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Activities of Moxifloxacin in Combination with Macrolides against Clinical Isolates of *Mycobacterium abscessus* and *Mycobacterium massiliense*

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Infections caused by *Mycobacterium abscessus* and *Mycobacterium massiliense* are on the rise among humans. Although macrolides, including clarithromycin (CLR) and azithromycin (AZM), are key antibiotics for the treatment of *M. abscessus* and *M. massiliense* infections, treatment regimens for these infections are still largely undefined. In this study, we evaluated the *in vitro*, *ex vivo*, and *in vivo* activities of moxifloxacin (MXF) in combination with macrolides against clinically isolated *M. abscessus* and *M. massiliense* strains. Overall, CLR, AZM, and MXF alone showed activity against both species *in vitro*, *ex vivo*, and *in vivo*. When MXF was combined with a macrolide against *M. abscessus* isolates, antagonism was observed in 65.4% (17/26) of the strains with CLR and 46.2% (12/26) of the strains with AZM *in vitro* as well as in 66.7% (10/15) of the strains with CLR and 40.0% (6/15) of the strains with AZM in macrophages as determined by the fractional inhibitory concentration index. In contrast, either indifferent or synergistic effects of the MXF-macrolide combinations were observed against only *M. massiliense* strains. Moreover, a murine infection model showed similar results. Antagonism between the MXF and macrolide combinations was observed in five out of seven *M. abscessus* strains, while indifferent and synergistic effects for these combinations were observed for three of the six *M. massiliense* strains tested, respectively. In conclusion, the activity of MXF in combination with a macrolide differed for *M. abscessus* and *M. massiliense* infections and the addition of MXF to macrolide therapy had no benefit for the treatment of *M. abscessus* infections.

Mycobacterium abscessus is the most common etiologic agent of lung diseases that are caused by the rapidly growing mycobacteria (RGM) (12, 13, 18), and it has emerged as an important pathogen in patients with cystic fibrosis (25, 33, 36). *M. abscessus* is resistant to most antibiotics currently available and thus is very difficult to treat (23, 24, 34). Isolates are usually susceptible only to some parenteral agents (amikacin, cefoxitin, and imipenem) and to oral macrolides (clarithromycin [CLR] and azithromycin [AZM]) (12, 13, 18). Combination therapy of intravenous amikacin with cefoxitin or imipenem and an oral macrolide for 2 to 4 months has been recommended by the American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) and many other experts (12, 13, 18). After initial therapy, macrolide administration plus at least one other antibiotic agent to which the organism is susceptible should be used for treatment. However, this option is limited because of high *in vitro* resistance rates to the various other oral agents used against *M. abscessus* isolates (12, 13, 18).

Oral fluoroquinolones have also been used in the treatment of RGM infection. Although fluoroquinolones cannot be used as a single-drug therapy due to the risk of developing resistance mutations (45), some experts have suggested that "holding" regimens of a macrolide plus a fluoroquinolone may be helpful during periods between pulsed intravenous antibiotic therapies (14). Fluoroquinolones have been used in many patients during combination treatment of *M. abscessus* lung disease in clinical practices (23, 24), but the combined activities of a fluoroquinolone with a macrolide against *M. abscessus* have not been evaluated systemically in experimental studies or clinical trials.

Recently, *M. abscessus* was divided into three separate subspecies: *Mycobacterium abscessus* sensu stricto, *Mycobacterium massiliense*, and *Mycobacterium bolletii* (1, 3). *M. massiliense* is now recognized as a separate species from *M. abscessus*, and treatment response rates to CLR-based antibiotic therapy are much higher in patients with *M. massiliense* than in those with *M. abscessus* lung disease (28, 31). To gain greater insight into the optimal therapeutic strategy for *M. abscessus* and *M. massiliense* infections, we evaluated the *in vitro*, *ex vivo*, and *in vivo* activities of moxifloxacin (MXF) in combination with macrolides against clinical isolates of *M. abscessus* and *M. massiliense*.

