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Adjunctive therapy of *Mycobacterium vaccae* vaccine in the treatment of multidrug-resistant tuberculosis: A systematic review and meta-analysis

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Abstract. A number of studies have suggested that the *Mycobacterium vaccae* (MV) vaccine as an adjunctive therapy has a positive effect in the treatment of multidrug-resistant tuberculosis (MDR-TB). However, the result is inconclusive. The aim of the present study was to systematically evaluate the effect and safety of MV as an adjunctive therapy in the treatment of MDR-TB. A computerized search of PubMed, Embase, Cochrane Central Register of Controlled Trials, CBM, CNKI and VIP until October 2014 was conducted to collect the relevant studies. The main outcome measures were the sputum smear positive-turned-negative rate, the absorption rate of TB foci and the closure situation of the TB cavity. Two investigators identified the eligible studies and extracted data independently. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated and pooled using the fixed effects model. A total of 25 studies involving 2,281 patients with MDR-TB were included. The pooled OR was 3.84 (95% CI, 3.84-4.73) for the sputum smear positive-turned-negative, 4.08 (95% CI, 3.08-5.45) for the absorption rate of TB foci, and 3.42 (95% CI, 2.68-4.37) for the closure situation of TB cavity. Therefore, MV has a significant effect as an adjunctive therapy in the treatment of MDR-TB. However, larger scale multicenter randomized controlled trials are required to confirm this evidence for limited latent bias at present.

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Introduction

Tuberculosis (TB) is a public health and social problem worldwide, and remains the main infectious disease with a serious effect on health in the current century. Since the 1960s, TB chemotherapy has replaced the previous negative health nutrition therapy and has become recognized worldwide as the main method for control of TB. However, TB deteriorated as a global trend from the middle of the 1980s, and currently, the majority of the TB epidemic is extremely low in developed countries. The TB epidemic situation is noticeably improving in numerous developing countries. In numerous countries and regions the occurrence of TB is associated with the human immunodeficiency virus infection, multiple drug-resistant Mycobacterium TB (MTB) infection, increased poverty, population growth and migration, and other objective factors. By contrast, the complexity of the TB epidemic has increased due to a lack of understanding of the vigilance and control of TB. The management of TB was also believed to be solved, and therefore, controlling TB and the input and control of other subjective factors was reduced. According to the World Health Organization (WHO), an estimate in 2008 indicates that the global annual incidence of drug-resistant TB is ~9.2 million, while the actual multidrug-resistant TB (MDR-TB) may approach >500,000. The number of patients in China with MDR-TB ranks second worldwide (1). MDR-TB treatment for TB prevention and control is an extremely difficult problem that is often clinically treated on the basis of anti-TB chemotherapy combined with immunotherapy.

Mycobacterium vaccae (MV) vaccine is an immunization of heat-killed MV that enhanced anti-TB mycobacterial infections in patients with cellular immune function, and combined with chemotherapy can enhance the efficacy of chemotherapy for the adjunctive treatment of TB. In 2011, Yang *et al* (2) performed a meta-analysis to evaluate the MV as an adjunctive therapy to anti-TB chemotherapy in never-treated TB patients. Certain studies have shown that MV can protect against MDR-TB (3-5). However, not all the studies reached the same or similar association, and no summary of the evidence of the effectiveness of MV as an adjunctive therapy in the treatment of MDR-TB exists. The

present study conducted a comprehensive systematic review and meta-analysis of the eligible studies on the adjunctive therapy of MV in the treatment of MDR-TB to indicate the critical effect of MV, further research and application.

Materials and methods

Literature search. The study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement (6). A computerized search was conducted in the English databases of PubMed, Embase, Cochrane Central Register of Controlled Trials, CBM, CNKI and VIP until October 2014 for original studies using the following keywords: ('*Mycobacterium vaccae*' OR '*M. vaccae*' OR MV) AND ('multidrug tuberculosis' OR MDR-TB OR 'refractory tuberculosis'). The performance was limited to human studies and manual retrieval was applied to the literature references or bibliographies of all the relevant studies.

Study selection. All the initial studies complied with the following criteria: i) Randomized controlled trials (RCTs) or clinical controlled trials with available data to investigating MV as an adjunctive therapy used as intervention versus general chemotherapy (without MV); ii) the general chemotherapy regimens in the MV group was the same as the control group (without MV); and iii) subjects without adverse disease, such as severe autoimmune disease, or pregnant women.

Data extraction. Two investigators (HW and SL) independently conducted the data extraction and quality assessment. Any disagreements were resolved by discussion. The first author, year of publication and the detailed information of PICOS (patient, intervention, comparison and study design) were recorded.

