The Synergetic Effect of Sitafloracin-Arbekacin Combination in the *Mycobacteroides abscessus* Complex

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Abstract

Background

Mycobacteroides abscessus (*M. abscessus*) is the most commonly isolated rapidly growing mycobacteria (RGM) and is one of the most antibiotic-resistant RGM with rapid progression, therefore, treatment of *M. abscessus* is still challenging. The genus mycobacterium has long been known to have both rough and smooth colony phenotypes. Rough morphotypes generally are more virulent than smooth morphotypes. Recently, the efficacy of sitafloxacin, new fluoroquinolone (FQ), containing regimens against *M. abscessus* complex have been reported. We here presented a new combination treatment with sitafloxacin that targeted rough morphotypes of *M. abscessus*, causing aggressive infections.

Results

Thirty-four clinical strains of *M. abscessus* were isolated from various clinical samples at the Juntendo university hospital from 2011 to 2020. The susceptibility to a combination of sitafloxacin and antimicrobial agents was compared to that of the antimicrobial agents alone. Ten isolates (90.9%) of *M. abscessus* subsp. *abscessus* were susceptible to both sitafloxacin and arbekacin when the combination is administered; while, 11 isolates (50.0%) of *M. abscessus* subsp. *massiliense* were susceptible. Synergistic effects against *M. abscessus* complex were shown in 8 strains (23.5%) treated with sitafloxacin-amikacin combination, 9 (26.5%) and 19 (55.9%) of sitafloxacin-imipenem combination, and sitafloxacin-arbekacin combination, respectively. Sitafloxacin-arbekacin combination also exhibited synergistic effects against 10 strains (45.5%) of *M. abscessus* subsp. *massiliense* and 8 strains (72.7%) of *M. abscessus* subsp. *abscessus*, a highly resistant subspecies of *M. abscessus*. The sitafloxacin-arbekacin combination revealed more synergistic effects in rough morphotypes of *M. abscessus* complex ($p = 0.008$).

Conclusion

We demonstrated the synergistic effect of the sitafloxacin-arbekacin combination against *M. abscessus* complex. Further, this combination regimen might be more effective against *M. abscessus* subsp. *abscessus* or rough morphotypes of *M. abscessus* complex.

Background

Nontuberculous mycobacteria (NTM) are environmental pathogens that can cause diverse types of infectious diseases in humans. NTM are classified into RGM where colony formation requires less than seven days and slowly growing mycobacteria (SGM) forming colonies at least seven days. *M. abscessus* is the most commonly isolated RGM and the third most common cause of respiratory NTM in the United States [1]. Pulmonary disease caused by *M. abscessus* mostly occur in the setting of structural lung

conditions such as bronchiectasis, chronic obstructive pulmonary disease (COPD), and cystic fibrosis (CF). These infections are often incurable and associated with rapid lung function decline [2, 3]. *M. abscessus* isolates are uniformly resistant to the standard antituberculous agents. New NTM treatment guidelines were published in 2020 [4]. The guidelines introduced new treatment options, including inhaled amikacin, tigecycline, and clofazimine [5–7]; however, the treatment benefits were limited to negative conversion of sputum, and radical cure for *M. abscessus* pulmonary disease was obtained with limited disease and a combination of surgical resection of involved lung and chemotherapy [1]. Recently, the efficacy of sitafloxacin, new fluoroquinolone (FQ), containing regimens against *M. abscessus* complex have been reported [8, 9]. FQs are recommended for use as second-choice drugs in treating TB, although they are only weakly efficacious against MAC infections [10–12]. Sitafloxacin, with a chloro substituent at the C-8 position, is a newly developed oral quinoline, exhibiting good antimicrobial activity against extracellular and intramacrophage MAC compared to levofloxacin *in vitro* and *in vivo* [13–16]. These previous papers suggest that fluoroquinolone combining regimens could have a potency for the effective treatment of *M. abscessus* complex.

Mycobacterium genus included *Mycobacterium tuberculosis* (TB), and some NTM have long been known to have both rough and smooth colony phenotypes [17, 18]. These phenotypes are formed by the expression levels of glycopeptidolipids (GPLs). GPLs are produced by several NTMs, including RGMs (*M. abscessus*, *M. chelonae*, and *M. smegmatis*) [19–21] and *Mycobacterium avium* complex (MAC) members [22–24]. *M. abscessus* can spontaneously change between a smooth form, which expresses GPLs, and a rough form, lacking GPLs. The smooth form can form biofilms and colonize surfaces; conversely, the rough morphotypes cannot form biofilms but can multiply in macrophages and cause persistent infection [25]. Rough morphotypes are generally more virulent than smooth variants for isolates lacking GPLs enhanced releasing TNF- α from macrophage [25–27]. Conversely, to form biofilms, smooth variants were related to protecting from surrounding factors such as other bacteria, acidic pHs, hydrogen peroxide (H₂O₂), and treatment with amikacin or azithromycin [28]. Here, we presented the new sitafloxacin-arbekacin combination regimens, which are more effective on rough morphotypes, causing aggressive infections, and could be a potential treatment option against *M. abscessus* subsp. *abscessus*.