MATERIALS AND METHODS

Clinical isolate sources, growth conditions, and inoculum preparation. A total of 62 clinical isolates consisting of 31 *M. abscessus* and 31 *M. massiliense* isolates were recovered from patients who were diagnosed at the Samsung Medical Center (Seoul, South Korea). All patients met the diagnostic criteria for nontuberculous mycobacterium (NTM) lung disease published by the American Thoracic Society (18). The data are part of an ongoing prospective observational cohort study investigating NTM lung disease (ClinicalTrials.gov identifier NCT00970801). The study pro-

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tocol was approved by the institutional review board of the Samsung Medical Center (IRB approval 2008-09-016), and written informed consent was obtained from all participants. Two reference strains, *M. abscessus* ATCC 19977^T (ATCC, Manassas, VA) and *M. massiliense* CIP108297^T (Institut Pasteur, Paris, France), were always included as controls for each set of experiments.

Precise species identification of *M. abscessus* and *M. massiliense* was performed using sequence analysis targeting the *rpoB* and *hsp65* genes as well as an analysis of the variable-number tandem repeat (VNTR) profile as previously described (2, 8). All strains were initially cultured in 7H9 broth (Becton, Dickinson, Franklin Lakes, NJ) supplemented with oleic acid-albumin-dextrose-catalase (OADC; Becton, Dickinson, Sparks, MD) for 10 days at 37°C. Single-cell suspensions of each strain were prepared as previously described with slight modifications (41). Seed lots of each strain were kept in small aliquots at −80°C until use. Tenfold serial dilutions from seed lots of each strain were plated on Middlebrook 7H10 agar (Becton, Dickinson) to quantify the number of organisms per milliliter.

Antimicrobials. MXF was kindly provided by Bayer Schering Pharma AG (Berlin, Germany). Two macrolides, CLR and AZM, were purchased from Tokyo Chemical Industry Co. (TCI, Tokyo, Japan) and LKT Laboratories, Inc. (St. Paul, MN), respectively. A common diluent (0.03% acetic acid solution) for all of the antibiotics was included as a control in all of the experiments. All of the stock drug solutions were freshly prepared for each experiment and filter sterilized using a 0.22-μm polycarbonate syringe filter (Millipore Corp., Bedford, MA).

***In vitro* susceptibility testing.** MICs of all tested drugs were determined by broth microdilution assays according to the protocol set by the Clinical and Laboratory Standards Institute (CLSI) (11). Briefly, the drugs at final concentrations ranging from 0.125 to 128 μg/ml in 7H9-OADC broth were added to 96-well plates in 2-fold serial dilutions. The final inoculum size was adjusted to 10^4 CFU/ml. Controls included the inoculum without drug added (no-drug control) and the 1:100-diluted inoculum (99% control). The MIC of each drug was defined as the lowest drug concentration that inhibited more than 99% of the bacterial growth observed in the drug-free medium after incubation at 37°C for 72 h. The MIC_{50} and MIC_{90} levels were interpreted as the lowest concentration of antibiotics that inhibited 50% and 90% of the isolates, respectively. All assays were independently performed three times in triplicate.

Mice and preparation of bone marrow-derived macrophages (BMDMs). Specific-pathogen-free female C57BL/6 mice at 5 to 6 weeks of age were purchased from Japan SLC, Inc. (Shijuoka, Japan), and maintained under barrier conditions in a biosafety level 2 (BL-2) biohazard animal facility at the Medical Research Center of Chungnam National University. The animals were fed a sterile commercial mouse diet and provided with water *ad libitum*. The animal experiments complied with the ethical and experiment regulations for animal care at Chungnam National University (approval no. 2008-04).

Murine bone marrow-derived macrophages (BMDMs) were differentiated for 6 days in macrophage colony-stimulating factor (M-CSF)-containing media, as described previously (27). Briefly, bone marrow cells from the femur and tibia were cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone, Logan, UT) containing 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10% fetal bovine serum, and 20 ng/ml of recombinant M-CSF (R&D Systems, Minneapolis, MN) at 37°C in the presence of 5% CO_2. After 6 days, nonadherent cells were removed, and the differentiated macrophages were incubated in antibiotic-free DMEM until use.

Antibiotic activities against intracellular bacteria within macrophages. The activities of drug combinations between macrolides and MXF were evaluated for a reference strain and 14 clinical isolates of each species, *M. abscessus* and *M. massiliense*, using a murine bone marrow-derived macrophage system. These clinical isolates of each species were selected based on the similarity of their MICs to that of MXF (ranging from 0.5 to 2 μg/ml).

