<sup>a</sup> All strains are clinical isolates.

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TABLE 2. Activity of SRI-224, SRI-286, and thiacetazone against *M. avium* in mice

| Treatment <sup>a</sup> | No. of mice | Mean no. of CFU/g of liver $\pm$ SEM | Mean no. of CFU/g of spleen $\pm$ SEM |
|------------------------|-------------|--------------------------------------|---------------------------------------|
| SRI-224                | 19          | $(8.5 \pm 1.5) \times 10^{8b}$       | $(2.4 \pm 0.4) \times 10^{9b}$        |
| SRI-286                | 20          | $(2.5 \pm 0.6) \times 10^{8b,c}$     | $(5.6 \pm 2.4) \times 10^{8b,c}$      |
| Thiacetazone           | 19          | $(9.7 \pm 1.7) \times 10^{8b}$       | $(1.4 \pm 0.2) \times 10^{9b}$        |
| None (4 wk)            | 10          | $(1.2 \pm 0.3) \times 10^9$          | $(2.2 \pm 0.5) \times 10^9$           |
| None (1 wk)            | 5           | $(7.9 \pm 2.1) \times 10^7$          | $(1.7 \pm 0.4) \times 10^8$           |

<sup>a</sup> SRI-224, SRI-286, and thiacetazone were given at doses of 40 mg/kg/day.

<sup>b</sup>  $P > 0.05$  compared with untreated control at 1 week.

<sup>c</sup>  $P < 0.05$  compared with untreated control at 4 weeks.

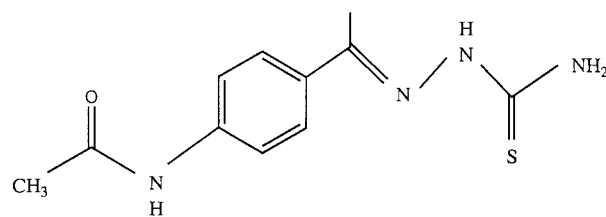
In some experiments, SRI-286 was also used at 80 mg/kg. Each drug treatment group contained at least 19 animals. A control group of 10 animals received the drug vehicle only. In addition, an experimental group of five mice was harvested after 7 days of infection in order to establish a baseline level of infection in tissues. In a subsequent series, mice were treated with either SRI-286 (40 mg/kg/day), moxifloxacin (100 mg/kg/day), or SRI-286 and moxifloxacin in combination. Mice received treatment for 4 weeks and were then bled for quantitative culture 48 h after their last dose and euthanized. Livers and spleens of all mice were aseptically dissected, weighed, and homogenized in 5 ml of Middlebrook 7H9 broth (Difco) containing 20% glycerol. Tissue suspensions were serially diluted and plated onto Middlebrook 7H11 agar plates to determine the number of viable organisms. Plates were incubated at 37°C for 10 days.

**Statistical analysis.** The statistical significance of the differences in tissue bacterial loads was assessed by analyses of variance. Differences between experimental groups were considered significant at  $P$  values of  $<0.05$ .

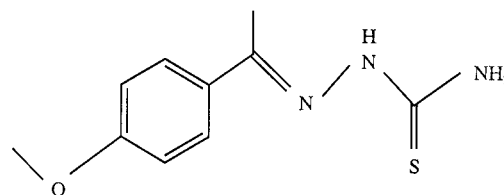
**Activity in vitro.** The inhibitory concentration of thiacetazone for five *M. avium* strains ranged from 0.25 to 32  $\mu$ g/ml (Table 1).

**Efficacy of antimicrobials.** The efficacies of thiacetazone, SRI-224, and SRI-286 were examined at the concentration of 40 mg/kg/day, the mouse dose corresponding to the human dose of thiacetazone (6, 8). At 40 mg/kg/day, treatment with SRI-286 was associated with significant inhibition of bacterial growth in liver and spleen. In contrast, treatment with SRI-224 or thiacetazone did not result in any reduction in bacterial load compared with the number of bacteria in livers and spleens of untreated mice (Table 2). The effect of SRI-286 was bacteriostatic.

