Activity of Clofazimine and TBI-166 against *Mycobacterium tuberculosis* in Different Administration Intervals in Mouse Tuberculosis Models

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ABSTRACT Clofazimine (CLO) and TBI-166 belong to the riminophenazine class of antimicrobial agent. TBI-166 exhibited promising antituberculosis activity *in vitro* and in animal models and is currently under phase I clinical development for the treatment of tuberculosis in China. To identify an optimal dosing regimen to support further clinical development of TBI-166, the efficacies of CLO and TBI-166 were evaluated in two aerosol infection models utilizing BALB/c and C3HeB/FeJNju mice. TBI-166 and CLO were dosed at 20 mg/kg daily for 2 weeks, followed by QD (once daily), TIW (thrice weekly), and BIW (twice weekly) for an additional 10 weeks at the same dose level. The bactericidal activities of TBI-166 and clofazimine via QD, TIW, and BIW dosing regimens were determined after treatment. Once-daily administration of CLO and TBI-166 appeared to be more efficacious than the two intermittent dosing regimens. Once-daily administration of TBI-166 increased the bactericidal activity by approximately 1 log₁₀ CFU in the lung and spleen compared with TIW or BIW dosing after 12 weeks of treatment, while once-daily administration of CLO increased the bactericidal activity by 1.27 to 1.90 log₁₀ CFU/lung and by 1.61 to 2.22 log₁₀ CFU/spleen in the BALB/c mouse model compared to the intermittent therapies. The differences between QD and TIW and between QD and BIW were significant ($P < 0.05$). The data suggest that accumulated total doses correlate with the log₁₀ CFU reductions. Therefore, intermittent administration of TBI-166 and CLO should be further evaluated at the same accumulated total doses in preclinical and clinical studies.

KEYWORDS *Mycobacterium tuberculosis*, TBI-166, clofazimine, mouse model

The emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) presents a major challenge to the eradication effort of tuberculosis, one of the leading causes of death in the world. The treatment of MDR-/XDR-TB requires an extremely long duration of multidrug therapy with a limited number of poorly tolerated drugs to choose from. WHO global tuberculosis report 2020 indicates that the treatment success rates for MDR-TB and XDR-TB were only 57% and 47%, respectively (1). There is an urgent need for safe and effective drugs for the treatment of MDR-/XDR-TB (2).

Recently, clofazimine (CLO), a member of the phenazine class originally developed for the treatment of leprosy, has received attention. In 2010, van Deun et al. reported that a new regimen containing CLO was able to shorten the duration of the treatment of MDR-TB with improved effectiveness (3). Further studies indicated that addition of CLO to both the first-line and the second-line TB drug regimens could dramatically

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TABLE 1 Accumulated dose of CLO and TBI-166 during the 2 studies

	Accumulated dose during the study, total mg/kg at the end of the study			
	1st 2 wks	wk 4	wk 8	wk 12 (study 2)
QD	200	400	800	1,200
TIW	200	320	560	800
BIW (study 2)	200	280	440	600

shorten the duration of therapy, indicating the potential of this drug class in the treatment of tuberculosis (3–5). In order to discover a better drug in the phenazine class for the treatment of tuberculosis, a large number of analogues of clofazimine have been designed, synthesized, and evaluated *in vitro* and *in vivo*. Among these compounds, TBI-166 was identified as a drug candidate and is currently under clinical development for the treatment of tuberculosis (6, 7).

Currently, CLO is administered orally once a day despite the fact that it has an extremely long half-life (estimated to be about 70 days). The accumulation of CLO in the body leads to significant skin pigmentation (8, 9). Therefore, it is reasonable to hypothesize that intermittent administration of CLO could potentially reduce skin pigmentation while preserving the efficacy. Clinical studies indicated that TBI-166 had a relatively short half-life (41.25 h) and reduced the potential for skin pigmentation. Intermittent administration of TBI-166 was also a question of high interest to guide further clinical development of this promising compound. In the current study, we evaluated the effects of various dosing regimens of CLO- and TBI-166-containing therapies in two well-established mouse models of tuberculosis (BALB/c mice and C3HeB/FeJNju mice).

RESULTS

Results from the C3HeB/FeJNju mouse model (study 1). The objective of this study was to compare the efficacies of CLO and TBI-166 (20 mg/kg) administered once a day or three times a week in C3HeB/FeJNju mice infected with *Mycobacterium tuberculosis* H37Rv. The design of this experiment and the accumulated doses are presented in Table 1 and 2.