Differentiated BMDMs were adjusted to a density of 5.0×10^5/well in a 24-well tissue culture plate (Corning Life Sciences, Acton, MA) and were infected with each strain of *M. abscessus* and *M. massiliense* for 4 h at the ratio of 1 bacterium to 1 macrophage. This infectious dose of mycobacteria did not affect the macrophage viability during the experiments, and thus, the drug activity could be measured only against intracellular bacteria. The infected BMDMs were washed twice with DMEM to remove extracellular bacteria and were cultivated for 24 h at 37°C in 5% CO_2 to allow the intracellular mycobacteria to adapt. To compare the antibiotic activities of each individual drug and the MXF-macrolide combinations against the intracellular bacteria, the mycobacterium-infected macrophages were treated with 10 μg/ml of each drug or MXF-macrolide combinations at the same concentrations followed by incubation for an additional 48 h. At the indicated times (before and after treatment), the macrophages were lysed with distilled water, and the number of CFU within the cell lysates was determined using the plate counting method for cultures grown on 7H10-OADC.

Evaluation of treatment efficacy in mice. Six clinical isolates of *M. abscessus* and 5 clinical isolates of *M. massiliense* as well as the two reference strains were used for the therapeutic evaluation of each drug and the MXF-macrolide combinations in mycobacterium-infected mice. Before conducting the experiments, the minimum lethal dose (MLD) of each strain was determined using six mice per strain, and 4-fold dilutions of the MLD (4.0×10^6 to 1.0×10^7 CFU) were subsequently used in the *in vivo* experiments. Six-week-old female C57BL/6J mice (36 mice per strain, 6 mice per drug) were infected with the selected dose of each strain by intravenous injection via the tail vein. At 4 days postinfection, treatment was initiated with the antimicrobial agents (100 mg/kg of body weight/day of each drug alone or in combination with 100 mg/kg/day of MXF). The dose of each antibiotic was selected based on the results of previous studies that determined the effective dose of each drug in murine infection models of other mycobacteria (5, 6, 32). Antimicrobial agents were administered daily by oral gavage for 12 days. Control mice for each strain received the same volume of 0.03% acetic acid in distilled water over the same period. At 16 days postinfection, six mice per group were euthanized, and their livers, spleens, and lungs were aseptically collected for bacterial counts. The numbers of viable bacteria in the organs were determined by plating serial dilutions of the organ homogenates onto 7H10-OADC agar plates. Colonies were counted after 5 days of incubation at 37°C. The differences detected when comparing the drug-treated groups and control groups as well as among the drug-treated groups (drug alone versus combinations) are represented as the means of the $\log_{10}$ CFU ± the standard deviation (SD) for each group of mice. This experiment was performed twice.

Evaluation of combined drug action. Evaluation of the antimycobacterial activities of each drug alone and of the MXF-macrolide combinations, the fractional inhibitory concentration (FIC) based on the MICs *in vitro*, and the FICs based on CFU counts in macrophages and mice are described below.

(i) Evaluation of combination activity *in vitro*. The effects of the antibiotic combinations were determined by the fractional inhibitory concentration (FIC) based on the MICs of each drug alone and of each drug combination as previously described with slight modifications (19, 20, 26, 39). The concentration range of each antibiotic in combination ranged from 1/16 the MIC to 4 times the MIC. The FIC index was calculated as follows: (MIC of drug A in combination)/(MIC of drug A alone). The following definitions were used: synergism, FIC index of ≤0.5; indifference, FIC index of >0.5 and <2; and antagonism, FIC index of ≥2. All tests were performed in triplicate, and the results were averaged. For the evaluations of the combined effects of MXF and macrolides, drug A was defined as CLR or AZM. Synergy has been defined as a 2-fold reduction in the MIC of the combination of antibiotics compared with each antibiotic alone. Antagonism has been defined as a 2-fold increase in the MIC when a combination of antibiotics is used. The *in vitro* interactions of the individual macrolides in combination with MXF are summarized in Table 2.