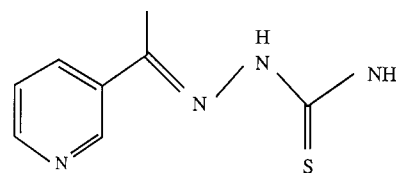
**Efficacy of SRI-286 at 40 and 80 mg/kg.** To determine whether the activity of SRI-286 could be enhanced by increasing the dose of the compound without toxicity, we treated mice



Thiacetazone



SRI-224



SRI-286

FIG. 1. Structures of thiacetazone, SRI-224, and SRI-286.

with 40 and 80 mg/kg. Table 3 shows that SRI-286 was significantly more active at 80 mg/kg/day than at 40 mg/kg/day in liver, spleen, and blood. SRI-286 also had greater activity than ethambutol based on results of 62 complete experiments over the years (1; data not shown).

**Efficacy of SRI-286 in combination with moxifloxacin.** Moxifloxacin is active against *M. avium* (1). Therefore, we investigated whether the combination of moxifloxacin and SRI-286 was more efficacious than each drug alone. SRI-286 was administered at 40 mg/kg. This dose was chosen because of the diminished chance of side effects from this dose compared with that from the dose of 80 mg/kg. The association of moxifloxacin and SRI-286 was significantly more active against *M. avium* than each drug by itself. Although the combination was bacte-

TABLE 3. Comparison of the anti-*M. avium* activities of SRI-286 in mice when administered at 40 and 80 mg/kg/day

| Regimen                  | No. of mice | Mean no. of CFU/g of liver $\pm$ SEM | Mean no. of CFU/g of spleen $\pm$ SEM | Log change in mean no. of CFU/ml of blood $\pm$ SEM |
|--------------------------|-------------|--------------------------------------|---------------------------------------|---|
| SRI-286 (40 mg/kg)       | 12          | $(8.9 \pm 1.4) \times 10^8$          | $(1.7 \pm 2) \times 10^9$             | $-0.25 \pm 0.17$                                    |
| SRI-286 (80 mg/kg)       | 11          | $(6.4 \pm 8) \times 10^{8a}$         | $(9.1 \pm 1.6) \times 10^{8a}$        | $-0.65 \pm 0.13^a$                                  |
| Control (4 wk)           | 10          | $(5.1 \pm 5) \times 10^9$            | $(1.0 \pm 0.07) \times 10^{10}$       | $+0.39 \pm 2.8$                                     |
| None (1 wk) <sup>b</sup> | 5           | $(5.8 \pm 0.3) \times 10^8$          | $(8.2 \pm 0.9) \times 10^8$           |   |

<sup>a</sup>  $P < 0.05$  compared to SRI-286 at 40 mg/kg. Comparison between the mean bacterial loads in the experimental group after treatment and at 1 week (before treatment) determines if the drug is bactericidal or bacteriostatic. Comparison between the experimental group and the control group at 4 weeks determines the significance of the inhibitory effect.

<sup>b</sup> Treatment was initiated after 1 week of infection.