Quality assessment. The methodological quality of each study was evaluated using the Cochrane collaboration's tool for assessing the risk of bias. The following four items were mainly assessed: Adequate sequence generation, allocation concealment, blinding and incomplete outcome data addressed.

Statistical analysis. All the data were pooled using Stata 12.0 (Stata Corp., College Station, TX, USA). For the binary outcomes, the odds ratio (ORs) and 95% confidence intervals (CIs) were calculated; and for the continuous outcomes, the standard mean differences (SMDs) and their 95% CIs were calculated. The heterogeneity test was performed for the included studies and P<0.1 was considered to indicate a statistical significant difference, and subsequently a quantitative measure of heterogeneity across studies was conducted using the I^2 statistic. Studies with an I^2 of 25-50% were considered low heterogeneity, 50-70% were considered moderate heterogeneity and >75% were considered high heterogeneity (7). The fixed effects model was applied to pool the effects for studies or subgroups without heterogeneity. Sensitivity analysis was carried out by removing studies, and using fixed and random effect models. Subgroup analysis was conducted to investigate the potential source of between-study heterogeneity. A two-side P<0.05 in the Z-test was considered to indicate a statistically significant difference.

Results

Description of studies and quality assessment. The initial search yielded 178 relevant studies, of which 150 were excluded for various reasons depending on the abstracts, and finally 25 studies (8-32) were included. These 25 studies involved 2,281 Chinese patients. The characteristics of the eligible studies are shown in Table I. The course of treatment ranged from 6 to 24 months. The quality assessment of the eligible studies is shown in Table II.

Meta-analysis of outcome measures. In total, 23 studies (8-29,32) reported the sputum smear positive-turned-negative rate (Table III). There was no heterogeneity between these studies (I^2=0.0%, P-value for heterogeneity=0.493). Subsequently, the fixed effects model was performed to pool the results. The aggregated result of these studies suggested that MV as an adjunctive therapy is associated with a significantly increased sputum smear positive-turned-negative (OR=3.84; 95% CI, 3.11-4.73). Following this, sensitivity analysis was carried out by changing the statistical model (OR=3.73; 95% CI, 3.01-4.61; I^2=0.0%; P-value for heterogeneity=0.493).

Fifteen studies (8-13,17-22,24,26,27) reported the absorption rate of TB foci (Table III). There was no heterogeneity between these studies (I^2=35.7%, P-value for heterogeneity=0.084). Following this, the fixed effects model was applied to pool the results. The pooled result suggested that MV as an adjunctive therapy is associated with a significantly increased absorption rate of the TB foci (OR=4.08; 95% CI, 3.06-5.45). Subsequently, sensitivity analysis was carried out by changing the statistical model (OR=4.08; 95% CI, 2.75-6.05; I^2=35.7%; P-value for heterogeneity=0.084).

Eighteen studies (9-23,25,26,28) reported the closure situation of the TB cavity (Table III). There was no heterogeneity between these studies (I^2=0.0%, P-value for heterogeneity=0.794). Subsequently, the fixed effects model was performed to pool the results. The summarized results of these studies suggest that MV as an adjunctive therapy is associated with a significant increase in the closure situation of the TB cavity (OR=3.42; 95% CI, 2.68-4.37). Following this, sensitivity analysis was carried out by changing the statistical model (OR=3.40; 95% CI, 2.65-4.36; I^2=0.0%; P-value for heterogeneity=0.794).

Four studies (26,27,30,31) reported the curative effects of the adjunctive therapy of MV. The aggregated results of these studies suggest that MV as an adjunctive therapy is associated with a significant increase in the curative effects (OR=2.44; 95% CI, 1.29-4.60; I^2=0.0%; P-value for heterogeneity=0.979) with the fixed effects model.

Four studies (10,13,17,21) reported the improvement of symptoms of the adjunctive therapy of MV. The aggregated results of these studies suggest that MV as an adjunctive therapy is associated with a significant increase in the improvement of symptoms (OR=6.15; 95% CI, 3.32-11.42; I^2=15.0%; P-value for heterogeneity=0.317) with the fixed effects model.

Four studies (11,13,18,22) reported the T-lymphocyte subsets counts including cluster of differentiation 3^+ ($CD3^+$), $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$. The pooled result of these

Table I. Characteristics of the studies included in the meta-analysis.

