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Gatifloxacin and Ethionamide as the Foundation for Therapy of Tuberculosis

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The use of gatifloxacin (GAT) in combination with ethionamide (ETA) with or without pyrazinamide (PZA) for a 12-week treatment period followed by an 8-week observation period was evaluated in a model of tuberculosis in mice. Mice treated with GAT at 300 mg/kg of body weight in combination with ETA (25 mg/kg) for 5 days per week had sterile lungs, whereas mice treated with GAT (100 mg/kg) and ETA (25 mg/kg) had about 10 CFU/lung; however, there was regrowth of the organisms in both groups at the end of the observation period. When PZA (450 mg/kg 5 days per week) was added to the high-dose GAT-ETA regimen, no viable mycobacteria were present after the 8-week observation period. GAT in combination with ETA and PZA has great promise for the treatment of tuberculosis.

The activities of gatifloxacin (GAT) alone and in combination with ethionamide (ETA) and pyrazinamide (PZA) were recently studied in a murine tuberculosis model (1); GAT at 100 mg/kg of body weight daily was used alone and in combination with ETA at 75 mg/kg daily and performed well. The addition of PZA at 150 mg/kg daily did not enhance the activity of the GAT-ETA regimen.

One of the newer 8-methoxyquinolones (GAT or moxifloxacin) with potent in vitro and in vivo (murine model) antituberculosis activities in combination with ETA might provide an effective regimen for the treatment of patients with multiple-drug-resistant tuberculosis (MDRTB). It would be useful to develop a short-course regimen against MDRTB whose efficacy is comparable to that of isoniazid (INH)-rifampin (RIF)-PZA. GAT, a DNA gyrase inhibitor, has a mechanism of action different from those of established antimycobacterial agents. ETA is active against most INH-resistant tuberculosis isolates. It is reasonable to evaluate regimens for the treatment of MDRTB caused by a pansusceptible strain if there is minimal potential for cross-resistance among the agents studied.

The aims of this study were to evaluate these agents in combination regimens by using a long-term treatment model (5) to evaluate their clinical potential for the treatment of MDRTB.

MATERIALS AND METHODS

Drugs. GAT was provided by Bristol-Myers Squibb Co., Princeton, N.J. INH, RIF, PZA, and ETA were purchased from Sigma Chemical Co., St. Louis, Mo. GAT was dissolved in 20% ethanol (80% distilled water), INH was dissolved in distilled water, while RIF, ETA, and PZA were dissolved in 20% dimethyl sulfoxide. INH-RIF and ETA-PZA were prepared, aliquoted, frozen at −20°C, and then thawed before use. The drug(s) was administered in a 0.2-ml volume by gavage. The mice were treated with ETA, ETA-PZA, or INH-RIF in the morning and with GAT in the afternoon. GAT was administered separately because the high-dose preparation was a fine suspension rather than a solution.

Isolate. *Mycobacterium tuberculosis* ATCC 35801 (strain Erdman) was obtained from the American Type Culture Collection, Manassas, Va. The MICs of the antimicrobial agents other than PZA were determined in 7H10 broth (pH 6.6; 7H10 agar formulation with agar and malachite green omitted) supplemented with 10% Middlebrook oleic acid-albumin-dextrose-catalase (OADC) enrichment (Difco Laboratories, Detroit, Mich.) and 0.05% Tween 80; the MICs were as follows: INH, 0.03 μg/ml; RIF, 0.06 μg/ml; ETA, 4 μg/ml; and GAT, 0.125 μg/ml. The MIC of PZA was determined in modified 7H10 broth (pH 5.8) and was 32 μg/ml.

Inoculum preparation. The organism was grown in modified 7H10 broth with 10% OADC enrichment and 0.05% Tween 80 on a rotary shaker for 7 days at 37°C. The cells were diluted in modified 7H10 broth to yield 100 Klett units per ml (Photoelectric colorimeter; Manostat Corp., New York, N.Y.) or approximately 5×10^7 CFU/ml. Aliquots of the cell suspension were stored at −70°C prior to use.

