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In Vitro and In Vivo Activities of Gatifloxacin against *Mycobacterium tuberculosis*

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Gatifloxacin (GAT) and moxifloxacin (MXF) were evaluated in vitro to determine their activities against *Mycobacterium tuberculosis*. GAT was subsequently compared in a dose range study to isoniazid (INH) in a murine tuberculosis model. GAT was somewhat less active than INH. GAT and MXF were evaluated in mice infected with *M. tuberculosis* and were found to have similar activities. GAT was studied alone and in combination with ethambutol, ethionamide (ETA), and pyrazinamide (PZA) and compared to INH and rifampin (RIF). GAT appears to have sufficient activity alone and in combination with ETA with or without PZA to merit evaluation for treatment of tuberculosis.

New antituberculosis regimens are needed to treat multidrug-resistant strains and to reduce the duration of therapy required for a durable cure. Quinolones have been used primarily for the treatment of patients with multidrug-resistant tuberculosis (2, 7, 17). The potential use of quinolones in initial treatment regimens for tuberculosis remains unclear (9). Several recent studies demonstrated enhanced antimycobacterial activity of 8-methoxyfluoroquinolones gatifloxacin (GAT) and moxifloxacin (MXF) in vitro (4, 6, 7, 18; B. Minassian, G. Warr, B. Kolek, B. Ryan, J. Fung-Tomc, and D. Bonner, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E181, 1998). Tuberculosis can be treated with MXF and GAT at concentrations that should severely restrict the selection of resistant mutants (5). The aim of this study was to evaluate the potential activity of GAT in regimens for the treatment of tuberculosis. GAT was initially evaluated alone and in combination with several established antituberculosis agents (ethambutol [EMB], pyrazinamide [PZA], and ethionamide [ETA]) and compared to isoniazid (INH) and rifampin (RIF) using short (4-week) and long (12-week) treatment periods.

GAT and MXF have been evaluated in vitro for their antituberculosis activities (14, 16). MXF has been studied at 100 mg/kg of body weight in a murine tuberculosis model and was found be slightly less active than INH at 25 mg/kg (8, 13). GAT has not previously been studied in a murine tuberculosis model.

MATERIALS AND METHODS

Drugs. GAT, MXF, and levofloxacin (LVX) were provided by Bristol-Myers Squibb Co., Princeton, N.J.; Bayer Corporation, Pharmaceutical Division, West Haven, Conn.; and the R.W. Johnson Pharmaceutical Research Institute, Raritan, N.J., respectively. INH, RIF, PZA, EMB, and ETA were purchased from Sigma Chemical Co., St. Louis, Mo. For use in broth dilution studies, stock solutions (10×) were prepared by dissolving LVX, GAT, and MXF in methanol and double-distilled water (1:4 [vol/vol]). Stock solutions were filter sterilized by passage through a 0.22-μm-pore-size membrane filter and stored at −20°C until use.

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Isolates. Twenty clinical *Mycobacterium tuberculosis* isolates were used in the study. Isolates were kindly provided by Leonid B. Heifets (National Jewish Center for Immunology and Respiratory Diseases, Denver, Colo.) and Betty A. Forbes (Upstate Medical University, Syracuse, N.Y.). *M. tuberculosis* ATCC 35801 (strain Erdman), ATCC 35828, and ATCC 27294 were obtained from the American Type Culture Collection, Manassas, Va. The MICs of the antimicrobial agents other than PZA were determined in modified 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 (3). The MIC of PZA was determined in modified 7H10 broth, pH 5.8 (11).

Inoculum preparation. The organisms were grown in modified 7H10 broth with 10% OADC enrichment and 0.05% Tween 80 on a rotary shaker for 5 to 10 days at 37°C prior to testing. The cell suspensions were diluted in modified 7H10 broth to yield 100 Klett units per ml (Klett-Summerson colorimeter; Klett Manufacturing, Brooklyn, N.Y.) or approximately 5×10^7 CFU/ml. The inoculum size was verified by plating serial dilutions of the bacterial suspension in triplicate onto 7H10 agar plates supplemented with 10% OADC enrichment. Plates were incubated at 37°C in ambient air for 4 weeks prior to the counting of *M. tuberculosis* colonies.