TABLE 1 *In vitro* antimicrobial activities of macrolides and moxifloxacin against 62 clinical isolates of *M. abscessus* and *M. massiliense* and each reference strain

Species (no. of isolates)	Agent	No. of strains distributed at each MIC (μg/ml)									Resistance rate, no. of strains (%)
		≤0.25	0.5	1	2	4	8	16	32	≥64	
M. abscessus (32)	AZM				4	5	15	2	3	3	6[a] (18.8)
	CLR	3	15	5	2	1	1	1	1	3	6[a] (18.8)
	MXF	6	7	10	9						
M. massiliense (32)	AZM			1	2	6	23				
	CLR		20	12							
	MXF	12	10	3	3	3[b]	1				4[b] (12.5)

[a] Six isolates of *M. abscessus* that were resistant to both macrolides due to a point mutation in the 23S rRNA were excluded from the subsequent experiments.
[b] Four isolates of *M. massiliense* that were resistant to moxifloxacin were excluded from the subsequent experiments.

(ii) Determination of combination activity in macrophages and in mice. To determine the effect of antibiotic interactions in macrophages and in mice, the antibiotic activity of the combination treatment was defined as the $\log_{10}$ CFU reduction as previously reported with slight modifications (32, 40). Briefly, the antimycobacterial activity was assessed by counting the CFU from the macrophage lysates or the homogenates of inoculated organs on 7H10 agar supplemented with 10% OADC and determining the $\log_{10}$ CFU per ml after 5 days.

In these calculations, x refers to the $\Delta\log_{10}$ CFU from the control obtained with the drug combination and y refers to the lowest $\Delta\log_{10}$ CFU from the control obtained with each drug used alone. The interaction between the drugs was assessed quantitatively by adopting the x/y quotient method described by Hoffner et al. (20). An x/y value of 1 indicated that there was no interaction between the two drugs and was interpreted as indifference, an x/y value of <0.5 indicated synergy, and an x/y value of >2.0 indicated antagonism.

Statistical analysis. The results in the text and tables are reported as the mean ± standard deviation or as the number (percentage) of strains. Comparisons between single drugs and drug combinations in the same strain were analyzed using Wilcoxon's matched pairs test. The differences between the treatment groups in the same species were determined using the Mann-Whitney nonparametric test. A comparison of treatment efficacies for *M. abscessus* and *M. massiliense* with each drug combination was performed with a chi-squared test. The differences were considered significant at a P value of <0.05. All statistical analyses were performed using GraphPad Prism software (version 4.03; GraphPad Software, San Diego, CA).

RESULTS

***In vitro* susceptibilities to macrolides and MXF.** The antimicrobial activities of the macrolides were tested against 31 *M. abscessus* and 31 *M. massiliense* isolates in addition to the two reference strains. The MIC data for CLR, AZM, and MXF against *M. abscessus* and *M. massiliense* are shown in Table 1. Overall, the MIC range of CLR was lower than that of AZM for both *M. abscessus* and *M. massiliense*. According to the CLSI, ≤2 μg/ml is regarded as susceptible and ≥8 μg/ml is regarded as resistant to CLR and ≤1 μg/ml is regarded as susceptible and ≥4 μg/ml is regarded as resistant to MXF (11). However, the breakpoint for AZM against *M. abscessus* and *M. massiliense* has not been established. In this study, *M. abscessus* and *M. massiliense* isolates were considered susceptible if the MIC of AZM was ≤16 μg/ml and were considered resistant at ≥32 μg/ml. These values were derived from both the distribution of MICs and the MIC_{90} of CLR.

Among the *M. abscessus* isolates, six strains showed initial drug resistance to both macrolides due to a point mutation in the adenine at position 2058 (A_{2058}) in the 23S rRNA gene (data not shown). In contrast, none of the *M. massiliense* strains were resistant to either macrolide. Interestingly, 4 of the 31 *M. massiliense* isolates and the reference strain displayed MXF resistance, while the MICs of MXF for all of the *M. abscessus* strains did not exceed 2 μg/ml. The strains that showed initial resistance to any drug were ruled out for subsequent experiments to prevent interference in the determination of the combination activities of each drug. Thus, *M. abscessus* and *M. massiliense* clinical isolates with no considerable variation in MIC values upon each antimicrobial agent were used to investigate the effects of the combined drugs. MXF exhibited the lowest range of MIC values against the clinical isolates tested in the study (≤0.25 to 2 μg/ml for *M. abscessus* and ≤0.25 to 8 μg/ml for *M. massiliense*), while AZM had a wider range of MIC values than did MXF or CLR.

