Review Article
Mcl-1 intervention regulating macrophages apoptosis in vivo and in vitro TB model: a systematic review and meta-analysis

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Abstract: Tuberculosis has seriously affected human health, and current situation of prevention and control tuberculosis is becoming more and more urgent. Intervention the expression of Mcl-1 can control latent and persistent TB infection effectively, including virulent and attenuated MTB strains, and has divergent effects between different host macrophages environment and between different intervention times and methods. Herein, a meta-analysis was performed by independently searching databases including the Cochrane Library, PubMed, Springer, Embase, and China National Knowledge Infrastructure, to analyze effects of Mcl-1 intervention on MTB infection. Compared to controls, MTB infection induced macrophages apoptosis significantly increase in vivo and in vitro macrophages infected with different virulence of MTB strains (P < 0.0002), and short time’s infection caused more host macrophages apoptosis in vivo TB model (P < 0.0002). After Mcl-1 intervention, compared with controls, Mcl-1 induction rate was significantly increase in H37Ra infection group (95% CI, 1.51, 3.86; Z = 2.69; P < 0.00002), Mcl-1 induction rate was also significantly increase in H37Rv infection group (95% CI, 9.51, 24.28; Z = 16.91; P < 0.00002), whereas the induction relative weaker in other MTB strains infection group. Short Mcl-1 intervention time significantly increased host macrophages apoptosis infected BCG and H37Rv (P < 0.00001), but the effect was relatively decrease in other MTB strains infection group (P = 0.006), while it have no significantly differences in H37Ra infection group (P = 0.15). However, Long Mcl-1 intervention time increased macrophages apoptosis in all MTB strains (P < 0.00001). These findings may provide a theoretical basis for the interaction between host macrophages and MTB and Mcl-1 intervention introduce to control latent and persistent TB infection.

Keywords: Mcl-1, macrophages apoptosis, systematic review and meta-analysis

Introduction

Tuberculosis (TB) is a worldwide infectious diseases caused by Mycobacterium Tuberculosis (MTB). Based on the Bulletin of the World Health Organization (WHO), one-third of the world’s population may be asymptotically infected with tuberculosis (TB) [1]. TB has been a remarkable public health issue in mainland China, and 80% of new TB cases worldwide have been reported in China each year. In 2013, an estimated 9.0 million individuals developed TB and 1.5 million died. China alone accounted for 11% of the total cases worldwide [2]. However, MTB remains to survival within infected macrophages for prolonged periods by evading the elimination of host immune responses [3, 4]. As such, the long-term latent infection and persistent infection in host macrophages becomes the main difficulties of control and prevention TB. In addition, its incidence and the prevalence of Multidrug Resistant-TB (MDR-TB) have increased [5], while our tools to combat MDR-TB are unsatisfactory and not ideal. However, studies have found that inhibit the expression of Mcl-1 can effectively promote the MTB infection of host macrophage apoptosis, so as to achieve the purpose of persistent infection with the tuberculosis control in recent years. So the research on the basis of the literature retrieval using meta-analysis was carried out on the experimental data published at
home and abroad in recent years, comprehensive analysis, aimed to explore inhibit Mcl-1 expression for MTB infected macrophage apoptosis regulation function, for mycobacterium tuberculosis and the principle of the interaction between the host and provides the reference for the prevention and control of tuberculosis.

Methods

Search strategy

Using the PICO principle [6], searches were performed using the following electronic databases: the Cochrane Library, PubMed, Embase, Springer, Web of Science, China Science and Technology Journal Database (CSTJ), and China National Knowledge Infrastructure (CNKI) (last search conducted in July 2017). The key search string was (Mcl-1) AND (MTB) OR (Macrophages) OR (Infection) OR (Apoptosis) OR (TB) OR (Tuberculosis).

Inclusion criteria

We systematically reviewed published studies according to the following inclusion criteria any animal and cells lines, category not limited, published in either Chinese or English. Mcl-1 intervened by any kind of method in TB model groups, and its compounds were used as the experimental groups, and the untreated served as control groups. If various intervention methods of Mcl-1 were used in the study, the best intervention was chosen for this analysis. If various intervention times of Mcl-1 were used in a study, the longest time was chosen for this analysis.