Intranasal model. Five- to 6-week-old female C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Maine) were anesthetized with tiletamine-zolazepam (Telazol; 45 mg/kg; Fort Dodge Animal Health, Fort Dodge, Iowa) and xylazine (7.5 mg/kg; Phoenix Pharmaceutical Inc., St. Joseph, Mo.), which were injected intramuscularly. The mice were infected intranasally with 20 μl of 7H10 broth containing approximately 10^6 viable *M. tuberculosis* organisms. Previous work with the intranasal infection model in our laboratory demonstrated that about 80% of the total inoculum is delivered to the lungs (unpublished data). The inoculum size was verified by plating serial dilutions of the bacterial suspension in triplicate on 7H10 agar plates.

Each treatment group contained eight mice. Treatment was started 1 week after infection. The members of a control group of untreated infected mice were killed at the start of treatment (early controls). A second group of untreated mice (late controls) was observed until the mice were moribund, and then the mice were killed. Therapy was given by gavage 5 days per week for 4 weeks (dose-response study) or 12 weeks (treatment observation study). In the 4-week study, GAT was evaluated at 100 and 300 mg/kg and ETA was evaluated at 25, 50, and 75 mg/kg (3, 4) in comparison to INH at 25 mg/kg. In the 12-week study, mice were given ETA at 25 mg/kg daily or 75 mg/kg twice a week, ETA at 25 mg/kg plus PZA at 450 mg/kg, or INH at 25 mg/kg plus RIF at 20 mg/kg in the morning and GAT at 100 or 300 mg/kg in the afternoon. In the last study the paired groups received treatment for 12 weeks, followed by a 2-month observation period for each treatment group. All mice were euthanized by CO_2 inhalation. The right lungs were aseptically removed and ground in a tissue homogenizer (Idea Works! Laboratory Devices, Syracuse, N.Y.) containing 0.05% Tween 80 in 0.9% NaCl. The number of viable organisms was determined by serial 10-fold dilution and subsequent inoculation onto 7H10 agar plates for the lungs from mice in the 4-week study groups and from some of the mice in the 12-week study groups. The whole lungs from mice in the following 12-week treatment groups were plated: GAT at 300 mg/kg plus ETA at 25 mg/kg and PZA at 450 mg/kg, GAT at 300 mg/kg plus ETA at 25 mg/kg, and INH plus RIF. The cultures were incubated at 37°C in ambient air for 4 weeks prior to counting of the organisms.

Statistical evaluation. Viable cell counts were converted to logarithms, which were then evaluated by Kruskal-Wallis one-way analysis of variance and the Mann-Whitney rank sum test to make pairwise comparisons among groups. A comparison of all groups noted statistically significant differences ($P < 0.001$)

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TABLE 1. Dose-response study of GAT and ETA against *M. tuberculosis* ATCC 35801 in mice

Treatment group or treatment (dose [mg/kg])[a]	No.[b]	Median $\log_{10}$ CFU/lung (95% CI)
Early control[c]	7[d]	8.01 (7.80–8.29)
GAT (100)	8	6.11 (5.74–6.43)
GAT (300)	8	4.11 (3.84–4.36)
ETA (25)	8	6.67 (6.43–6.83)
ETA (50)	8	6.79 (6.52–7.00)
ETA (75)	7[d]	6.58 (6.41–6.70)
INH (25)	8	5.59 (5.45–5.71)

[a] Four weeks of treatment was started 1 week after mice received 1.6×10^6 viable mycobacteria intranasally. The drugs were used at the indicated doses. Each group was treated for 5 days/week.
[b] No., number of mice per group.
[c] In the late control group, 2 mice died 16 days after infection. The remaining mice were euthanized on the same day due to their moribund state.
[d] Data for one mouse in each group are missing due to a technical error.

between groups. The organ counts were expressed as the $\log_{10}$ CFU per lung and the median 95% confidence interval (CI).

