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Effect of Thymoquinone on Th1 and Th2 Balance in Rats Infected with *Mycobacterium tuberculosis*

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Abstract

BACKGROUND: Mycobacterium tuberculosis (TB) could alter the Th1 and Th2 balance by stimulating phagocyte IL-1β production, and subsequent Th2 differentiation. Thymoquinone (TQ) is an active compound of Nigella sativa which has a potential immunomodulatory effect in Th1 and Th2 balance.

AIM: We aim to evaluate the effect of TQ in restoring the Th1 and Th2 balance in MTB infection.

METHODS: Four groups of rats were infected with virulent MTB strain H37Rv. Different doses of TQ (25, 50, and 75 mg/kg BW) were given to three groups, and one group was left without treatment. Additional one group was neither infected nor treated with TQ. We measure interleukin (IL)-1β, IL-4, and interferon gamma (IFN-γ) levels using ELISA 14 days after treatment.

RESULTS: We found there were increased IL-1β, IL-4, and IFN-γ levels after MTB infection, but we observed no significant effect of TQ treatment to Th1 and Th2 balance.

CONCLUSION: We conclude that TQ could not restore Th1 and Th2 balance in rats infected with MTB.

Introduction

Tuberculosis (TB) is still a major health problem worldwide. In 2018, it affects an estimated 10 million people and caused 1.2 million deaths among HIV-negative and additional 250,000 among HIV-infected patients. The global cumulative incidence reduction of only 6.3% does not meet expected 20% reduction of the 2020 milestone of end TB strategy [1].

Despite 69% TB treatment coverage, the success rate was 85% for drug-sensitive TB (DS-TB) and 56% for multidrug-resistant (MDR-TB), suggesting there is still a gap needed to be addressed. Long period of oral anti-TB (OAT) treatment often causes patients' non-adherence. Furthermore, the emergence of drug-resistant strain of *Mycobacterium TB* contributes to treatment failure to first-line OAT. The burden of MDR-TB with 18% of previously treated cases, compared to 3.4% of new cases, suggesting the failure to exterminate MTB in previous treatment is more likely to induce drug-resistant strain. However, more than 50 years of new TB drugs research has resulted only a few agents used as second-line TB treatment. Alternative treatments such as agents expected to improve patients' immune response have been sought to help TB treatment more effective.

The study showed that during MTB infection, the microorganism could alter T cell polarization by increasing Th2 cytokines through stimulation of interleukin (IL)-1β production of dendritic cells, as well as inhibiting Th1 cytokines, which results in MTB escape from elimination within macrophage [2], [3]. Thus, immunomodulators that could increase Th1 cytokines and decrease Th2 cytokines are expected to make this bacteria elimination more effective.

Thymoquinone (TQ) is an active agent of *Nigella sativa* which is traditionally used for many diseases. It has been found that this active agent could inhibit Th2 inflammation in asthma models [4], [5], [6]. However, the effect of TQ on MTB infection has never been studied.

We aimed to study the effect of TQ on Th1 and Th2 cytokines in rats infected with MTB.

Methods

Design and ethics

The animal experimental study was performed after ethical approval was obtained from the Research Ethics Commission of Brawijaya University (Approved ID: 924-KEP-UB). All rats used in this experiment were ethically killed by intramuscular injection of ketamine-xylazine cocktail.

Animals

We used two months old female *Rattus norvegicus* rats for this study. The animals were maintained at the animal facility of the Institute of Tropical Disease Universitas Airlangga, Surabaya. The rats were placed within separated cages each of five rats. Food and water were supplied adequately.

Inoculation

MTB strain H37Rv was obtained from the Institute of Tropical Disease Universitas Airlangga, Surabaya. Aliquot contained 10^5 cfu/mL. Rats were anesthetized using intramuscular injection of ketamine-xylazine 1:1.

TQ

TQ was purchased from Sigma Aldricht Co. The solution was constituted with dimethyl sulfoxide (DSMO) to make 10 mg/mL solution.

Grouping

Rats were divided into five groups, each consisted of five rats. Animals in all groups were infected by 0.2 mL of MTB strain H37Rv, except those in the negative control group.

After 3 weeks, groups 1-3 were given TQ 25, 50, and 75 mg/kg body weight (BW) respectively. The positive (group 4) and negative (group 5) control groups were treated with DSMO only.