Exclusion criteria

The exclusion criteria were as follows: (1) repeat publications; (2) incomplete information; (3) insufficient or insignificant statistical data; (4) unrelated to the study objectives; (5) lack of appropriate controls; and (6) review articles.

Outcome indicators

Host macrophage apoptosis rate: including apoptosis rate detected by FCM (Flow of cytometry), and TUNEL technology (Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling) detected apoptosis rate. Mcl-1 induc-
Data collection

Two reviewers (Xiao-fang Wang and Xin-min Wang) independently extracted data and cross-checked their data after aggregating the results. The following information was extracted from the complete manuscripts of each qualified study: publication characteristics (title of the study, first author, publication date, and journal/magazine title), baseline data (mean and standard deviation [SD]) for the experimental and control groups, subject characteristics (source of cells and tissue, arsenic doses and exposure times), outcome indicators, and the source of indicator estimates. This information is presented in Table 1. When the two reviewers’ opinions differed, Disagreements were resolved by discussion with Professor Le Zhang, was asked to make the final decision regarding the results.

Data analysis

Ten articles were analyzed in Review Manager Version 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, 2012, Portland Oregon, OR, USA). Significant heterogeneity was detected (p < 0.05, I²>75%) and a random effects model was therefore applied for the meta-analysis. A multivariate meta-regression analysis was performed to determine the source of heterogeneity and continuous variables were estimated as standardized mean differences (SMDs) with 95% confidence intervals (CIs) between the arsenic treated groups and control groups. All reported p-values are two-sided and a significance level of 0.05 was used. For additional insight, subgroup analyses were performed based on TB models (MTB infection in vivo; MTB infection in vitro) and intervention time (≤24 h or >24 h), based on the median of the indexes reported in the papers, to determine the factors associated with differences in the outcome indicators across different studies. Small-study effects were explored using funnel plots and Egger’s tests and study sensitivities were assessed. Sensitivity analyses were performed using Stata 12.0 (StataCorp, College Station, Texas, TX, USA).

Results

Search result

Figure 1 shows the study selection process, 10 relevant articles were identified. Initially, 162 articles were included in our search strategy. Finally, a total of 12 articles were included in the analysis, based on the inclusion and exclusion criteria. A total of 9 studies assessed Mcl-1 intervention in vitro TB model, 6 studies examined Mcl-1 intervention in vivo TB model (Table 1).

Study characteristics

Using the search strategy described in Section 2, 162 relevant articles were identified (Figure 1), of which 10 were used for the meta-analysis based on the eligibility and exclusion criteria (Table 1). Various cell lines and animals were used as TB model groups in these studies, and in each study, the effect of Mcl-1 intervention on the MTB infection was assessed. The Mcl-1 intervention groups were primarily cell lines and animals treated with various MTB strains (e.g. BCG, H37Ra, H37Rv, XJ-MTB, Pneumococcus), and the control models were blank controls. Alone MTB infection was categorized as either in vivo TB model (n = 8) or in vitro TB model (n = 6). Mcl-1 intervention time varied among the studies and was categorized as ≤24 h (n = 9) or >24 h (n = 7), Mcl-1 intervention methods was categorized as RNAi (n = 5) or signaling pathway intervention (n = 5). Mcl-1 induction rate also varied among the studies, and was categorized as either in vivo TB model (n = 7) or in vitro TB model (n = 4).

Results of the meta-analysis

The influence of MTB infection alone on host macrophages apoptosis

A total of 8 studies were included in this study, and of these, 8 estimated the impact of alone MTB infection by host macrophages apoptosis. A pooled analysis showed that BCG, H37Ra, H37Rv, and other MTB strains infection were 28.64, 56.24, 42.07, 28.09-fold higher than the control group (BCG group: 95% CI, 17.04-40.24; Z = 4.84; p < 0.00001; H37Ra group: 95% CI, 26.25-86.23; Z = 3.68; p = 0.0002; H37Rv group: 95% CI, 26.71-57.43; Z = 5.37; p
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< 0.00001; Other MTB strains group: 95% CI, 9.67-46.51; Z = 2.99; p = 0.003) in vivo TB model, while only BCG group with no significant heterogeneity (p = 0.11; I² = 60%; Figure 2).