The colony counts of *M. tuberculosis* in the lung and spleen were 6.81 ± 0.67 and $4.16 \pm 0.44 \log_{10}$ CFU, respectively, on the day of treatment initiation (day 0). After 4 weeks of treatment, all treatment groups resulted in a 1.57 to 2.04 $\log_{10}$ CFU/lung and a 1.14 to 1.68 $\log_{10}$ CFU/spleen reduction. For both CLO and TBI-166, there was no statistical difference in the CFU counts of spleen and lung between the TIW and the QD groups. After 8 weeks of treatment, most of the treated mice converted to culture negative in both the lungs and spleens as indicated in Table 3.

The level of pigmentation of the subcutaneous adipose tissue of the C3HeB/FeJNju

TABLE 2 Experimental design for C3HeB/FeJNju mouse model. Dosing schedule: loading doses (5/7) for 2 weeks for all treatment groups, dosing days per week (5/7 for QD, 3/7 for TIW) after loading dose

Groups	Drug	No. of mice sacrificed in each group					Total no. of mice
		D-28 ^a	D-14	D0	W4 ^b	W8	
Control	Untreated	3	3	3 + 3	5 + 3	5 + 3	28
QD	CLO				5 + 3	5 + 3	16
TIW	CLO				5 + 3	5 + 3	16
QD	TBI-166				5 + 3	5 + 3	16
TIW	TBI-166				5 + 3	5 + 3	16
Total no. of mice		3	3	6	40	40	92

^aD, day sacrificed.

^bW, weeks treated.

TABLE 3 Lung and spleen CFU counts at the indicated time points (values are means $\pm$ SD, standard deviation, $n = 5$)

Groups	Log CFU		Log CFU (4 wk)		Log CFU (8 wk)	
	Lung	Spleen	Lung	Spleen	Culture negative ^a	
D-28	2.77 $\pm$ 0.56	0				
D-14	5.20 $\pm$ 0.24	3.29 $\pm$ 0.45				
D0	6.81 $\pm$ 0.67	4.16 $\pm$ 0.44				
Control			5.76 $\pm$ 0.14	4.86 $\pm$ 0.33		
TBI-166, QD			4.05 $\pm$ 0.23	3.72 $\pm$ 0.27	4/5	3/5
TBI-66, TIW			4.18 $\pm$ 0.21	3.22 $\pm$ 0.62	5/5	3/5
CLO, QD			3.72 $\pm$ 0.17	3.18 $\pm$ 0.17	3/5	3/5
CLO, TIW			4.19 $\pm$ 0.50	3.31 $\pm$ 0.34	5/5	3/5

^anumber of mice with culture negative lung and spleen by 8 weeks.

mice treated by different regimens of CLO and TBI-166 for 8 weeks is shown in Fig. 1. Compared to no treatment control, the omental fat and abdominal fat of both treatment groups are shown in orange.

Pathology. In this experiment, pathological examination and hematoxylin and eosin (H&E) staining were carried out in the lungs of infected mice to assess the pathological changes among various treatment groups (Fig. 2 and 3). Three mice were dissected in each group at various time points. Pathological observation and scoring were carried out after H&E staining in the lung. Inspection of the lungs on day 0 (the day treatment started) demonstrated severe granulomatous alveolar pneumonia and pulmonary nodular lesions in the lungs of aerosol-infected C3HeB/FeJNju mice (Fig. 2), indicating that the tuberculosis infection was successfully established after 6 weeks of aerosol inoculation.

After 8 weeks of treatment (Fig. 3), there was a wide range of nodular lesions in the control group, including multiple foam-like cells proliferation and granulomatous alveolar pneumonia, while only a small number of foam-like cells and granulomatous alveolar pneumonia showed locally small nodular lesions in group 166 QD and CLO QD. The pathological results illustrated that there were fewer lung lesions in the treatment group than in the control group, which was consistent with the CFU counts of each group (Table 3).

Results from the BALB/c mouse model (study 2). The objective of this study was to compare the efficacies of CLO and TBI-166 (20 mg/kg) administered once a day, three times a week, or twice a week in BALB/c mice infected with *Mycobacterium tuberculosis* H37Rv. The design of the experiment is shown in Table S1 and the results are shown in Table S2. The changes of the log₁₀ CFU of the lung and spleen during the treatment are shown in Fig. 4 and 5, respectively.