Broth dilution method. First, solutions of the highest concentration to be evaluated were prepared by 10-fold dilution in modified 7H10 broth. Subsequently, serial twofold dilutions were prepared from this stock to provide a final test range of 8 to 0.003 μg/ml. These tubes and a control tube (containing no drug) were inoculated with 0.1 ml of a suspension of mycobacteria (0.1 Klett unit/ml of suspension containing about 5×10^5 organisms/ml) to 1.9 ml of broth to yield a final concentration of approximately 2.5×10^4 viable organisms/ml (range, 0.65×10^4 to 7.1×10^4 organisms/ml). Tubes were incubated on a rotary shaker at 37°C for 14 days prior to reading. The MIC was defined as the lowest concentration of the antimicrobial agent yielding no visible turbidity.

Intravenous model. Six-week-old female CD-1 mice (Charles River, Wilmington, Mass.), were infected intravenously through a caudal vein. Each mouse received approximately 10^7 viable *M. tuberculosis* ATCC 35801 suspended in 0.2 ml of modified 7H10 broth.

There were eight mice in each group unless indicated otherwise. Treatment was started 1 week after infection. A control group of untreated infected mice was sacrificed at the start of treatment (early controls). A second group of untreated mice was sacrificed at the completion of the treatment period (late controls). Therapy was given by gavage 5 days per week for 4 weeks, except in the long-term GAT combination experiment where treatment was given for 12 weeks followed by a 3-month observation period. All mice were euthanized by CO_2 inhalation. The spleens and right lungs were aseptically removed and ground in a tissue homogenizer (IdeaWorks! Laboratory Devices, Syracuse, N.Y.). The number of viable organisms was determined by serial 10-fold dilutions and subsequent inoculation onto 7H10 agar plates. Cultures were incubated at 37°C in ambient air for 4 weeks prior to counting.

In the first study, a dose range study, GAT was evaluated at doses ranging from 5, 25, 75, and 150 mg/kg in comparison to the 25-mg/kg dose of INH. In the next study, GAT (100 mg/kg) was compared to MXF (100 mg/kg) alone and in

TABLE 1. MICs of GAT, MXF, and LVX against 23 *M. tuberculosis* isolates[a]

Antimicrobial agent	MIC (μg/ml)		
	50%	90%	Range
GAT	0.031	0.031	0.007–0.12
MXF	0.062	0.125	0.031–0.12
LVX	0.5	1	0.12–1

[a] Four isolates were resistant to one drug, (INH or PZA) and one isolate was resistant to both INH and RIF.
[b] 50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

combination with RIF (20 mg/kg) plus INH (25 mg/kg), PZA (150 mg/kg) plus EMB (100 mg/kg) plus ETA (75 mg/kg), INH, INH plus RIF (INF-RIF), and PZA-EMB-ETA. Subsequently, GAT alone and in combination with EMB, EMB-ETA, and EMB-ETA-PZA were compared to INH-RIF. In this study, there were 16 mice per treatment group initially. Eight mice per group were killed at the end of the 12-week treatment period. The remaining mice were monitored during the 12-week observation phase.

GAT and MXF were prepared each day prior to administration. GAT and MXF were dissolved in 20% ethanol (80% distilled water). INH was dissolved in distilled water, while RIF, PZA, EMB, and ETA were each dissolved in 20% dimethyl sulfoxide. INH, INH-RIF, and PZA-EMB-ETA were prepared and combined (for the last two), aliquoted, frozen at 20°C, and then thawed before use. The drug(s) were administered in 0.2-ml volumes by gavage.

Intranasal model. Six-week-old female C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Maine) were anesthetized with telazol (45 mg/kg) (Fort Dodge Animal Health, Fort Dodge, Iowa) and xylazine (7.5 mg/kg) (Bayer Corp., Shawnee Mission, Kans.) injected intramuscularly. Mice were infected intranasally with 20 μl of 7H10 broth containing approximately 10^6 viable mycobacteria (15). The inoculum size was verified by plating serial dilutions of the bacterial suspension in triplicate on 7H10 agar plates.