***In vitro* activities of macrolide-MXF combinations.** The activities of the drug combinations CLR-MXF and AZM-MXF were compared with those of single drugs for each of the strains that were found to be susceptible to each of the three drugs (Table 2). When antagonism was defined as an FIC index of >2.0, 17 (65.4%) and 12 (46.2%) of 26 *M. abscessus* isolates showed antagonism for the CLR-MXF and AZM-MXF combinations, respectively. Notably, synergism resulting from the combination of MXF with either CLR or AZM was observed for one strain only.

However, when the macrolide and MXF combinations were tested against the 28 *M. massiliense* strains, both CLR-MXF and AZM-MXF combinations generally resulted in an indifferent interaction for the same 16 isolates (57.1%). The synergistic effects of CLR-MXF and AZM-MXF were observed for 11 isolates (39.3%) and 10 isolates (35.7%), respectively. However, antago-

TABLE 2 *In vitro* antimicrobial activities of the combinations of a macrolide and moxifloxacin against *M. abscessus* and *M. massiliense*

Species and agents	No. (%) of isolates with combination activity[a]		
	Synergism	Indifference	Antagonism
M. abscessus ($n = 26$)			
CLR-MXF	1 (3.8)	8 (30.8)	17 (65.4)
AZM-MXF	1 (3.8)	13 (50.0)	12 (46.2)
M. massiliense ($n = 28$)			
CLR-MXF	11 (39.3)	16 (57.1)	1 (3.6)
AZM-MXF	10 (35.7)	16 (57.1)	2 (7.1)

[a] The effects of antibiotic combinations were determined by the fractional inhibitory concentration (FIC): synergism, FIC index of ≤0.5; indifference, FIC index of >0.5 and <2.0; antagonism, FIC index of ≥2.0.

TABLE 3 Evaluation of the antimicrobial activities of drug combinations against intracellular bacteria in bone marrow-derived macrophages

Species and agents	No. (%) of isolates with combination activity[a]		
	Synergism	Indifference	Antagonism
M. abscessus ($n = 15$)			
CLR-MXF	1 (6.6)	4 (26.7)	10 (66.7)
AZM-MXF	1 (6.6)	8 (53.3)	6 (40.0)
M. massiliense ($n = 15$)			
CLR-MXF	5 (33.3)	9 (60.0)	1 (6.7)
AZM-MXF	3 (20.0)	10 (66.7)	2 (13.3)

[a] The effects of antibiotic combinations were determined by adopting the *x*/*y* quotient method. Synergism, an *x*/*y* value of ≤0.5; indifference, a value of >0.5 and <2.0; antagonism, a value of ≥2.0.

nism was observed for one isolate (3.6%) when combining MXF with CLR and for two isolates (7.1%) when MXF was combined with AZM. A statistical analysis using a chi-squared test showed a significant difference in the responses of the *M. abscessus* and *M. massiliense* species to the MXF-macrolide combinations ($P < 0.005$).

Activities of macrolide-MXF combinations against intracellular bacteria. Next, we investigated the effect of the drug combinations on intracellular bacteria in comparison with the effects of the single drugs using the *x*/*y* quotient method (Table 3). Among the 15 *M. abscessus* strains susceptible to macrolides and MXF, 10 (66.7%) of the strains showed antagonism for the CLR-MXF combination, and 6 (40.0%) of the strains showed antagonism for the AZM-MXF combination when the antagonistic effect was defined as an *x*/*y* value of >2.0. Synergism for both drug combinations was also observed in one strain, which was the same strain that showed synergism *in vitro*. In contrast, a large proportion of the *M. massiliense* strains revealed indifference for CLR-MXF (60.0%) and AZM-MXF (66.7%). A synergistic effect was observed in five *M. massiliense* (33.3%) strains when treated with CLR-MXF and three strains (20%) when treated with AZM-MXF. A statistical analysis using a chi-squared test showed a significant difference between the *M. abscessus* and *M. massiliense* groups for the effects of the MXF-macrolide combinations ($P < 0.005$).