FIG 1 Drug-induced discoloration in infected C3HeB/FeJNju mice. Photographs of the subcutaneous adipose tissue were taken at 8 weeks. Photographs appear in the following order: control, CLO QD, CLO TIW, 166 QD, 166 TIW.

FIG 2 Histopathology of lesions in untreated C3HeB/FeJNju mice at day 0 (D0) (representative pictures).

After 4 weeks of treatment, the QD group of 166 and CLO performed as expected in this model, resulting in a reduction of approximately 2 $\log_{10}$ CFU/lung. For TBI-166, there was no significant difference in the number of CFU in spleens and lungs among the three dosing regimens (QD, TIW, and BIW). For CLO, there was no significant difference between various dosing regimens in the lungs and spleens with the exception that the number of CFU in spleen was different between the QD and BIW dosing regimens ($P=0.04$).

However, after 8 weeks of treatment, the QD dosing group of TBI-166 was significantly more bactericidal than the TIW and BIW groups, by 0.71 to 0.97 $\log_{10}$ CFU/lung, respectively (QD versus TIW, $P=0.005$; QD versus BIW, $P=0.004$). For CLO, the lung and spleen CFU counts were significantly different between QD dosing and TIW dosing (lung, $P=0.011$; spleen, $P=0.047$) and between QD dosing and BIW dosing (lung $P=0.003$, spleen $P=0.04$). However, there was no significant difference between the BIW and the TIW dosing regimens in spleen and lung CFU counts for both drugs by 8 weeks.

After 12 weeks of treatment, all treated mice remained culture positive. The QD dosing group had an even larger effect for both drugs than the intermittent regimens. QD administration of TBI-166 increased the bactericidal activity by 0.87 to 0.98 $\log_{10}$ CFU/lung and by about 0.85 to 1.04 $\log_{10}$ CFU/spleen at 12 weeks compared with the TIW and the BIW dosing regimens (QD versus TIW, $P=0.001$ for lung, $P=0.046$ for spleen; QD versus BIW, $P=0.001$ for lung, $P=0.016$ for spleen). However, there was no significant difference between the TIW and the BIW dosing regimens. A similar trend was observed for CLO. QD dosing of CLO increased the bactericidal activity by 1.27 to 1.90 $\log_{10}$ CFU/lung and by 1.61 to 2.22 $\log_{10}$ CFU/spleen (QD versus TIW, $P=0.002$ for lung, $P=0.002$ for spleen; QD versus BIW lung, $P<0.001$, spleen $P=0.003$). The lung CFU counts were significantly different between the BIW and the TIW dosing regimens ($P=0.029$).

At 4 weeks, the mice received 400, 320, and 280 mg/kg doses of drugs in the QD, TIW, and BIW dosing groups, respectively. The difference of total doses received in the 3 groups was below 120 mg/kg, and as a result, the CFU counts were not significantly different. However, at 8 weeks, the mice received 240 and 360 mg/kg lower doses of drugs in the TIW and the BIW dosing groups than in the QD dosing group, respectively. The CFU counts were significantly different between the QD and the intermittent regimens. At 12 weeks, the mice received 1,200, 800, and 600 mg/kg doses of drugs in the QD, TIW, and BIW dosing groups, respectively. An even more significant difference was observed between the QD and the TIW dosing regimens and the QD and the BIW dosing regimens. In addition, the CFU counts of the CLO treatment groups showed a significant difference between the TIW and the BIW groups.

In order to understand the characteristics of drug accumulation of various dosing regimens and the relationships with skin pigmentation potential, we evaluated the concentrations of both drugs in the blood, skin, and fat tissue (Table S3).

FIG 3 Histopathology of lesions in the control, TBI-166 QD, and CLO QD groups, respectively, after 8 weeks of treatment.

FIG 4 Lung CFU counts of BALB/c mice before and during treatment. Data points represent mean values, and error bars represent the standard deviation (SD) (5 mice per group per time point).

As expected, the concentrations of CLO and TBI-166 in the blood, skin, and fat tissue were dose-dependent. The plasma concentration of TBI-166 reached the steady state after 4 weeks of treatment. The skin and fat tissue concentrations of TBI-166 at 12 weeks were 2-fold higher than those at 4 weeks. The plasma, skin, and fat concentration of CLO did not increase significantly at 12 weeks. The plasma and fat concentrations of TBI-166 were higher than those of CLO.