Cytokines

Cytokines IL-1β, IL-4, and interferon gamma (IFN-γ) was measured using Rat IL-1β ELISA Kit, Rat IL-4 ELISA Kit, and Rat IFN-γ ELISA Kit (Bioassay Technology Laboratory, Shanghai, China) in Laboratorium of Parasitology, Faculty of Medicine, Universitas Brawijaya.

Results

The level of IL-1β of positive control was significantly higher than negative control (0.9 ± 0.29 vs. 0.5 ± 0.19 ng/mL, p = 0.007). After treatment with TQ, the level of IL-1β of treatment groups was not significantly different compared to that of the positive control group. However, groups treated with TQ tended to have a lower level of IL-1β compared to the positive control group, especially at dose 50 mg/kg BW (Figure 1).

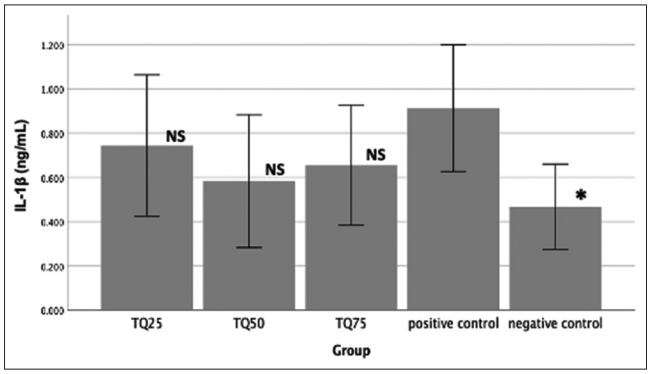

*Figure 1: Level of interleukin-1β at 14 days after treatment with Thymoquinone at doses 25, 50 and 75 mg/kg body weight. Statistical difference between positive control and other groups: *p = 0.007; NS not significant*

The level of IL-4 of positive control was significantly higher than negative control (24.6 ± 9.46 vs. 12.6 ± 5.16 ng/mL, p = 0.022). Although not significantly different, groups treated with TQ at dose 50 and 75 mg/kg BW tended to have a lower level of IL-4 than that of the positive control group (Figure 2).

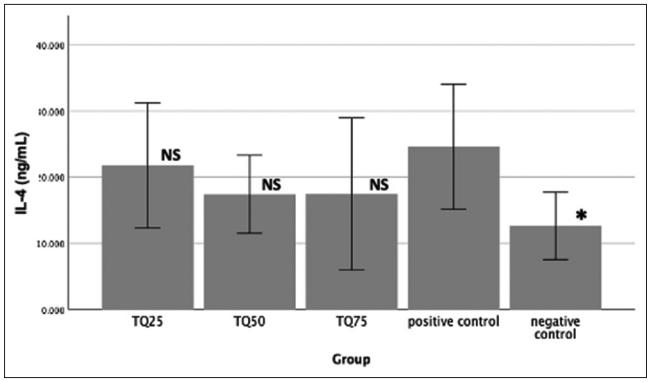

*Figure 2: Level of interleukin-4 at 14 days after treatment with Thymoquinone at doses 25, 50 and 75 mg/kg body weight. Statistical difference between positive control and other groups: *p = 0.022; NS not significant*

The level of IFN-γ of positive control was significantly higher than the negative control (24.4 ± 4.16 vs. 16.1 ± 2.50 ng/mL, p = 0.033). Similar to that of IL-4, the level of IFN-γ of groups treated with TQ at dose 50 and 75 mg/kg BW tended to be lower than that of positive control group (Figure 3).

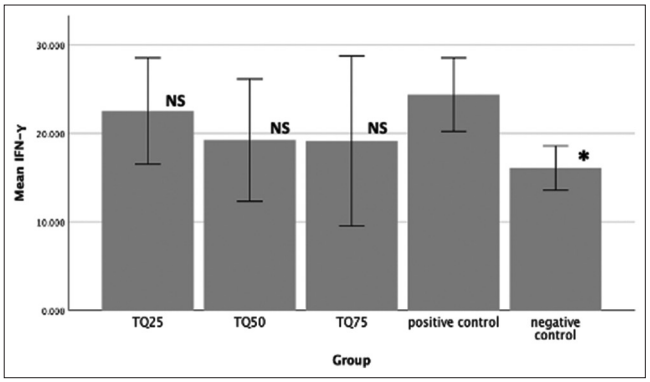

*Figure 3: Level of interferon gamma at 14 days after treatment with Thymoquinone at doses 25, 50 and 75 mg/kg body weight. Statistical difference between positive control and other groups: *p = 0.033; NS not significant*

The ratio of IFN-γ/IL-4 was not significantly different between groups. However, the ratio tended to be similar between the group treated with 75 mg/kg BW and negative control (Figure 4).