Figure 2. Effects of alone MTB infection in vivo TB model. Forest plot showing the impact of Mcl-1 intervention by different methods compared with controls. Abbreviations: SMD = standardized mean difference, IV = independent variable, 95% CI = 95% confidence interval.

Figure 3. Effects of alone MTB infection in vitro TB model. Forest plot showing the impact of Mcl-1 intervention by different methods compared with controls. Abbreviations: SMD = standardized mean difference, IV = independent variable, 95% CI = 95% confidence interval.
Table 2. The apoptosis rate comparison of different models in MTB infection

<table>
<thead>
<tr>
<th>Index</th>
<th>In Vivo TB model</th>
<th>In Vitro TB model</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>n</td>
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<tr>
<td>≤24 h</td>
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<td>BCG group</td>
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<tr>
<td>H37Ra group</td>
<td>3</td>
<td>25</td>
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<tr>
<td>H37Rv group</td>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td>Other MTB group</td>
<td>3</td>
<td>27</td>
</tr>
</tbody>
</table>

N = the number of documents; n = the number of samples. 95% CI = 95% confidence interval. SMD = standardized mean difference.

Pooled analysis showed that there were 60.56, 85.81, 20.76-fold higher than the control group (BCG group: 95% CI, 35.86–85.75; Z = 4.71; p = 0.0001; H37Ra group: 95% CI, 37.48–134.13; Z = 3.48; p = 0.0005; H37Rv group: 95% CI, 10.58–30.94; Z = 4.0; p < 0.00001) in vitro TB model, but only BCG group with significant heterogeneity (p = 0.04; I² = 77%; Figure 3).

In subgroup analyses, the analysis based on the source of macrophages (in vivo TB model vs. in vitro TB model) and MTB infection time...
The effects of Mcl-1 intervention on host macrophages apoptosis infected MTB

Considering special role of Mcl-1 in MTB infection, firstly, we pooled analyzed Mcl-1 induction rate in alone MTB infection. The meta-analysis results showed that H37Rv infection induced 16.91-fold Mcl-1 expression higher than the control group (BCG group: 95% CI, 9.54-24.28; Z = 4.5; p < 0.00001), and with significant heterogeneity (p = 0.000001; I^2 = 82%; Figure 5).

However, BCG, H37Ra, and other MTB strains infection either induced less Mcl-1 expression compared with H37Rv infection group. The next analyses were the effects of Mcl-1 intervention.

The subgroup analysis based on Mcl-1 intervention time (>24 h vs. ≤24 h) and Mcl-1 intervention method (RNAi vs. Signaling pathway intervention) was conducted. The analysis showed that Mcl-1 intervention in short BCG, H37Rv, and other MTB strains infection time (≤24 h) caused 26.84, 38.11, 46.49-fold host macrophages apoptosis higher than the control group (BCG group: 95% CI, 17.37-36.11; Z = 5.59; p < 0.00001; H37Ra group: There was no significant difference; p = 0.15; H37Rv group: 95% CI, 24.61-51.62; Z = 5.53; p < 0.00001; Other MTB strains group: 95% CI, 13.27-79.72; Z = 2.74; p = 0.003), while only H37Rv group with no significant heterogeneity (p = 0.16; I^2 = 38%; Figure 6; Table 3). Mcl-1 intervention in long BCG, H37Ra, H37Rv, and other MTB infections (>24 h vs. ≤24 h) was conducted. The analysis demonstrated that short infection time (≤24 h) promote macrophages apoptosis in vitro TB model (95% CI, 26.59-86.99; Z = 3.69; p = 0.0002), and there were 56.79-fold higher than the control group, with significant heterogeneity (p < 0.00001; I^2 = 92%; Figure 4; Table 2).