Pigmentation of the skin was visible after 4 weeks of drug treatment. Dissection revealed visible pigmentation of the subcutaneous. The pictures of the TBI-166- and the CLO-treated mice in different dosing regimens after 12 weeks of treatment are shown in Fig. 6. The subcutaneous fat discoloration of BIW the dosing group is lighter than that of the QD and the TIW dosing group, which correlates with the measured drug concentration (Table S3).

DISCUSSION

For the treatment of MDR/rifampin-resistant TB (RR-TB) patients, at least four potentially effective drugs are needed at the beginning of the treatment regimen, which include all group A drugs and at least one group B drug (10). CLO has been recom-

FIG 5 Spleen CFU counts of BALB/c mice before and during treatment. Data points represent mean values, and error bars represent the SD (5 mice per group per time point).

FIG 6 Clofazimine and TBI-166 induced discoloration in infected BALB/c mice. Photographs of the subcutaneous adipose tissue were taken at 12 weeks. Photographs appear in the following order: control, CLO QD, CLO TIW, CLO BIW, 166 QD, 166 TIW, 166 BIW.

mended by WHO as a group B drug by consolidated drug-resistant TB treatment guidelines in 2020 and plays an important role in the treatment of drug-resistant tuberculosis (11, 12). Although CLO was recommended at 100 mg or 200 mg QD in the treatment of drug-resistant tuberculosis, an optimized dosage and administration interval should be further studied and identified because of its long half-life and tissue accumulation (8). As a structural analogue of CLO, TBI-166 is currently in the phase I clinical trial. TBI-166 showed at least the same activity as CLO in acute and chronic tuberculosis mouse models. In addition, TBI-166 demonstrated less potential for skin pigmentation than CLO (13). In this study, we explored whether an intermittent administration of TBI-166 is optimal to support further clinical development of this compound. Skin pigmentation is a concern for both CLO and TBI-166; the previous data indicated a positive correlation between the dosage and adverse reactions (14, 15). The purpose of our study is to evaluate whether intermittent administration of CLO and TBI-166 have the same activity as dosing every day but reduce the potential of skin pigmentation.

Our previous work showed that TBI-166 had delayed antimicrobial activity in the chronic infection model, similar to that of CLO (13), but lacked early bactericidal activity (EBA) during the first 2 weeks of treatment. In the current study, we decided to use a loading dose. TBI-166 and CLO were dosed at 20 mg/kg daily for 2 weeks, followed by QD, TIW, and BIW for an additional 10 weeks at the same dose level. In the first experiment, the antituberculosis activity of TBI-166 and CLO once daily and three times a week were compared in the chronic infected C3HeB/FeJNju mouse model. The lung pathological sections were evaluated. Pathological findings showed that H&E staining of lung slices showed pulmonary nodule formation, nodular lung lesions, granulomatous alveolar pneumonia, and a small amount of foam cells around pulmonary nodules after 6 weeks of infection. After 8 weeks of treatment, the lung lesions were more severe in the control group than in the treatment groups. The CFU counts were consistent with those of pathological findings. The CFU counts showed that there was no significant difference between the QD and the TIW dosing groups after 4 weeks and 8 weeks of treatment, although the total dose differences between two groups were 20% and 30% (Table 1). After 8 weeks of treatment, the lung and spleen of 60% to 100% mice reached culture negative in treatment groups. However, the external lesions of the lung did not show a synchronous recovery after 8 weeks of treatment, which indicated that the pathological changes were slower than the changes of the CFU counts. In the second study, we utilized the BALB/c mouse acute infection model and increased the initial inoculum, because the majority of the mice had reached culture negative after 8 weeks of treatment in the first study. The treatment was initiated 10 days after infection. Compared with study 1, a twice-weekly dosing regimen (BIW) was added, and the duration of treatment was extended to 12 weeks. At 4 weeks of treatment, the efficacy of the TIW dosing group of TBI-166 was similar to that of the QD dosing group. However, after 8 and 12 weeks of treatment, the efficacy of the TIW dosing group of TBI-166 was less than that of the QD dosing group. The accumulated total doses at the end of week 12 were 1,200 mg/kg for QD, 800 mg/kg for TIW, and 600 mg/kg for BIW, respectively. The gap between the total number of doses of the

drug in the TIW and QD groups increased with the prolongation of the treatment. These data suggest that accumulated total doses correlate with the $\log_{10}$ CFU reductions. We speculated that the difference between the BIW and the TIW groups will be more significant with prolonged treatment.