***In vivo* activities of macrolide-MXF combinations.** The therapeutic efficacies of single drugs and drug combinations were evaluated in a murine model of *M. abscessus* and *M. massiliense* infection. Treatment with each drug alone, CLR-MXF, and AZM-MXF was initiated 3 days postinfection and administered daily for 12 days. All experiments were performed twice, and similar results were obtained for both experiments. Although the activity of each drug differed depending on the tested strains, most mice that received macrolides or MXF alone revealed a significant decrease in the bacterial load in all of the organs examined ($P < 0.001$) compared to the control mice. The therapeutic effects of each drug alone and the drug combination treatments against each *M. abscessus* and *M. massiliense*-infected mice are shown in Tables S1 and S2 in the supplemental material. Similar to the results obtained from the *in vitro* and *ex vivo* investigations, the combination of a macrolide and MXF was not superior to either drug alone in the treatment of *M. abscessus* infection. Among the 7 strains tested, 5 and 2 strains showed antagonism and an indifferent effect for both macrolide-MXF combinations, respectively (Fig. 1). In contrast, *in vivo* synergism between CLR-MXF and AZM-MXF was observed against 3 of the *M. massiliense* strains (Fig. 2). Indifferent effects in the *M. massiliense*-infected mice were observed for 3 strains for both of the macrolide-MXF combinations (Fig. 2). A statistical analysis using a chi-squared test showed a significant difference between the *M. abscessus* and *M. massiliense* groups for treatment with the MXF-macrolide combinations ($P < 0.005$).

DISCUSSION

Treatment of the most common NTM pathogens improved with the introduction of macrolide-containing regimens, but the treatment outcome for *M. abscessus* lung disease remains disappointing (22–24, 34), because of the relatively few therapeutic alternatives for the treatment compared to other mycobacteria. Thus, novel therapeutic approaches are needed because clinical failures are frequently encountered.

Fluoroquinolones such as MXF showed good *in vitro* activity against *M. abscessus* clinical isolates in some studies (21, 38). In fact, MXF is an attractive treatment option because it can be administered orally for a long duration. However, there is only limited evidence regarding the clinical efficacy of MXF against *M. abscessus* and *M. massiliense*.

Our results clearly demonstrated two findings: (i) MXF hinders the activity of macrolides and (ii) the antagonism between MXF and the macrolide was relatively common against the *M.*

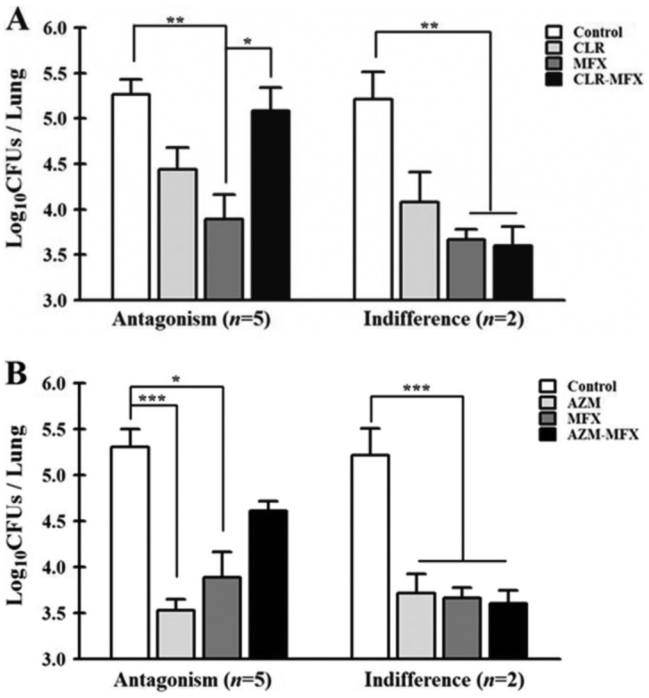

FIG 1 Activities of clarithromycin, azithromycin, and moxifloxacin alone and macrolide-moxifloxacin combinations against 7 *M. abscessus* isolates in mice. (A) Significant differences are shown in the in the $\log_{10}$ CFU/lung between treatments with clarithromycin alone and with the clarithromycin-moxifloxacin combination. (B) Significant differences in the $\log_{10}$ CFU/lung between treatments with azithromycin alone and with the azithromycin-moxifloxacin combination. The *P* value versus control mice was evaluated using Wilcoxon's matched pairs test. *, $P > 0.05$; **, $P > 0.005$; and ***, $P > 0.001$.