First author, year	Sample size, n (m/f)	COT, months	Interventions	Comparison	Outcomes	Refs.
Luo, 2000	28/28	6	Chemotherapy regimens + 0.1 mg MV	Km, Am, P, Cm, Ofx, Lfx and Pto	1-5	(8)
Song, 2002	36/35	9	Chemotherapy regimens + 22.5 μg MV	3Pa-Pto-Am-E-Lfx/6Pa-Pto-E-Lfx	1-3, 5	(9)
Zheng, 2004	50/47	6	Chemotherapy regimens+MV	H, Z, K, Pto, P and RFT	1-3, 5	(11)
Wu, 2004	43/41	18	Chemotherapy regimens + 22.5 μg MV	Pa, RFT, Lfx, Pto and Am (or Z, P, E and Cm)	1-3	(10)
Yao, 2005	30/30	18	Chemotherapy regimens + 22.5 μg MV	3Pa-RFT-Am-Z-Pto-Lfx/ 15Pa-RFT-Z-Pto-Lfx	1-3, 5	(13)
Chen, 2005	38/41	12	Chemotherapy regimens + 22.5 μg MV	2RFT-Lfx-Am-Pa-E/10RFT-Lfx-Pa	1-3, 5	(12)
Wang, 2006	36/35	12	Chemotherapy regimens + 22.5 μg/ml MV	3Cp-Ofx-Pa-Z-Pto/9Pa-Ofx-Pto	1-3	(15)
Liu, 2006	34/36	18	Chemotherapy regimens + 22.5 μg MV	3Pa-RFT-Pto-Lfx-Am/ 15Pa-RFT-Pto-Lfx	1, 3, 5	(14)
Lan, 2007	35/33	18	Chemotherapy regimens + 22.5 μg MV	3Pa-Pto-Lfx-Am-Z/15Pa-Pto-Lfx	1, 3, 5	(16)
Zhao, 2008	52/50	12	Chemotherapy regimens + 22.5 μg MV	3Pa-Cp-Pto-Lfx-E/9Pa-Pto-Lfx-E	1-3, 5	(17)
Xi, 2009	43/41	18	Chemotherapy regimens + 22.5 μg MV	3P-Lfx-Pto-Am-E/15P-Lfx-Pto-E	1-5	(22)
Chu, 2009	98/91	18	Chemotherapy regimens + 22.5 μg MV	3Pa-RFT-Lfx-Pto-Am-Cm/ 15Pa-RFT-Lfx-Pto-Cm	1-6	(18)
Hong, 2009	47/45	12	Chemotherapy regimens + 22.5 μg MV	3Pa-Am-Pto-Lfx-E/9Pa-Pto-Lfx-E	1-3, 5, 6	(20)
Gu, 2009	34/36	18	Chemotherapy regimens + 22.5 μg MV	3Pa-RFT-Am-Z-Pto-Lfx/1 5Pa-RFT-Z-Pto-Lfx	1-3, 5	(19)
Wang, 2009	120/120	18	Chemotherapy regimens + 22.5 μg MV	3Pa-RFT-Am-Z-Pto-Lfx/ 15Pa-RFT-Z-Pto-Lfx	1-3, 5	(21)
Mei, 2010	40/41	18	Chemotherapy regimens + 22.5 μg MV	3Pa-Lfx-Am-Z/15Pa-Lfx	1, 2, 5	(24)
Zhang, 2010	56/52	12	Chemotherapy regimens + 22.5 μg MV	3Pa-Pto-Z-Lfx-Am/15Pa-Pto-Z-Lfx	1, 3, 6	(25)
Liu, 2010	32/34	9	Chemotherapy regimens + 22.5 μg MV	3Am-Pa-Z-Lfx-E/6Pa-E-Lfx	1, 3	(23)
Zhou, 2011	72/71	21	Chemotherapy regimens + 22.5 μg MV	3Pa-Lfx-Z-Pto-S/18Pa-Lfx-Z-Pto	1-3, 5, 6	(29)
Wang, 2011	60/60	12	Chemotherapy regimens + 22.5 μg MV	RFT, Pa, and Lfx	1-3, 5, 7	(27)
Yang, 2011	30/30	12	Chemotherapy regimens + 22.5 μg MV	Pa, Pto, Z, Lfx, and Am	1-3	(28)
Sheng, 2011	36/36	21	Chemotherapy regimens + 22.5 μg MV	3H-P-Cm-Pto-Lfx/18Pa-Pto-Lfx	1-3, 5, 7	(26)
Yin, 2012	17/18	24	Chemotherapy regimens + 22.5 μg MV	6Z-Am-Lfx-Pto-P/18Z-Lfx-Pto-P	7	(31)
Chen, 2012	43/40	12	Chemotherapy regimens + 22.5 μg MV	Pro, RFT, Lfx, and Pa	7	(30)
Zhang, 2013	72/62	24	Chemotherapy regimens + 22.5 μg MV	6Mfx-Z-Pto-Km-P/18Mfx-Z-Pto-P	1, 4, 5	(32)