RESULTS

Dose-response study. Each of the treatments reduced the mycobacterial cell counts in the lungs ($P < 0.001$) compared to those in the lungs of the mice in the early control group (Table 1). The late controls were dead by day 16: two mice were found dead and the remaining mice (6) were euthanized on the same day due to their moribund state. The counts in the group that received GAT at 300 mg/kg (4.11 $\log_{10}$ CFU/lung; 95% CI, 3.84 to 4.36 $\log_{10}$ CFU/lung) were different from those in the group that received GAT at 100 mg/kg (6.11 $\log_{10}$ CFU/lung; 95% CI, 5.74 to 6.43 $\log_{10}$ CFU/lung [$P < 0.001$]) and the INH group (5.59 $\log_{10}$ CFU/lung; 95% CI, 5.45 to 6.43 $\log_{10}$ CFU/lung [$P < 0.001$]). The cells counts for mice that received INH were different from those for mice that received GAT at 100 mg/kg ($P = 0.002$). There were no differences in the cell counts in the three groups that received ETA; however, INH was more active than ETA ($P < 0.001$).

Twelve-week model. (i) Treatment phase. Each of the treatments reduced the mycobacterial cell counts in the lungs ($P < 0.001$) compared to those in the lungs of the mice in the early control group (Table 2). The late controls were euthanized at 18 days postinfection, when they all looked moribund. The cell counts in the lungs of the mice treated with INH-RIF were different from those in the lungs of the mice in each of the other treatment groups ($P = 0.021$). The counts in the lungs of the mice in the group that received GAT at 100 mg/kg plus ETA at 25 mg/kg were not different from those in the lungs of the mice that received GAT at 300 mg/kg plus ETA 75 mg/kg two times per week ($P > 0.05$). No mycobacteria could be cultivated from mice in either the group that received GAT at 300 mg/kg plus ETA at 25 mg/kg or the group that received GAT at 300 mg/kg plus ETA at 25 mg/kg and PZA 450 at mg/kg after 12 weeks of therapy.

(ii) Observation phase. In the groups treated with INH-RIF, GAT at 100 mg/kg plus ETA at 25 mg/kg, and GAT at 300 mg/kg plus ETA at 75 mg/kg, there was about a 2-log increase in the viable cell counts during the observation period. The group treated with GAT at 300 mg/kg plus ETA at 25 mg/kg had regrowth of about 2.5 logs during the observation period. It is interesting that during the observation phase the noncultivatable state was maintained for the group that received GAT at 300 mg/kg plus ETA at 25 mg/kg and PZA at 450 mg/kg.

DISCUSSION

There is a lot of interest in improving the therapy for tuberculosis. The majority of tuberculosis patients are infected with drug-susceptible organisms. In some parts of the world, MDRTB is a significant problem (2). It is likely that the development of successful new regimens for tuberculosis will involve shortening the length of therapy or increasing the times between treatments. Both approaches provide important operational benefits for tuberculosis control programs. In a small clinical trial with patients with MDRTB, sparfloxacin in combination with ETA and kanamycin converted the sputum of all nine treated patients to negativity within 6 months (and did so within 3.5 months for seven patients) (10). These results suggest that an appropriate quinolone combined with ETA could be the basis for effective therapy of pansusceptible tuberculosis as well as MDRTB.