Each treatment group contained six mice. Treatment began 1 week postinfection and was given by gavage 5 days per week for 4 weeks. The drugs were utilized at the concentrations given above.

Statistical evaluation. Viable cell counts were converted to logarithms, which were then evaluated by one- or two-variable analyses of variance. Statistically significant effects from the analyses of variance were further evaluated by Tukey's honestly significant difference tests (10) to make pairwise comparisons among means.

RESULTS

In vitro evaluation of LVX, GAT, and MXF. The activities of LVX, GAT, and MXF were evaluated in vitro (Table 1). The LVX, GAT, and MXF MICs at which 50 and 90% of the isolates tested are inhibited were 0.5 and 1 μg/ml, 0.031 and 0.031 μg/ml, and 0.062 and 0.125 μg/ml, respectively. GAT had somewhat better in vitro activity against *M. tuberculosis* than MXF, and both were much more active than LVX.

The MICs of LVX, GAT, MXF, RIF, INH, PZA, EMB, and ETA against *M. tuberculosis* ATCC 35801 were 0.125, 0.125, 0.062, 0.06, 0.03, 32, 1, and 4 μg/ml, respectively.

In vivo activity of GAT. The activity of GAT in vivo was determined (Table 2). The colony counts in the lungs and spleens for the higher-dose GAT groups (75 and 150 mg/kg) and INH were significantly different ($P < 0.05$) than those for the early control group (infected but untreated mice sacrificed at initiation of therapy), although there was no significant difference in the values for these treatment groups. The values for the two lower-dose GAT groups were not different from those of the early controls.

Mortality in the late control group was 50%, with deaths occurring on days 14, 16, 17, and 21 postinfection. For the GAT treatment groups, there were three deaths in the 5-mg/kg treatment group (days 14, 18, and 29 postinfection), two deaths in the 75-mg/kg treatment group (days 10 and 11 postinfection); one death each in the 25-mg/kg and 150-mg/kg treatment groups (days 9 and 11 postinfection, respectively). There were no deaths in the early control group or in the INH treatment group.

TABLE 2. Activity of GAT and INH against *M. tuberculosis* ATCC 35801 in mice

Treatment group[a]	n[b]	Log_{10} CFU/organ (mean ± SD)	
		Spleen	Lung
Early control	8	7.58 ± 0.54	6.74 ± 0.98
Late control	4[c]	7.97 ± 0.36	8.80 ± 0.18
GAT			
5 mg/kg	5[d]	7.40 ± 0.20	8.32 ± 0.18
25 mg/kg	7[e]	6.66 ± 0.47	7.56 ± 0.80
75 mg/kg	6[f]	5.22 ± 0.50	5.85 ± 0.52
150 mg/kg	7[e]	4.79 ± 0.54	5.36 ± 0.48
INH, 25 mg/kg	8	4.91 ± 0.63	4.71 ± 0.89

[a] Treatment was started 1 week after mice received 3.6×10^7 viable mycobacteria intravenously.
[b] Number of mice per group.
[c] Four mice found dead.
[d] Three mice found dead during therapy.
[e] One mouse found dead during therapy.
[f] Two mice found dead during therapy.

In vivo comparison of GAT with MXF alone and in combination. The activities of GAT with MXF alone and in combination were examined (Table 3). The values for the GAT, MXF, and INH groups were statistically significantly different ($P < 0.01$) than those for both spleens and lungs in the early control group. GAT, MXF, and INH had similar activities against *M. tuberculosis,* and there was no significant difference between these groups ($P > 0.05$).

There was one death in the late control group occurring on day 15 postinfection. The mortality in each of the treatment groups was as follows: GAT (day 30 postinfection), MXF (day 23 postinfection), and INH (day 22 postinfection). There were no deaths in the early control group.