Results

Clinical features of three subspecies of *M. abscessus* complex

Thirty-four clinical strains of *M. abscessus* were isolated from various clinical samples at the Juntendo university hospital from 2011 to 2020. The characteristics of patients isolated from *M. abscessus* are shown in Table 1. Twenty-seven of 29 (79.4%) patients were diagnosed with *M. abscessus* complex from the culturing of sputum or bronchial lavage. Of the isolates, 11 (32.4%) were of *M. abscessus* subsp. *abscessus*, 22 (64.7%) of *M. abscessus* subsp. *massiliense*, and 1 (2.9%) of *M. abscessus* subsp. *bolletii* as determined by multi-locus sequence analysis. Nine of 34 (26.5%) patients had received immunosuppressive treatment, including corticosteroids, before the culture.

Susceptibility to antimicrobial agents in combination with sitafloxacin

Methods of incubation time and susceptibility testing were used as a reference to our previous report [29]. The difference of MICs between both colony phenotypes was evaluated (Figure 1), and MICs of sitafloxacin and amikacin in rough phenotypes were significantly lower than smooth phenotypes (p values of sitafloxacin and amikacin were 0.0004 and 0.002, respectively). Therefore, we investigated the best combination partners of sitafloxacin as the potential regimens for *M. abscessus*, especially rough morphotypes. The susceptibility to a combination of sitafloxacin and antimicrobial agents was compared to that of the antimicrobial agents alone, categorized into each subspecies of *M. abscessus* complex (Figure 2). The MICs of three antimicrobial agents (amikacin, imipenem, and arbekacin) were measured with or without sitafloxacin. Ten isolates (90.9%) of *M. abscessus* subsp. *abscessus* were susceptible to both sitafloxacin and arbekacin in the combination administration; while, 11 isolates (50.0%) of *M. abscessus* subsp. *massiliense* were susceptible. The median MICs of sitafloxacin and arbekacin were significantly lower in the combination administration (Table S2). Figure 2 also showed the colony phenotype and antimicrobial resistance gene status. We next evaluated the most synergistic combinations by using the fractional inhibitory concentration (FIC) index as described in previous paper (Figure 3 and Table 2) [30]. Susceptibility was divided into two classes, synergy and additive as a synergistic effect and indifference and antagonism as an antagonistic effect. Synergistic effects were shown in 8 strains (23.5%) treated with sitafloxacin-amikacin combination, 9 (26.5%) of sitafloxacin-imipenem combination, and 19 (55.9%) of sitafloxacin-arbekacin combination, respectively. Sitafloxacin-arbekacin combination also exhibited the synergistic effects against 10 strains (45.5%) of *M. abscessus* subsp. *massiliense* and 8 strains (72.7%) of *M. abscessus* subsp. *abscessus*, a highly resistant subspecies of *M. abscessus*.

Association of clinical features with susceptibilities to the sitafloxacin-arbekacin combination

We investigated whether susceptibility to the sitafloxacin-arbekacin combination might associate with clinical or isolate status. The rough colony morphotypes revealed more synergistic effects than antagonistic effects ($p = 0.008$) (Table 3). The other clinical parameters such as age, sex, smoking history, bronchiectasis lesion, and treatment history of antibiotics did not influence the sitafloxacin-arbekacin combination.

Discussion

We demonstrated here the efficacy of the new sitafloxacin-arbekacin combination regimen. The regimen revealed that both sitafloxacin and arbekacin MICs reduction when the combination administration and significantly high synergistic effect against *M. abscessus* complex. The combination regimen showed a higher rate of susceptibility and synergistic effects against *M. abscessus* subsp. *abscessus* than *M. abscessus* subsp. *Massiliense*. These results might suggest that the combination therapy was more effective against *M. abscessus* subsp. *abscessus*, showing a high level of antimicrobial resistance.

Furthermore, the combination revealed higher efficacy for the treatment of *M. abscessus* complex in rough morphotypes associated with aggressive infections.