The long infection time (>24 h) were 47.95-fold higher than the control group (95% CI, 26.06-69.85; Z = 4.29; p < 0.00002) in vivo TB model, with significant heterogeneity (p = 0.0005; I^2 = 80%; Figure 4; Table 2).

![Forest plot showing the impact of Mcl-1 intervention by different methods compared with controls. Abbreviations: SMD = standardized mean difference, IV = independent variable, 95% CI = 95% confidence interval.](image-url)
**Table 3.** The comparison of different Mcl-1 intervention time

<table>
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<tr>
<td></td>
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<td>n</td>
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<tr>
<td>BCG treatment group</td>
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<tr>
<td>H37Ra treatment group</td>
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<tr>
<td>H37Rv treatment group</td>
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<td>Other MTB treatment group</td>
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</tr>
<tr>
<td>Total effect</td>
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<td>110</td>
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</table>

N = the number of documents; n = the number of samples. 95% CI = 95% confidence interval. SMD = standardized mean difference.

Strains infection time (>24 h) caused 30.46, 61.96, 46.37, 40.11-fold higher than the control group (BCG group: 95% CI, 18.06-42.85; Z = 4.82; p < 0.00001; H37Ra group: 95% CI, 42.50-81.42; Z = 6.24; p < 0.00001; H37Rv group: 95% CI, 27.55-65.19; Z = 4.83; p < 0.00001; Other MTB strains group: 95% CI, 27.01-53.21; Z = 2.74; p < 0.000001) in vitro TB model, but BCG and H37Rv group with no significant heterogeneity (H37Ra group: \( I^2 = 0\% \); Other MTB strains group: \( I^2 = 0\% \); Figure 7: Table 3). Obviously, the effect of signaling pathway intervention method was weak than RNAi, and RNAi included in every studies that we chose articles, while take into account the intersection of the two, so we didn’t have any in-depth analysis.

**Sensitivity analysis**

As H37Rv infection for example, we conducted a sensitivity analysis for the TB model, Mcl-1...
intervention time, and Mcl-1 induction rate of H37Rv infection group, respectively (see Appendix 1). All of the included studies were distributed evenly from the central line, with no significant deviation. Therefore, no individual study affected the pooled effect results.

Discussion

Multidrug-resistant MTB in recent years as the popularity of mixed infection with HIV, TB control situation is more serious, tuberculosis (TB) has become a threat to people’s life and health of all infectious disease leading killer [17, 18]. However, the detail interaction mechanism of Mycobacterium tuberculosis (MTB) with host macrophages is unclear. In our study found that MTB infection in a short time will cause more macrophages apoptosis in vitro TB model, and BCG induced host macrophages apoptosis was higher than other MTB strains in vivo TB model. It explained the consequences of mycobacterium tuberculosis infection and TB occurs or not is closely related to the host macrophages environment and infection time. These results will provide more theoretical basis for elucidating the mechanism of MTB interaction with host macrophages and prevention and control of tuberculosis.

Mcl-1 (Myeloid cell leukelma-1) gene belongs to the members of the family of the Bcl-2, Mcl-1 protein by combined with promoting apoptosis proteins Bax delay cells apoptosis, increase cell survival time to play the role of resistant to apoptosis [19, 20], Mcl-1 is the key factor of the upstream regulation of apoptosis. Researchers suggest that Mcl-1 is a new target for control and prevention Tuberculosis [7-16]. The present study found that H37Rv infected host macrophages can induce higher expression of Mcl-1, and the role of other MTB strains are relatively weak. This may be the reported that the expression level of Mcl-1 is closely related to the MTB strains removal within the host mac-
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While to Mcl-1 is the anti-apoptotic protein, so the expression level of Mcl-1 will fluctuate with the level of apoptosis. That indicates that by inhibiting Mcl-1 expression to promote host macrophage apoptosis infected MTB is a possible treatment for tuberculosis disease. These results provide a theoretical basis for the introduction of Mcl-1 to the treatment of latent infection of tuberculosis.