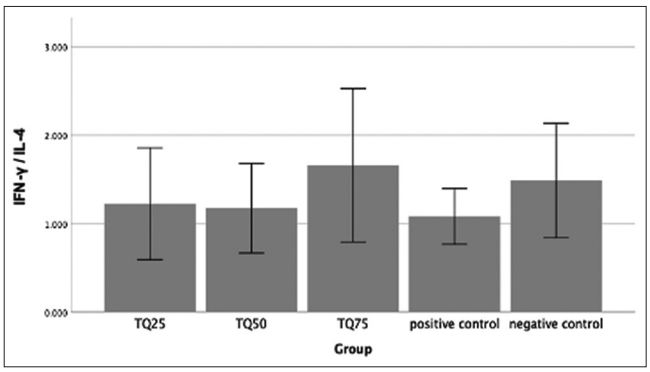

Figure 4: Ratio of interferon gamma to interleukin-4 at 14 days after treatment with Thymoquinone at doses 25, 50 and 75 mg/kg body weight. There was no statistical difference between group (p = 0.584)

The level of IL-4 had strong correlation with the level of IL-1β (r = 0.505; p < 001) and moderate correlation with the level IFN-γ (r = 0.467; p = 001) (Figure 5a and b).

Discussion

TQ is an active agent of *N. sativa*, which has traditionally been used for the treatment of various diseases. Wide studies have proven the ability of TQ to affect the immune system and this agent has been known to have antioxidative, anti-inflammatory, and immunomodulatory activities [7]. In addition to its anti-bacterial effect, TQ also has anti-tubercular activity *in vitro* [8], [9], [10]. An *in vitro* study had demonstrated TQ inhibitory effect on intracellular replication of MTB in mouse macrophage culture [11]. Furthermore, TQ has been believed to have an immunomodulatory effect on Th1 and Th2 balance [12]. So far, there is no studies have reported the effects of TQ on the pathogenesis of MTB infection.

The balance of Th1-Th2 plays important role in macrophage polarization. Macrophages polarize into M1 in response to IFN-γ released by infected macrophage as well as Th1 CD4 T-cell; and into M2 in response to Th2 cytokine such as IL-4 and IL-13 [13], [14], [15]. One study showed that MTB could alter T cell polarization by increasing Th2 cytokines through stimulation of IL-1β production of dendritic cells, as well as inhibiting Th1 cytokines, which results in MTB escape from elimination within macrophage [2]. Other study revealed the role of increased SOCS3 produced by macrophages infected with MTB [16].

TQ has been known as potential immunomodulator in several diseases. Studies had shown that TQ could modulate Th1 and Th2 balance in ovalbumin sensitized guinea pig [4], [5], [6]. The study by Xuan *et al.* in 2010 showed that TQ inhibits LPS-induced differentiation and maturation, as well as cytokine release of DC cultured from mice bone marrow [17]. Hence, there is a possibility that treatment with TQ could decrease IL-4 levels, through inhibition of IL-1β by dendritic cells, and restore the balance toward Th1.