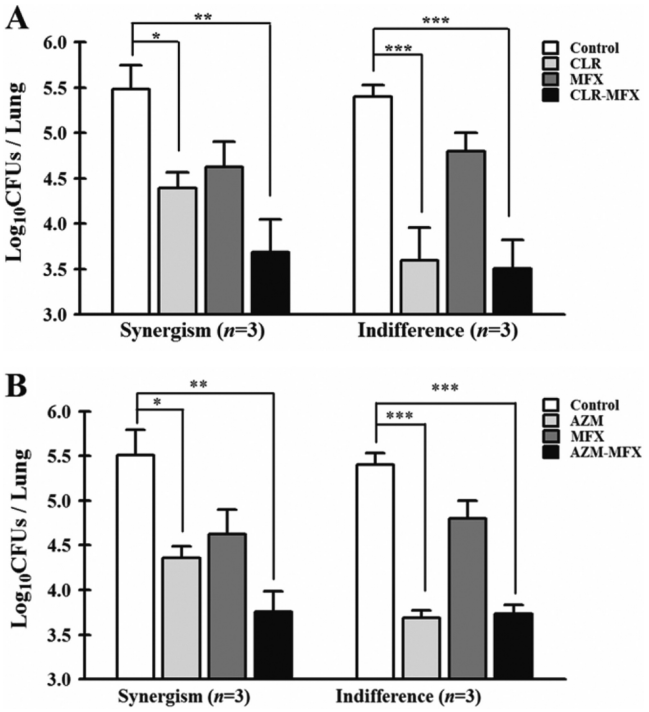

FIG 2 Activities of clarithromycin, azithromycin, and moxifloxacin alone and macrolide-moxifloxacin combinations against 6 *M. massiliense* isolates in mice. (A) Significant differences in the $\log_{10}$ CFU/lung between treatments with clarithromycin alone and with the clarithromycin-moxifloxacin combination. (B) Significant differences in the $\log_{10}$ CFU/lung between treatments with azithromycin alone and with the azithromycin-moxifloxacin combination. The *P* value versus control mice was evaluated using Wilcoxon's matched pairs test.

abscessus isolates compared to the *M. massiliense* isolates. Thus, our study demonstrated that combining macrolides with MXF provided no advantage for the treatment of *M. abscessus* lung disease.

MXF has been reported to have clinical efficacy for *M. abscessus* infection. A recent prospective observational study demonstrated that CLR-MXF combination therapy for postacupuncture cutaneous *M. abscessus* infections resulted in a more rapid resolution of the cutaneous lesions than did the use of a CLR-amikacin combination (9). Moreover, MXF has been shown to be effective against experimentally induced *M. abscessus* keratitis and in human cases of that disorder (7, 10, 37). However, MXF obviously interferes with CLR activity in over 65% of the tested strains of *M. abscessus* in our models, indicating that many factors should be considered in explaining such a different result. For example, the pharmacokinetic and pharmacodynamic properties of MXF may differ according to the type of disease (cutaneous infection versus pulmonary infection), and differences in the drug dose and therapeutic regimens could also influence the results.

In contrast to the efficacy of medication regimens for nonpulmonary diseases such as skin infection, no antibiotic regimen based on *in vitro* susceptibilities has been shown to produce effective treatment for patients with *M. abscessus* lung disease (18). A lack of correlation between the *in vitro* results and the *in vivo* effects regarding drug activity was also observed in this study. For example, MXF showed activity *in vitro* superior to that of CLR and AZM against both *M. abscessus* and *M. massiliense*. However, monotherapy with MXF was less effective than that with AZM in the murine infection model. Thus, our results indicate that it may be difficult to predict the *in vivo* therapeutic effects of MXF based on its *in vitro* MIC test result. These results are in agreement with those of a similar study conducted on the *Mycobacterium avium* complex (32). Factors that could account for this may be differences in the ability of the strain to grow in the medium versus *in vivo* or differences in the penetrating power of the antibiotics. Similarly, many studies have demonstrated that MXF and macrolides are more effective during bacterial replication than when the bacterial numbers approach the maximal values in stationary phase (15, 16, 42).