Mcl-1 are expressed in a variety of malignant tumor cells, high Mcl-1 expression not only inhibits tumor cell apoptosis, also increased the chemotherapy drug resistance [9, 21], reduce the Mcl-1 expression can lead to cell cycle arrest and apoptosis increase [21-23]. Mcl-1 lower expression can enhance the hypoxia induced by lung cancer cell apoptosis [23, 24]. Mcl-1 against Fas mediated apoptosis plays an important role in melanoma, the application of RNAi technology cut the Mcl-1 expression can increase cell apoptosis in the melanoma cells [24]. In recent years, studies have shown that inhibit Mcl-1 expression can effectively promote the host macrophage apoptosis, to protect against a latent MTB infection [7-16].

The study found that intervention Mcl-1 expression can significantly increase the MTB infected host macrophage apoptosis, but the effects of Mcl-1 intervention is closely related to MTB infection time. MTB infection for a short period of time, the effects of Mcl-1 intervention induced host macrophages apoptosis was weak in BCG, H37Ra, H37Rv infection group compared with other MTB strains group. However, as the time of infection increases, which host macrophage apoptosis rate were increased significantly, especially H37Ra and H37Rv infection group. These results suggest that the cell apoptosis pathways may be activated as the infection time increases. Considering that the function of the Mcl-1 is mainly involved in maintaining the stability of the mitochondrial membrane, inhibit the release of Cytochrome-c, so as to promote cell survival, prevent cell apoptosis [25]. So we speculate that intervention Mcl-1 expression for a long time, the stability of the mitochondrial membrane are destroyed, resulting in the extrinsic apoptosis pathways or the intrinsic apoptotic pathways was activated, so that the host macrophage apoptosis rate increased significantly, thus, latent infection and persistent infection of tuberculosis are controlled. These results indicate that the removal of mycobacterium tuberculosis within the host macrophages is closely related to the time of Mcl-1 intervention. Therefore, the effective use of the regulatory function of Mcl-1 intervention in mycobacterium tuberculosis infected host macrophage apoptosis might provide new ideas and targets for TB prevention and control, to speed up the pace of people against TB.

Of course, Mcl-1 may play double regulatory role or have no regulatory role on the same host macrophage in different intervention methods. Whereas the amount of studies we included this section is limited, hence the meta-analysis results did not show this inference. However, it should be noted that in the treatment of tuberculosis (TB) with Mcl-1 intervention, the dosage of intervention treatment, the intervention way and time is particularly important, an inappropriate choice may lead to an increase in the degree of infections.

To sum up, the study found that the consequences of mycobacterium tuberculosis infection and TB occurs or not is closely related to the host macrophages environment and infection time. By inhibiting Mcl-1 expression to promote host macrophage apoptosis infected MTB is a potential treatment for tuberculosis disease, but the removal of mycobacterium tuberculosis within the host macrophages is closely related to the time of Mcl-1 intervention. These results provide a theoretical basis in the Mcl-1 intervention treatment of latent tuberculosis infection and persistent infection and prevention and control Tuberculosis for our future. However, current study concluded articles exists heterogeneity, in addition to the associated with the factors of subgroup analysis, may be also related to the researchers choose the type of object of study, the type of MTB, and many other factors, but none of the existing studies on these factors to do a detailed description or part description only 1 paper, unable to conduct subgroup analysis, remains to be further research in the future.

Conclusion

As described in the present study results demonstrate that inhibiting Mcl-1 expression to promote host macrophage apoptosis infected MTB is a potential treatment for tuberculosis disease, but the removal of mycobacterium tuberculosis within the host macrophages is closely related.
related to the time of Mcl-1 intervention. These findings contribute to the latent infection and persistent infection of prevention and control Tuberculosis, and provide a theoretical basis for the implementation of new TB control strategy.

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Disclosure of conflict of interest

None.

Abbreviations

HIV, Human immuno-deficiency virus; MDR, Multi drug resistant; MDR-MTB, Multidrug resistant Mycobacterium tuberculosis; MDR-TB, Multidrug resistant tuberculosis; MTB, Mycobacterium tuberculosis.

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