In 2020, the new treatment guidelines for NTM pulmonary disease had been published [4], and new treatment strategies recommended changing the regimens based on macrolide resistance gene status. The systematic review described that treatment of macrolide containing regimens for *M. abscessus* subsp. *massiliense* led to a better culture conversion rate than *M. abscessus* subsp. *abscessus* [31]. However, the treatment options for macrolide resistance species of *M. abscessus* complex were lacking. We focused on a novel, highly effective FQ, sitafloxacin, as a potential treatment option for the refractory *M. abscessus* infections.

Sitafloxacin is approved in Japan, and it has been clinically used against most mycobacterial infections, mainly MAC, by its exceedingly low MIC level compared to other FQs. Eighteen patients with pulmonary MAC disease who received sitafloxacin for at least 4 weeks for pulmonary MAC disease were evaluated; 10 patients (55.6%) showed improved radiological characteristics, and 8 patients (44.4%) showed negative sputum cultures at 6 months [32]. Thirty-one patients with refractory MAC-lung disease, who received sitafloxacin-containing regimen for ≥ 4 weeks, were evaluated, 26% and 19% of patients showed symptomatic and radiological responses, respectively 12 months after receiving, and low sitafloxacin MIC ($\leq 1 \mu\text{g/mL}$) were significant predictors of negative sputum culture conversion [33]. In a recent study, sitafloxacin efficacy against *M. abscessus* has been reported. Bedaquiline-clofazimine-sitafloxacin combination revealed a synergistic effect against 11 isolates of 70 *M. abscessus* subsp. *abscessus* (15.7%) [8]. In a Japanese retrospective study of 13 *M. abscessus* pulmonary disease, all 4 patients who received sitafloxacin-containing regimens achieved negative sputum conversion after 1 year of treatment and improved radiological findings [9]. Together with our data and previous reports, sitafloxacin could be an effective antimicrobial combination partner against refractory *M. abscessus* complex.

M. abscessus exist in two distinct morphotypes, smooth and rough, that differ in their gross colony appearances when grown on solid media due to their differing amounts of cell wall GPLs. The smooth morphotype initially colonizes the airway mucosa, and had generally lower pathogenicity in this state. Subsequently switching from smooth morphotypes to rough morphotypes, aggressive pulmonary disease cause. Smooth morphotypes have an advantage in survival due to biofilm formation, leading to inhibit bacteria-induced apoptosis [34]. Conversely, rough morphotypes, without biofilms, induce the invasion ability mediated by apoptotic cell death [35, 36]. Several clinical data have revealed increased pathogenicity from the rough morphotype. The rates of isolation of rough morphotype is higher in the CF patients with clinical symptoms [37], and case reports describe that the CF patients with rough morphotypes lead to dramatic declines of respiratory function and/or death [26, 38]. Thus, the development of new treatment targeted rough morphotypes has become imperative. Our study revealed that sitafloxacin-arbekacin combination had the higher synergy in rough morphotypes, the combination could be useful treatment for the patients who are isolated with rough morphotypes and/or whose disease have progressed. On the other hands, the combination had the less synergy in smooth morphotypes by biofilm activity. Interestingly, in 17 out of 19 smooth morphotypes (89.5%), sitafloxacin

susceptibility in the combination treatment improved as compared to alone. This data suggested that sitafloxacin-arbekacin combination could be also partially effective as the treatment for smooth morphotypes of *M. abscessus*.

Conclusion

Our *in vitro* study demonstrated the synergistic effect of the sitafloxacin-arbekacin combination against *M. abscessus* complex. Further, this combination regimen might be more effective against not only rough morphotype of *M. abscessus*, causing severe disease, but also *M. abscessus* subsp. *abscessus*, which is thought to reveal high resistance to antibiotics. Further studies are required to assess the clinical efficacy and adverse reactions of the sitafloxacin-arbekacin combination; however, we expect that the combination could be a potential treatment option for the patients who are refractory to the standard combination therapy.

Materials And Methods

Determination of *M. abscessus* complex

Methodological details are described in the Supplementary Materials and Methods.

PCR amplification, DNA sequencing, and MALDI-TOF MS analysis

The conditions of each analysis and primer sequences used in PCR to detect transcripts are described in the Supplementary Materials and Methods.

Antimicrobial susceptibility testing

The MIC breakpoints, indicating susceptible, intermediate, and resistant strains, were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) criteria for amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, moxifloxacin, trimethoprim/sulfamethoxazole, and tobramycin (Table 4) [39]. Sitafloxacin breakpoints were treated as the same as moxifloxacin breakpoints, and arbekacin as the same as tobramycin. The effect of each agent combined with sitafloxacin was evaluated using FIC index analysis [30].