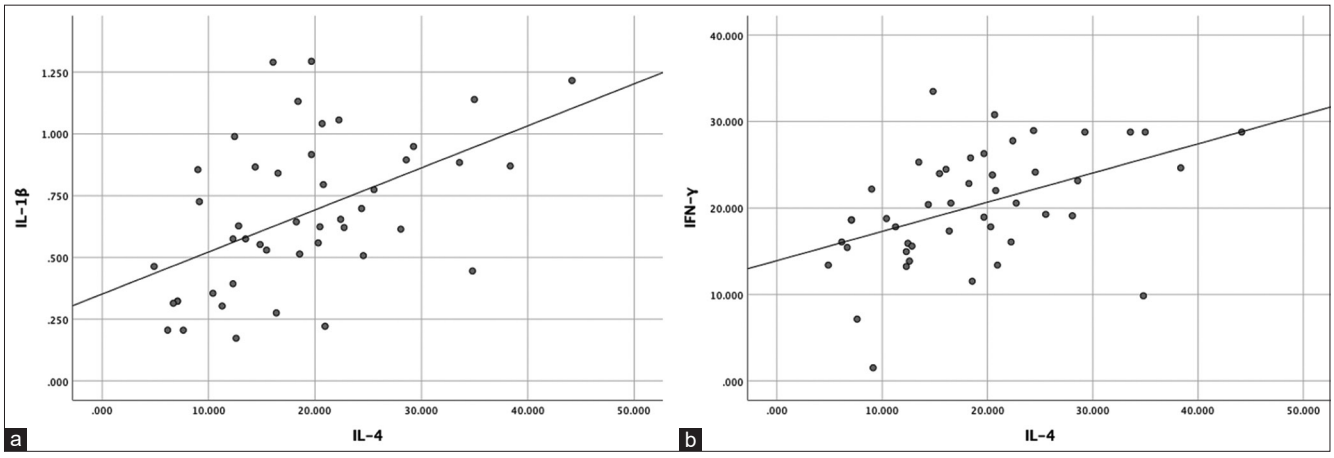

Figure 5: The correlation between interleukin (IL)-4 and IL-1β level. There was positive correlation (r = 0.505; p < 001) between IL-4 level and IL-1β (a); the correlation between IL-4 level and interferon gamma (IFN-γ). There was positive correlation (r = 0.467; p = 001) between IL-4 level and IFN-γ (b)

To the best of our knowledge, this study was the first to study the effect of TQ on TB disease. TQ was expected to restore Th1 and Th2 balance by inhibiting the production of IL-1β and IL-4, and increasing the ratio of IFN-γ/IL-4 in rats infected with MTB.

In this study, we found IL-1β level increase after infection of MTB. Upon virulent MTB strain H37Rv infection, copious amount of IL-1β is secreted by macrophage which is independent to the caspase-1 pathway and by dendritic cells, in which RD-1 antigen of MTB plays an important role [2], [18]. We found that the increase of IL-4 level was correlated with that of IL-1β level. This suggests that the increase of IL-1β, which might be produced by macrophage and dendritic cells after the infection of MTB, would affect the differentiation of CD4 toward Th2 cells. This finding is in concordant to the work of Dwivedi *et al.*, which found that MTB infection could alter the balance toward Th2 differentiation dependent to IL-1β produced by dendritic cells [2].

We found in this study that the increase of IFN-γ level following MTB infection. This cytokine is produced by activated macrophages upon phagocytosis of MTB. As inflammation progress and adaptive immune response is involved, IFN-γ also secreted by Th1 $CD4^+$ cell and play important role in sustaining macrophage activation last longer [19], [20].

Although our study did not demonstrate a significant difference in IL-1β, IL-4 and IFN-γ levels after TQ treatment of MTB infected rats, there was a tendency of decreased IL-1β and IL-4 levels in groups treated with TQ at dose of 50 mg/kg BW. Likewise, an increase of IFN-γ/IL-4 ratio tended to increase in groups treated with TQ at a dose of 75 mg/kg BW. However, we demonstrate that the IL-4 level was correlated to the IL-1β level.

Our finding was not in accordance with the results of studies which showed decreased IL-4 in sensitized allergic models treated with TQ [4], [6], [17]. The study by Miliani *et al.*, 2018, showed increased production of IFN-γ and IFN-γ/IL-4 ratio in necrotic Jurkat T cell line lysates-pulsed macrophage co-culture [21]. It seems that in infection models, particularly MTB infection, the alteration of Th1-Th2 balance is not as affected as in allergic disease by TQ treatment. Indeed, in this study, the IFN-γ level was elevated in line with increase of IL-4 level. However, further research is required to explore the higher safe dose and longer duration of treatment, as well as its combination with standard anti TB drugs.

Our study had several limitations. We did not explore the serial observations of these cytokines before day-14 of TQ treatment, which may pose a dynamics concentration. Likewise, a longer duration of treatment may reveal different findings concerning these Th1 and Th2 cytokines. Second, we did not explore the effect of TQ treatment to MTB load in lung tissue, which may have been beneficial despite unchanged Th1-Th2 cytokine afer TQ treatment.

Conclusion

We demonstrate the increase of IL-1β, IL-4, and IFN-γ levels of rats infected with MTB. Treatment with TQ could not restore Th1 and Th2 balance. However, there is a chance to evaluate the potential of TQ as adjunctive treatment in TB.

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Authors' Contributions

E.O; A.T.E; S.S; H.M.S.C.: Study concept and design. E.O.: Acquisition of data. E.O; A.T.E.: Analysis and interpretation of data. E.O.: Drafting of the manuscript. A.T.E; S.S; H.M.S.C; K.H.: Critical revision of the manuscript for important intellectual content. E.O.: Statistical analysis.

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