TABLE 4. Activity of SRI-286 and moxifloxacin against *M. avium* in mice

| Treatment                | No. of mice | Mean no. of CFU/g of liver $\pm$ SEM | Mean no. of CFU/g of spleen $\pm$ SEM | Log change in mean no. of CFU/ml of blood $\pm$ SEM |
|--------------------------|-------------|--------------------------------------|---------------------------------------|---|
| SRI-286 (40 mg/kg)       | 17          | $(2.1 \pm 0.5) \times 10^{8a}$       | $(4.9 \pm 0.7) \times 10^{8a}$        | $(-)0.81 \pm 0.27^a$                                |
| Moxifloxacin (100 mg/kg) | 18          | $(2.4 \pm 0.4) \times 10^{8a}$       | $(6.0 \pm 0.9) \times 10^{8a}$        | $(-)0.71 \pm 0.25^a$                                |
| SRI-286 and moxifloxacin | 16          | $(1.2 \pm 0.2) \times 10^{8a}$       | $(2.2 \pm 0.3) \times 10^{8a}$        | $(-)2.55 \pm 0.42^a$                                |
| None (4 wk)              | 17          | $(1.2 \pm 0.2) \times 10^9$          | $(4.0 \pm 0.7) \times 10^9$           | $(+)0.29 \pm 0.22$                                  |
| None (1 wk)              | 10          | $(1.1 \pm 0.1) \times 10^8$          | $(3.7 \pm 0.6) \times 10^8$           |   |

<sup>a</sup>  $P < 0.05$  compared with untreated control at 4 weeks.

riostatic in the liver, bactericidal activity was observed in the spleen (Table 4).

**Summary.** *M. avium* infection is common in individuals with chronic lung diseases (such as emphysema and chronic bronchitis) and in patients with AIDS with CD4 T-cell counts lower than  $100/\text{mm}^3$  (9, 12, 15, 16). The introduction of new macrolides had a remarkable positive impact on the management of *M. avium* infection (5, 17). However, the emergence of resistance to clarithromycin and azithromycin during treatment represents a challenge for the clinician, since no oral drug with efficacy comparable to that of these macrolides is available. Therefore, there is a need to establish alternative regimens without macrolides.

We describe the first thiosemicarbazole with activity against *M. avium* in vivo. Although the MICs of thiacetazone and SRI-224 are comparable for the MAC 101 strain of *M. avium* in vitro, treatment with those compounds had no effect on the infection. The activity of SRI-286 was comparable to the activity of moxifloxacin, the most potent quinolone against *M. avium* in vivo, and better than that of ethambutol, a drug used in patients with *M. avium* infection (1). The combination of SRI-286 and moxifloxacin had additive effect.

A previous study indicated that thiacetazone was active against *M. avium* in vitro (7), a result that was equivalent to our results for two of five strains tested. However, it is interesting that the MICs of all three compounds that we evaluated (thiacetazone, SRI-224, and SRI-286) were below  $1 \mu\text{g}/\text{ml}$  for strain MAC 101 while only SRI-286 showed activity in vivo. Since the structures of the three compounds are very similar, it would be interesting to investigate what modification in the side chains is responsible for the activity in vivo. The possibility that differences in pharmacokinetics would explain the activity in vivo is certainly not strong, because of the activity of thiacetazone in humans (8). Therefore, two plausible hypotheses are that SRI-286 has the ability to maintain activity in the environment where *M. avium* is in vivo and that it is active against the phenotype of *M. avium* in the host. A recent study showed that *M. avium* within cells can develop a phenotype of resistance to drugs that are active in vitro (13).

Another aspect to be considered is the potential side effects of this class of compounds. Phototoxicity has been reported in patients receiving thiacetazone, although it did not prevent the extensive use of this drug in Africa and South America. Since we do not know what portion of the molecule is associated with toxicity, it is possible that analogs may not have similar side effects.

Thiacetazone has been found to be a very effective drug for the treatment of tuberculosis when administered in combination with isoniazid (8). In fact, the World Health Organization uses a preparation in both Africa and South America which contains both compounds in the same tablet. The results ob-

tained with the combination of moxifloxacin and SRI-286 suggest that SRI-286 may also be useful when used in a similar strategy against *M. avium*.

In summary, we report the identification of the first thiosemicarbazole with activity against *M. avium* in vivo. Further investigation will specifically address some of the issues raised by this study.

We thank Karen Allen for preparing the manuscript.

This work was supported by the contract NO1-AI-25140 from the National Institute of Allergy and Infectious Diseases.

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