Further methodological details are described in the Supplementary Materials and Methods.

Statistical analysis

Categorical variables were compared using the chi-square test or Fisher's exact test. The evaluation of changes in MIC was performed using the Wilcoxon signed-rank test. Differences were considered significant at $p < 0.05$. When the chi-square test results were statistically significant, adjusted residuals were calculated to determine which particular associations were significant. Adjusted residuals were significant at $p < 0.05$ level if they were less than -1.96 or more than 1.96 and were significant at $p < 0.01$

level if they were less than -2.58 or more than 2.58. All statistical analyses were performed using the SPSS software program (version 20, IBM Japan, Japan).

Abbreviations

M. abscessus: *Mycobacteroides abscessus*; NTM: nontuberculous mycobacteria; RGM: rapidly growing mycobacteria; SGM: slowly growing mycobacteria; COPD: chronic obstructive pulmonary disease; CF: cystic fibrosis; *M. abscessus* subsp. *massiliense*: *M. abscessus* subspecies *massiliense*; TB: *Mycobacterium tuberculosis*; GPL: glycopeptidolipid; MAC: *Mycobacterium avium* complex; MALDI-TOF MS: matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry; CLSI: Clinical and Laboratory Standard Institute; STFX: sitafloxacin; AMK: amikacin; IPM: imipenem; ABK: arbekacin; HIV: human immunodeficiency virus; FIC: fractional inhibitory concentration; FQ: fluoroquinolone; MBEC: minimum biofilm eradication concentration

Declarations

Ethics approval and consent to participate

The study was approved by the Independent Ethics Committee at Juntendo University Hospital (approval no. 18-010 and 19-038) and adhered to the tenets of the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceived and designed the experiments: JW, HI, ST1, ST2, and AN. Performed the experiments: ST1. Analyzed the data: JW, HI, ST1, ST2, and AN. Collected the data and/or samples: JW, ST1, YF, TH, KK, YA, KS, IS, YO, and SM. Contributed reagents/materials/analysis tools: HI, ST1, IS, YO, and SM. Reviewed the

initial and final drafts of the manuscript: TM and KT. Wrote the paper: JW, HI, ST1, ST2, and AN. All authors read and approved the final manuscript.

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Tables

Due to technical limitations, Table 1,2,3 and 4 are only available as a download in the Supplemental Files section.

Figures

Figure 1

: The comparison of MIC of each antimicrobial, sitafloxacin, amikacin, imipenem, and arbekacin, compared with rough and smooth colony morphotypes. * p value <0.05, ** p value <0.01. Abbreviations: STFX, sitafloxacin; AMK, amikacin; IPM, imipenem; ABK, arbekacin.

<i>M. abscessus</i> subsp. <i>abscessus</i>	Colony	Alone				Combination		Combination		Combination	
		STFX	AMK	IPM	ABK	STFX	AMK	STFX	IPM	STFX	ABK
Strain 1	Smooth	4	8	8	4	0.12	4	4	16	0.5	2
Strain 2	Rough	4	4	16	2	0.12	4	4	16	0.12	2
Strain 3	Smooth	2	8	16	4	1	4	4	16	0.5	2
Strain 4	Smooth	1	4	8	2	0.12	4	0.12	4	0.12	2
Strain 5	Rough	2	4	8	4	0.12	4	1	2	0.12	2
Strain 6	Rough	0.5	4	4	4	0.12	2	1	4	0.12	2
Strain 7	Rough	1	2	4	2	0.12	1	0.12	2	0.12	1
Strain 8	Smooth	2	8	16	4	0.5	4	4	16	0.12	2
Strain 9	Rough	0.5	4	4	4	0.12	4	0.12	2	0.12	2
Strain 10	Smooth	2	8	8	4	2	2	4	16	0.25	2
Strain 11	Smooth	2	8	8	4	4	8	4	16	4	4
<i>M. abscessus</i> subsp. <i>massiliense</i>											
Strain 12	Rough	2	16	16	8	0.25	4	0.25	8	0.25	2
Strain 13	Rough	1	4	8	4	0.12	4	0.12	8	0.25	2
Strain 14	Smooth	8	8	8	4	8	8	0.12	8	8	4
Strain 15	Rough	2	4	8	4	0.124	4	0.12	4	0.25	2
Strain 16	Smooth	2	8	16	4	0.5	4	2	2	0.5	2
Strain 17	Smooth	8	16	8	4	8	8	8	16	8	4
Strain 18	Smooth	4	16	16	8	4	16	4	16	4	8
Strain 19	Smooth	8	16	8	8	8	16	8	16	8	8
Strain 20	Smooth	8	8	16	4	8	16	8	16	8	4
Strain 21	Rough	1	4	8	4	0.12	4	0.12	2	0.12	2
Strain 22	Smooth	1	4	8	4	0.12	4	0.12	4	0.25	2
Strain 23	Smooth	4	8	8	4	4	8	4	16	4	4
Strain 24	Rough	1	4	32	4	0.12	4	1	2	0.25	2
Strain 25	Smooth	4	16	8	8	4	16	4	16	4	8
Strain 26	Smooth	2	8	16	4	4	8	4	16	4	4
Strain 27	Rough	1	4	16	4	0.12	4	1	2	0.12	2
Strain 28	Smooth	4	16	32	8	4	16	4	32	4	8
Strain 29	Smooth	2	16	16	8	2	4	2	2	4	8
Strain 30	Smooth	2	8	16	4	4	8	2	2	4	4
Strain 31	Rough	2	4	4	2	0.12	4	0.12	4	0.12	2
Strain 32	Rough	1	16	16	8	1	1	1	2	0.25	2
Strain 33	Rough	2	16	16	8	0.5	4	2	2	0.5	2
<i>M. abscessus</i> subsp. <i>bolletii</i>											
Strain 34	Rough	1	4	16	8	0.12	4	0.12	2	0.12	2