INH-RIF is more active than GAT at 100 mg/kg plus ETA at 25 mg/kg in mice. In humans, the former regimen is likely to have activity similar to or less than that of the latter regimen because the activity of the 20-mg/kg dose of RIF used in mice likely overestimates the activity of the standard dose of 600 mg

TABLE 2. Activity of GAT-ETA against *M. tuberculosis* ATCC 35801 in mice

Treatment group or treatment (dose [mg/kg])[a]	Median $\log_{10}$ CFU/lung (95% CI)	
	12 wk of treatment	12 wk of treatment followed by 8 wk without treatment
Early controls	7.72 (7.20–8.20) (8)[b]	
INH (25)–RIF (20)	0.81 (0.39–0.99) (8)	2.78 (1.60–3.26) (8)
GAT (100)–ETA (25)	1.15 (0.50–1.51) (8)	3.97 (3.34–4.17) (6)[c]
GAT (300)–ETA (25)	0 (8)	2.47 (2.21–3.51) (8)
GAT (300)–ETA (75) (twice/wk)	1.35 (1.00–1.69) (8)	3.94 (3.54–4.39) (8)
GAT (300)–ETA (25)–PZA (450)	0 (8)	0 (7)[d]

[a] Treatment was started 1 week after mice received 2.4×10^6 viable mycobacteria intranasally. The drugs were used at the indicated doses. Each group except the group treated with GAT at 300 mg/kg plus ETA at 75 mg/kg was treated for 5 days/week; the group receiving GAT at 300 mg/kg plus ETA at 75 mg/kg was treated twice a week. Six late controls were found dead at 18 days postinfection, and the two remaining mice were euthanized on the next day due to their moribund state.
[b] The values in the second sets of parentheses indicate the number of mice per group.
[c] Data for two mice in the group are missing due to a technical error.
[d] Data for one mouse in the group are missing due to a technical error.

per day used in humans. The more appropriate dose for mice is probably 10 mg/kg. The higher dose was selected to be consistent with the dose used in our previous studies with this mouse tuberculosis model. It is likely that a dose of GAT modestly higher than 400 mg could be tolerated during daily therapy. A higher dose, perhaps 600 mg daily, would enhance its efficacy. Although ETA is often difficult to tolerate at doses between 500 and 1,000 mg/day, doses of 250 to 500 mg/day may be better tolerated and may be as effective as the higher doses usually used. The regimen of GAT at 300 mg/kg plus ETA at 75 mg/kg twice a week was as effective as GAT at 100 mg/kg plus ETA at 25 mg/kg daily, suggesting that intermittent therapy would be feasible with these agents.

Both GAT (300 mg/kg)–ETA (25 mg/kg) and GAT (300 mg/kg)–ETA (25 mg/kg)–PZA (450 mg/kg) were able to achieve a noncultivatable state after 12 weeks of treatment, but only the latter regimen yielded a sustained noncultivatable state (durable cure) after the 8-week observation period. This was due to the contribution of PZA. We have previously been unable to demonstrate a benefit of PZA (150 mg/kg/day) when it was added to regimens consisting of INH and rifamycin (rifampin, rifapentine, or rifalazil) (6, 8); perhaps this was due to the use of a dose of PZA that was too low. Theoretically, one should be able to demonstrate in mice the enhanced antituberculosis activity that PZA provides to INH-RIF in humans (with a decrease in the duration of therapy from 9 to 6 months). Additional experiments to evaluate a higher dose of PZA with INH-RIF and GAT (100 mg/kg)–ETA (25 mg/kg) are in progress.

While the exposure provided by GAT at 300 mg/kg/day may not be achievable in humans, it is very likely that a regimen of GAT-ETA with or without PZA would be effective for the treatment of tuberculosis in humans. Moxifloxacin, a closely related 8-methoxyquinolone, has recently been evaluated at daily doses of 100 to 400 mg/kg in two different murine tuberculosis models (7, 13). The authors of those papers cited recent pharmacokinetic data that suggest that a 400-mg dose of moxifloxacin in mice (11) has an area under the concentration-time curve equivalent to that of a dose of 7.5 mg/kg (400 mg/day) in humans (9, 12). On the basis of the data from the mouse model presented in this paper, in conjunction with previously published pharmacokinetic data, one could conjecture that a short-course (6-month) regimen of GAT-ETA-PZA has the potential to achieve a durable cure of tuberculosis. Clinical trials will be necessary to determine the appropriate dosing and duration of therapy for this regimen.

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