It is worth noting that the accumulation of CLO and TBI-166 in the tissues can lead to drug carryover from the homogenized lungs and spleens (16); we used 7H10 agar supplemented with 0.04% activated charcoal to absorb the drug. At the same time, the mice were dissected 3 days after the last administration to minimize the effects of drug carryover. The efficacies of the TIW and the QD dosing regimens differed between the two mouse models after 8 weeks of treatment. This difference may be related to the inoculum of the infection, acute versus chronic infection, and the strain of the mice.

The plasma, skin, and fat tissue concentrations of CLO and TBI-166 in different dosing regimens were dose dependent and consistent with the pharmacokinetics/pharmacodynamics (PK/PD) study of CLO (17). Although the plasma and fat tissue concentrations of TBI-166 were higher than those of CLO, the color of skin and subcutaneous fat in the TBI-166 group was less apparent than that in the CLO group, indicating that TBI-166 could potentially reduce the potential for skin pigmentation compared to CLO despite achieving a higher concentration in the relevant tissues (13). TBI-166, with a lower lipophilicity ($\log P$ 4.52) and a shorter half-life ($t_{1/2}$, 41.25 h) than CLO ($\log P$ 5.34; $t_{1/2}$, 65.38 h), had higher accumulation in tissues. This may be attributed to its unique physicochemical properties. We found that the plasma and fat tissue concentration of CLO decreased at 8 weeks of treatment compared to that at 4 weeks of treatment. Baik et al. also reported that serum CLO concentrations were lower after 8 weeks of treatment than after 3 weeks of treatment, and these changes over time paralleled its concentration in fat tissue. They observed that CLO redistributed from adipose tissue to liver, spleen, gut, and lungs between 3 and 8 weeks of continuous exposure (18). Redistribution of accumulated CLO can cause a bactericidal effect. This also partly explained why the *M. tuberculosis* CFU counts of CLO-treated mice decreased more at 8 and 12 weeks of treatment.

The PK/PD study of CLO showed that the antituberculosis activity of CLO does not depend on the concentration of the drug in the tissue (16, 17). Its efficacy was associated with the time that serum CLO levels remained at or above the MIC 0.25 $\mu\text{g/ml}$ for *M. tuberculosis* (11). Elimination of CLO was extremely slow, ensuring the long-term effective concentration *in vivo* during treatment. Yoon et al. suggested that CLO therapy is accompanied by the formation of drug biocrystals that accumulate within resident tissue macrophages (19). The quantitative, mechanistic information about the distribution of CLO during chronic exposure or during long-term treatment will need to be further studied. The hollow fiber infection model might be an adequate tool to estimate the activity of the different dosage schemes, which could further strengthen the conclusion of mouse experiments.

The limitations of this study include the following. (i) The treatment duration for the tuberculosis patients is 6 to 9 months, and there were 8- to 12-week periods in the two mouse models. The treatment duration was not long enough, which resulted in the differences among the observed CFU decline not being significant. (ii) CLO and TBI-166 were evaluated as a single drug or candidate in the study. It is necessary to further evaluate them in various combination regimens in the future. (iii) The drug concentration measurement was only done at a single time point. The blood and tissues concentration at multiple time points can be used to further analyze the relationship between the concentration and drug efficacy or pigmentation.

Based on the results of this study, the accumulated total doses correlate with the $\log_{10}$ CFU reductions. To achieve the same treatment effect as daily treatment, longer duration of treatment is needed for intermittent treatment. Prolonging the treatment period will reduce the patients' compliance, which is also inconsistent with the recommendation of short-term treatment. Our work will be an important source of information

for intermittent administration. Although CLO and TBI-166 both have a long half-life, they will be not suitable for intermittent administration in the case of the same daily dose with once-daily administration. TBI-166 and CLO should be further evaluated at the same accumulated total doses in preclinical and clinical studies.

MATERIALS AND METHODS

The animal experiments were approved by the animal ethics committee of Beijing Chest Hospital, Affiliate of Capital Medical University. All animal procedures were performed according to the Animal Care Guidelines of the Institutional Animal Care and Use Committee of Capital Medical University (Beijing, China).

Antimicrobial agents. Clofazimine (CLO) was purchased from Hanxiang bio-company (Shanghai, China). TBI-166 was a donation from Institute of Materia Medica, Chinese Academy of Medical Sciences (Beijing, China).

Mycobacterial strain. *M. tuberculosis* H37Rv strain (ATCC 27294) was grown in Middlebrook 7H9 broth supplemented with oleic acid-albumin-dextrose-catalase (OADC) enrichment (Difco, USA), 0.2% glycerol, and 0.05% Tween 80. Log-phase cultures incubated at 37°C with 5% CO₂ were used in the study.