Each of the other treatment groups was statistically significantly different ($P < 0.05$) than the early controls. GAT, MXF,

TABLE 3. Evaluation of MXF alone and in combination compared to GAT against *M. tuberculosis* ATCC 35801

Treatment group[a]	n[b]	Log_{10} CFU/organ (mean ± SD)	
		Spleen	Lung
Early control	8	7.70 ± 0.16	6.93 ± 0.45
Late control	7[c]	8.83 ± 0.44	8.93 ± 0.42
GAT	7[c]	4.84 ± 0.52	4.52 ± 0.80
MXF	7[c]	4.66 ± 0.70	4.80 ± 0.40
INH	7[c]	5.02 ± 0.40	4.19 ± 0.29
INH-RIF	7[c]	3.06 ± 0.71	3.05 ± 0.71
INH-RIF-MXF	7[c]	2.65 ± 0.46	2.83 ± 0.47
PZA-EMB-ETA	6[d]	5.32 ± 0.25	4.76 ± 0.37
PZA-EMB-ETA-MXF	6[d]	4.46 ± 0.27	3.29 ± 0.16

[a] Treatment was started 1 week after mice received 3.6×10^7 viable mycobacteria intravenously. The drugs were evaluated at the following doses: GAT, 100 mg/kg; MXF, 100 mg/kg; INH, 25 mg/kg; RIF, 20 mg/kg; PZA, 150 mg/kg; EMB, 100 mg/kg; and ETA, 75 mg/kg.
[b] Number of mice per group.
[c] One mouse found dead during therapy.
[d] Two mice found dead during therapy.

TABLE 4. Reduction of *M. tuberculosis* ATCC 35801 cell counts by GAT alone and in combination after 12 weeks of treatment

Treatment group[a]	n[b]	Log_{10} CFU/organ (mean ± SD)	
		Spleen	Lung
Early control	8	8.45 ± 0.23	7.56 ± 0.30
Late control	0[c]		
GAT	8	2.93 ± 0.61	3.37 ± 0.80
GAT-EMB	8	3.06 ± 0.54	3.32 ± 0.68
GAT-EMB-ETA	8[d,e]	1.74 ± 0.24	1.60 ± 0.26
GAT-EMB-ETA-PZA	8[e]	0.26 ± 0.34	0.92 ± 0.59
INH-RIF	8[e]	0.49 ± 0.27	0.27 ± 0.32
INH-RIF-GAT	8[e]	0.08 ± 0.14	0.30 ± 0.36

[a] Treatment was started 1 week after mice received 3.2×10^7 viable mycobacteria intravenously. The drugs were evaluated at the following doses: GAT, 100 mg/kg; EMB, 100 mg/kg; ETA, 75 mg/kg; PZA, 150 mg/kg; INH, 25 mg/kg; and RIF, 20 mg/kg.
[b] Number of mice per group.
[c] All mice died before the completion of the treatment phase.
[d] Data for lungs based on six samples due to contamination.
[e] Entire organs were plated.

INH and PZA-EMB-ETA had comparable activity against *M. tuberculosis.* Treatment with MXF alone was statistically significantly different ($P < 0.01$) than the INH-RIF, INH-RIF-MXF, and PZA-EMB-ETA-MXF combinations in lungs, while in spleens this difference was not demonstrated ($P > 0.05$). Moreover, the PZA-EMB-ETA-MXF combination in lungs was statistically significantly different ($P < 0.05$) than PZA-EMB-ETA; however, there was no enhancement of activity in spleens ($P > 0.05$).

One of 8 mice died in the late control group and in the PZA-EMB-ETA group (days 15 and 11 postinfection, respectively). In the PZA-EMB-ETA-MXF group, deaths occurred on days 9 and 17 postinfection, respectively. No deaths occurred in the rest of the groups studied.

GAT alone and in combination in a long-term model. The activity of GAT alone and in combination was examined in a long-term model (Table 4) The values for each treatment group were statistically significantly different ($P < 0.01$) from that of the early control group. GAT alone and GAT-EMB had comparable activities against *M. tuberculosis*. The value for GAT-EMB-ETA was statistically significantly different ($P < 0.01$) from that for GAT or GAT-EMB. In lungs, the value for the GAT-EMB-ETA combination was not significantly different from the GAT-EMB-ETA-PZA value ($P > 0.05$). The GAT-EMB-ETA value was statistically significantly different from the INH-RIF value ($P < 0.01$). The GAT-EMB-ETA-PZA value was not significantly different from the value for INH-RIF or INH-RIF-GAT ($P > 0.05$). The addition of GAT to INH-RIF did not improve the activity of INH-RIF.