Another factor that could affect the response to antibiotics is the anatomic location of infection or between strains isolated from hosts in different geographical regions. Notably, the *in vitro* susceptibilities for MXF against *M. abscessus* varied with the isolation source as well as between countries and patients infected with the same strain. A previous study conducted using 21 strains of *M. abscessus* in Japan found that the MIC of MXF ranged from 2 to 32 μg/ml with an MIC_{90} of 32 μg/ml (35). Similarly, a study conducted in Taiwan with 98 *M. abscessus* clinical isolates reported an MIC range of 0.064 to 32 μg/ml, also with an MIC_{90} of 32 μg/ml (22). However, a study performed in South Korea using 74 *M. abscessus* isolates showed that the MIC_{90} of MXF was 2 μg/ml and that only five strains exceeded an MIC of 4 μg/ml of MXF (38). Interestingly, the MICs of MXF of *M. abscessus* isolates from 12 patients varied significantly more than the MICs of other antibiotics, such as CLR and amikacin, in an outbreak of postacupuncture cutaneous infections caused by *M. abscessus* species belonging to the same genotype (9). Thus, there are significant geographic differences and patient variations in the MICs of MXF against *M. abscessus*, and comparisons of the activity of MXF are complicated by differences in the drug regimens and/or strains used in the studies. Additional studies on different *M. abscessus* and *M. massiliense* strains are needed to substantiate these observations.

In our study, the MICs of different antibiotics are reported according to the species. To date, differential identification of *M. abscessus* and *M. massiliense* has not been intensively studied because they cause the same spectrum of diseases and because it is difficult to discriminate between the two species using traditional molecular methods (e.g., PCR-restriction fragment length polymorphism or sequence analysis of *rpoB* and *hsp65*) due to a lack of polymorphisms in the region being tested (8, 17, 29). However, recent studies suggest that the antibiotic susceptibilities and treatment outcomes differ significantly between the two species (31). In this study, *M. massiliense* showed marked susceptibility to CLR, while six isolates of *M. abscessus* were resistant to macrolides, results which are similar to previously reported findings (30). In contrast, MXF showed a wider range of MICs against *M. massiliense* than did *M. abscessus* according to CLSI criteria for RGM. In the same context, treatment efficacy for *M. massiliense* with MXF alone was less than that for *M. abscessus* in our murine infection model (Fig. 1 and 2). Thus, it is important to discriminate between these two species prior to treatment because an empirical addition of antibiotics to treat *M. abscessus* and *M. massiliense* may be potentially counterproductive.

The exact reasons for the antagonistic effect of MXF on the actions of the macrolide are not known, but one possible explana-

tion has been suggested in previous studies. Tomioka et al. showed that CLR decreases the activities of gatifloxacin and levofloxacin against *M. avium* complex strains *in vitro* (44). Kohno et al. also reported that the activity of CLR against *M. avium* complex strains was attenuated when combined with fluoroquinolones, including MXF, using both *in vitro* and *in vivo* models (32). Another study by Bermudez et al. revealed that the MXF-AZM combination was significantly less active than AZM alone for the treatment of mice infected with *M. avium* (4). In addition, protein synthesis inhibitors, such as CLR, interfere with the lethal antibacterial activities of fluoroquinolones (43).

A limitation of this study is that we did not investigate whether the combinations at various concentrations of each drug provided better antimycobacterial activity. An additional shortcoming of the present study is that we employed a systemic infection model via intravenous injection rather than an aerosol infection model.

Conclusively, the present study provides evidence that MXF negatively influences the treatment outcome in experimental models of *M. abscessus* infection when combined with macrolides. Our study demonstrates, for the first time, that MXF has significantly different effects on *M. abscessus* and *M. massiliense*.

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