Establishment of infection in mice. Study 1: specific-pathogen-free (SPF) C3HeB/FeJNju female mice ($n=92$), aged 6 weeks, were purchased from GemPharmatech Company. Mice were aerosol infected with *M. tuberculosis* H37Rv by Inhalation Exposure System (Glas-Col 099C A4224, Terre Haute, IN, USA). Mice were infected with 2×10^6 CFU/ml *M. tuberculosis* H37Rv bacteria. Three untreated mice were sacrificed 14 days and 28 days after the infection to determine the baseline counts of bacteria in the lungs and spleens at the start of the treatment, respectively. After 4 weeks and 8 weeks of drug administration, 5 mice from each of the five groups were taken out for treatment evaluation, and the other 3 mice were euthanized for pathological analysis.

Study 2: 102 female BALB/c mice (18 to 20 g), were purchased from Beijing Vital River Laboratory Animal Technology Company. Mice were aerosol infected with 3×10^6 CFU/ml *M. tuberculosis* H37Rv. Treatment started after 10 days of infection. After 4, 8, and 12 weeks of therapy, the CFU was measured. Three untreated mice from each infection run were sacrificed at the start of treatment and 4, 8, and 12 weeks after the infection, respectively. Compared to study 1, we extended the treatment period to 12 weeks and added a BIW treatment group in study 2.

Experimental design and treatment. Mice were divided into control group, TBI-166 daily administration group (QD), TBI-166 three times a week administration group (TIW, daily administration in the first 2 weeks, followed by thrice weekly), TBI-166 twice-weekly administration group (BIW, daily administration in the first 2 weeks, and then twice weekly), CLO daily administration group (QD), CLO three times a week administration group (TIW, daily administration in the first 2 weeks, followed by thrice weekly), and CLO twice-weekly administration group (BIW, daily administration in the first 2 weeks, and then twice weekly). To further clarify the correlation between the total dose and CFU count, total dose of CLO and TBI-166 in three groups were calculated.

Treatment was initiated 42 days after infection in study 1 and 10 days after infection in study 2. The drugs were prepared weekly by suspension in 0.5% carboxymethylcellulose (CMC) and administered by gavage in a 200- μ l suspension. Mice were dosed for 5 consecutive days each week in the QD group. The vehicle control mice received equal volumes of 0.5% CMC and the positive control mice received CLO (20 mg/kg) or TBI-166 (20 mg/kg).

Assessment of treatment activity. Efficacy was assessed based on the lung and spleen CFU counts during treatment. Five mice from each group were sacrificed at each time point. The lungs and spleens were removed and homogenized in 3.0 ml sterilizing saline. Tissue suspension was diluted 10-fold in PBS, and 0.1 ml of each dilution was plated on selective 7H10 agar plates enriched with 10% OADC enrichment (Difco, USA). To limit the consequence of CLO and TBI-166 carryover, lung and spleen homogenates from the CLO- and TBI-166-treated mice were plated on 7H10 selective agar supplemented with 0.4% (weight/volume) activated charcoal, which could absorb the residual CLO and TBI-166. All plates were incubated at 37°C with 5% CO₂ for 4 weeks before the CFU were enumerated.

Pathology. The lungs were placed in 10% formalin for 1 week. Then the tissue sections were embedded in paraffin and cut to 5- μ m thickness on a microtome. Subsequent tissue sections were mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E). Sections were scanned and visualized for evaluation.

Drug concentration. Infected BALB/c female mice received TBI-166 or CLO at 20 mg/kg in 0.5% CMC for up to 12 weeks. Groups of 3 mice were sacrificed 72 h after last dose after 4, 8, and 12 weeks of administration. At each time point, in addition to counting CFU in spleen and lung homogenate, plasma, subcutaneous fat, and skin were selected to measure drug concentrations. Skin was taken from the backs of mice after shaving off hair. After methanol extraction, the samples were centrifuged, and the supernatants were taken for detection. The concentrations of CLO and TBI-166 in the samples were determined by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) as previously described (6) (Table S2).

Statistical analysis. CFU counts were log-transformed before analysis, and data were analyzed using one-way analysis of variance (ANOVA) and followed by Tukey multiple comparisons (equal variances) or Welch's ANOVA test followed by Dunnett's T3 test (unequal variances). The significance level was 0.05. SPSS program (SPSS 19.0 version for Windows, Chicago) was used for all statistical analyses.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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