Rough

Smooth

Susceptible

Intermediate

Resistant

Figure 2

MIC distributions for amikacin, imipenem, and arbekacin combined with sitafloxacin, categorized into three subspecies of *M. abscessus* complex on day 7. Light blue color indicates rough colony morphotype, and orange color smooth colony morphotype. Green color indicates susceptibility, yellow color intermediate, and red color resistance to *M. abscessus*. Abbreviations: STFX, sitafloxacin; AMK, amikacin; IPM, imipenem; ABK, arbekacin.

<i>M. abscessus</i> subsp. <i>abscessus</i>	Colony	STFX/AMK	STFX/IPM	STFX/ABK
Strain 1	Smooth	0.53	3	0.625
Strain 2	Rough	1.03	2	1.03
Strain 3	Smooth	1	3	0.75
Strain 4	Smooth	1.12	0.62	1.12
Strain 5	Rough	1.06	0.75	0.56
Strain 6	Rough	0.74	3	0.74
Strain 7	Rough	0.62	0.62	0.62
Strain 8	Smooth	0.75	3	0.56
Strain 9	Rough	1.24	0.74	0.74
Strain 10	Smooth	1.25	4	0.625
Strain 11	Smooth	3	4	3
<i>M. abscessus</i> subsp. <i>masiliense</i>				
Strain 12	Rough	0.375	0.625	0.375
Strain 13	Rough	1.12	1.12	0.75
Strain 14	Smooth	2	1.015	2
Strain 15	Rough	1.062	0.56	0.625
Strain 16	Smooth	0.75	1.125	0.75
Strain 17	Smooth	1.5	3	2
Strain 18	Smooth	2	2	2
Strain 19	Smooth	2	3	2
Strain 20	Smooth	3	2	2
Strain 21	Rough	1.12	0.37	0.62
Strain 22	Smooth	1.12	0.62	0.75
Strain 23	Smooth	2	3	2
Strain 24	Rough	1.12	1.063	0.75
Strain 25	Smooth	2	3	2
Strain 26	Smooth	3	3	3
Strain 27	Rough	1.12	1.125	0.62
Strain 28	Smooth	2	2	2
Strain 29	Smooth	1.25	1.125	3
Strain 30	Smooth	3	1.125	3
Strain 31	Rough	1.06	1.06	1.06
Strain 32	Rough	1.063	1.125	0.5
Strain 33	Rough	0.5	1.125	0.5
<i>M. abscessus</i> subsp. <i>bolletii</i>				
Strain 34	Rough	1.12	0.245	0.37

Rough	FIC index ≤ 0.5	Synergy
Smooth	$0.5 < \text{FIC index} \leq 1$	Additive
	$1 < \text{FIC index} \leq 2$	Indifference
	$2 < \text{FIC index}$	Antagonism

Figure 3

FIC index of amikacin, imipenem, and arbekacin combined with sitafloxacin categorized into three subspecies of *M. abscessus*. Light blue color indicates rough colony morphotype, and orange color smooth colony morphotype. Light green color indicates synergy, green color indicates additive, yellow color indicates indifference, and red color indicates antagonism in each combination. Abbreviations: STFX, sitafloxacin; AMK, amikacin; IPM, imipenem; ABK, arbekacin; FIC index, fractional inhibitory concentration index

Supplementary Files

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