Twelve weeks after cessation of therapy, regrowth was detected in all groups (data not shown). In both spleens and lungs, the log CFU values for the GAT-EMB-ETA-PZA, INH-RIF, and INH-RIF-GAT combinations were not significantly different from each other but were statistically significantly lower than the values for the other groups.

No deaths occurred in the early control group nor in the treatment groups evaluated. Mortality in the late control group was 100%, with deaths occurring in weeks 4 (two mice), 7 (four mice), and 8 (two mice) postinfection.

GAT alone and in combination in intranasal model. The activity of GAT alone and in combination in an intranasal model was examined (Table 5) Each of the treatment groups had statistically significantly lower lung counts than that for the early control group ($P < 0.01$). The INH treatment was more effective than GAT-PZA ($P < 0.01$) but was not significantly different from the values for the other treatments. Each of the treatment groups containing GAT-ETA were more effective than GAT alone ($P < 0.05$). GAT-PZA was less effective than GAT-ETA, GAT-ETA-PZA, or GAT-ETA-PZA-EMB ($P < 0.01$).

DISCUSSION

GAT was evaluated alone and in combination with ETA, PZA, and EMB. ETA appeared to be the most promising single agent for use in combination with GAT. It is not clear whether EMB and/or PZA would substantially enhance GAT-ETA for treatment of clinical tuberculosis. Their addition to GAT plus ETA would likely decrease the emergence of resistance in the clinical setting; however, PZA in combination with ofloxacin was not well tolerated in humans (1).

It is difficult to extrapolate from the doses used in humans to those used in mice and vice versa. A 400-mg dose of GAT given orally once daily yielded a maximum concentration of drug in serum and area under the concentration-time curve of 4.2 μg/ml and 51.3 μg h/ml, respectively (package insert; Bristol-Myers Squibb Company). These parameters should be comparable to those achieved in mice with 100 mg/kg (12).

Based on the results of these experiments, GAT-ETA-PZA and/or EMB would likely be an effective regimen for treatment of multidrug-resistant tuberculosis. It is not clear whether this regimen could be enhanced by increasing the dose of GAT. Likewise, it is unclear how much of a detriment it would be to decrease the dose of ETA. Clearly, reducing the dose of ETA would enhance its tolerability. Additional experiments in mice designed to study the efficacy of higher doses of GAT would be of interest to understand the dose-response profile of this agent. Furthermore, the evaluation of lower doses of ETA in combination with GAT would further our understanding of

TABLE 5. Reduction of *M. tuberculosis* ATCC 35801 cell counts in the lungs by GAT alone and in combination after 4 weeks of treatment

Treatment group[a]	n[b]	Log_{10} CFU/lung (mean ± SD)
Early control	6	6.08 ± 0.34
Late control	3[c]	7.99 ± 0.63
INH	6	3.70 ± 0.61
GAT	5[d]	4.21 ± 0.23
GAT-ETA	5[d]	2.94 ± 0.15
GAT-PZA	6	4.80 ± 0.59
GAT-ETA-PZA	5[d]	3.02 ± 0.66
GAT-EMB-ETA-PZA	4[e]	3.09 ± 0.40

[a] Treatment was started 1 week after mice received 4.6×10^5 viable mycobacteria intranasally. The drugs were used at the following doses: GAT, 100 mg/kg; EMB, 100 mg/kg; ETA, 75 mg/kg; PZA, 150 mg/kg; INH, 25 mg/kg; and RIF, 20 mg/kg.
[b] Number of mice per group.
[c] Two mice died 25 days postinfection, and one mouse died 27 days postinfection.
[d] One mouse missing from each group due to technical error.
[e] Two mice missing due to technical errors.

potential limitations of this combination. Based on our results and the findings of Miyazaki et al. (13) GAT and MXF have sufficient in vivo activity to justify their further evaluation for the treatment of clinical tuberculosis. Future clinical studies involving 8-methoxyfluoroquinolones will be required to determine the role of these agents for the treatment of multidrug-resistant tuberculosis.

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