Outcome 1, the sputum smear positive-turned-negative rate; 2, the absorption rate; 3, the cavity closure rate; 4, T cell subgroup counts; 5, adverse effects; 6, improvement of symptoms; 7, the effective rate. COT, course of treatment; COD, course of disease; MV, *Mycobacterium vaccae*; H, isoniazid; R, rifapentine; RFT, rifapentine; Am, amikacin; Z, pyrazinamide; E, ethambutol; S, streptomycin; Pa, pasinizid; P, p-aminosalicylic acid; Km, kanamycin; Cm, capreomycin; Ofx, ofloxacin; Lfx, levofloxacin; Mfx, moxifloxacin; Pto, protionamide; m, male; f, female.

Table II. Quality assessment of the included studies.

First author, year	Adequate sequence generation	Allocation concealment	Blinding	Incomplete outcome data addressed	Refs.
Luo, 2000	Unclear	Unclear	Unclear	Yes	(8)
Song, 2002	Unclear	Unclear	Unclear	Yes	(9)
Zheng, 2004	Unclear	Unclear	Unclear	Yes	(11)
Wu, 2004	Unclear	Unclear	Unclear	Yes	(10)
Yao, 2005	No	Unclear	Unclear	Yes	(13)
Chen, 2005	Unclear	Unclear	Unclear	Yes	(12)
Wang, 2006	Unclear	Unclear	Unclear	Yes	(15)
Liu, 2006	Unclear	Unclear	Unclear	Yes	(14)
Lan, 2007	Unclear	Unclear	Unclear	Yes	(16)
Zhao, 2008	Unclear	Unclear	Unclear	Yes	(17)
Xi, 2009	Unclear	Unclear	Unclear	Yes	(22)
Chu, 2009	Yes	Unclear	Unclear	Yes	(18)
Hong, 2009	Unclear	Unclear	Unclear	Yes	(20)
Gu, 2009	Unclear	Unclear	Unclear	Yes	(19)
Wang, 2009	No	Unclear	Unclear	Yes	(21)
Mei, 2010	Unclear	Unclear	Unclear	Yes	(24)
Zhang, 2010	Unclear	Unclear	Unclear	Yes	(25)
Liu, 2010	Unclear	Unclear	Unclear	Yes	(23)
Zhou, 2011	Unclear	Unclear	Unclear	Yes	(29)
Wang, 2011	Unclear	Unclear	Unclear	Yes	(27)
Yang, 2011	Unclear	Unclear	Unclear	Yes	(28)
Sheng, 2011	Unclear	Unclear	Unclear	Yes	(26)
Yin, 2012	Yes	Unclear	Unclear	Yes	(31)
Chen, 2012	Unclear	Unclear	No	Yes	(30)
Zhang, 2013	Unclear	Unclear	Unclear	Yes	(32)

Table III. Sensitivity analysis of the pooled ORs of adjunctive therapy of MV vs. general chemotherapy (without MV).

	SSP		ATF		CSTBC	
Variable	Total, n	OR (95% CI)	Total, n	OR (95% CI)	Total, n	OR (95% CI)
Model						
FE	23	3.84 (3.11-4.73)	15	4.08 (3.06-5.45)	18	3.42 (2.68-4.37)
RE	23	3.73 (3.01-4.61)	15	4.08 (2.75-6.05)	18	3.40 (2.65-4.36)
COT, weeks						
<12	4	4.21 (2.49-7.12)	3	5.28 (2.89-9.66)	3	4.32 (2.42-7.71)
12	7	5.89 (3.79-9.16)	4	5.10 (2.27-11.45)	6	4.34 (2.72-6.92)
>12	12	3.15 (2.41-4.12)	8	3.31 (1.77-6.19)	9	2.79 (1.99-3.89)

MV, *Mycobacterium vaccae*; SSP, sputum smear positive-turned-negative; ATF, absorption of tuberculosis foci; CSTBC, closure situation of tuberculosis cavity; OR, odds ratio; CI, confidence interval; FE, fixed effect model; RE, random effect model; COT, course of treatment.

studies suggested that MV as an adjunctive therapy is associated with a significant increase in $CD4^+$ counts (SMD=0.98; 95% CI, 0.04-1.93; I^2=94.6%; P-value for heterogeneity <0.001) and specific value of $CD4^+/CD8^+$ (SMD=0.76; 95% CI, 0.06-1.45; I^2=90.6; P-value for heterogeneity <0.001) with the random effects model, and MV as an adjunctive therapy had no statistical significance for increasing $CD3^+$ counts (SMD=0.31; 95% CI, -0.05-0.68; I^2=68.2%, P-value for heterogeneity=0.024) and $CD8^+$ counts (SMD=-1.14; 95% CI, -2.81-0.53; I^2=98.0%; P-value for heterogeneity <0.001).

The adverse effects of MV mainly include induration at the vaccine sites (8-9,13,16,17,19-22,24,26), fever (8,13,16,19,21,22) and local swelling (9,12,17,20,21,26). For the purified protein

derivative test, only 1 study (15) reported that no significance occurred for the changes of the scleroma diameter between the MV and control groups.

Discussion

The main aim of the present study, which included 25 eligible studies involving 2,281 subjects, was to critically evaluate the effects of MV as an adjunctive therapy in the treatment of MDR-TB patients. Based on the available studies, the meta-analysis showed that MV is an effective adjuvant when combined with general chemotherapy for treating MDR-TB. MV can improve the sputum smear positive-turned-negative, the absorption of TB foci and the closure situation of the TB cavity for some of the poor quality RCTs reported. In addition, MV as an adjunctive therapy has fewer adverse effects compared to the general anti-TB drugs. The clinical effects of MV have been confirmed presently using the previous studies.

Particularly in low-income countries and developing nations, MDR-TB is spreading with a series of negative consequences. MDR-TB had been reported in Eastern Europe, Western Europe and Central Asia (33,34). Control of TB by chemotherapy is mainly measured according to 'the long-term, combined, in moderation, appropriate and whole course', but resistance is frequently due to unreasonable chemotherapy regimens, poorer compliance and worse immune function of patients, resulting in TB bacterium that are resistant of MDR, and subsequently, searching for the correct treatment is the focus of clinical physicians and pharmacists. The T-cell [T-helper cells (Th cells)] immunity of patient immune function is closely associated with the onset and outcomes of TB disease; Th1 cells decrease in peripheral blood and increase in lesions, and furthermore, they are associated with the course of dynamic changes. Therefore, according to the immunological characteristics of TB, the study protocol of immune therapy application combined with chemotherapy has been more frequently applied in anti-TB treatment in clinical practice. In an early study by Stanford *et al* (35), MV as immune agents improved the pathological changes around the immune cell activity, reduced tissue necrosis, weight gain, shortened the chemotherapy treatment, and reduced the case fatality rate.

MV, a type of immune enhancer, mainly affects the immune response to adjust the human immune function and achieve therapeutic purposes. MV can remove and restrain MTB by activating the Th1 cytokine-mediated immune response, improving Th1/Th2, and activating the macrophage phagocytosis of MTB. From the perspective of pharmacoeconomics, although the treatment cost of MV combined with chemical therapy increased for MDR-TB, its curative effect significantly improved and the incremental cost was reduced (12). It is the only recommended immunization agents by the WHO in the Tuberculosis Strategic Development Plan of the 1990s (36).

The result of the present meta-analysis with little heterogeneity was in accordance with the study reporting that immunotherapy with MV offers hope for the treatment of MDR-TB pulmonary TB in Estonia, Iran, Kuwait, New Zealand, Romania, Vietnam and the United Kingdom (37). There are certain limitations that originated from the limited studies present in this meta-analysis, which should be taken into consideration. Firstly, these studies (8-32) were not targeted globally (all studies were Chinese populations) and the course of treatment varied from 6 to 24 months, and the chemotherapy regimens in the control group were different forms in each trials. Secondly, the studies were all carried out in China and their qualities had a generally high risk of bias; all did not address the allocation concealment, which would magnify >40% effects of the results (38-40). Only 2 studies (18,31) reported the method of adequate sequence generation, including stratified random and using a random numbers table. Thirdly, the sample size of the included studies was relatively small. On the basis of the course of treatment in the subgroup analysis, a notable finding was that the effects of the sputum smear positive-turned-negative, the absorption of TB foci and the closure situation of TB cavity were reduced as the course of treatment increased. This was possibly due to the reducing effect of the general chemotherapy as the extension of treatment. Furthermore, the treatment of MDR-TB should always be prioritized above chemotherapy, and immunotherapy is a type of auxiliary treatment. Therefore, multi-central large RCTs are required to investigate the safety of MV as an adjunctive therapy in MDR-TB treatment in the future.

In conclusion, the present study demonstrates that MV has a significant effect and safety as an adjunctive therapy in the treatment of MDR-TB. Further studies are required to validate this conclusion.

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