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The Role of Zinc Supplementation in The Lung Tissue Damage of Tuberculosis Rats

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KEYWORDS

Perivasculitis
Granuloma
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Abstract Infection by *Mycobacterium tuberculosis* (Mtb) is a chronic contagious disease with a high mortality rate in various parts of the world, especially in developing countries with high poverty rates. The objective of the present study was to examine the effects of zinc supplementation on the lung tissue damage of the male tuberculosis rats. This study was conducted based on true experimental design, using a post-test control group design. Peribronchiolitis was found to have a higher rank in (K0, K1, K2) groups compared to the Mtb group treated with the addition of Zinc supplements (P1, P2, P3). Zinc supplementation in this study shows that there is a reduction in lung organ damage and having a positive impact on increasing the perivasculitis and granuloma. Zinc supplementation by its optimal dose of 50 mg/kg.bb/day given as additional nutrition in Mtb rats.

Introduction

Infection by *Mycobacterium tuberculosis* (Mtb) is a chronic contagious disease with a high mortality rate in various parts of the world, especially in developing countries with high poverty rates. Indonesia, as one of the largest developing countries in the world, found around 1.3 million cases of tuberculosis occurred in 2012 (Sulis *et al*., 2014), and around 8.6 million new cases in the last 10 years. Mortality in tuberculosis cases in 2016 reached 1,020,000 cases with an average of 391 cases per 100,000 population (WHO, 2016).

Nutritional status plays a role in determining the outcome of Mtb patients. Mtb patients with malnutrition are associated with delayed healing, increased mortality, risk of recurrence. One important component in nutrient intake is the content of micronutrients. Micronutrients are important elements for human health. Secondary immunodeficiency is caused by deficiencies in micronutrients, vitamins and trace metals. Trace metals play an important role in the metabolic pathway, cellular function and immune response (Kant *et al.,* 2016). Trace metal is needed for the defense of Mycobacterium tuberculosis in macrophages. Mycobacterium tuberculosis has the ability to take trace metals for defense and replication in macrophages. The host immune system responds by increasing trace metal levels, one of which is Zn, in phagosomes and trying to kill Mycobacterium tuberculosis with trace metal intoxication.

Zinc plays a role in the synthesis of nucleic acids (DNA), lymphocyte differentiation and macrophage potentiation. Mtb infection results in redistribution of zinc from plasma to tissues due to decreased production of macroglobulin-cr2 protein which plays a role in the circulation of zinc in the blood. Zinc deficiency decreases the action of phagocytosis macrophage and the number of T cell in blood serum. Zinc levels in plasma decrease during the intensive phase of OAT. The incident was associated with the use of zinc by macrophages to kill Mtb, increased absorption of zinc into tissues and elimination of zinc through urine by ethambutol (Pratomo *et al.,* 2012).

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Zinc acts as a membrane stabilizer, inhibits the enzyme nicotinamide adenine dinucleotide phosphate oxidase, a pro-oxidant enzyme and triggers the synthesis of metallothionein which decreases hydroxyl radicals and cuts the reactive oxygen species created during oxidative stress (Marreiro *et al.,* 2017).

Nutritional supplementation is expected to be a new approach in order to cure Mtb patients faster. Improving the nutritional status of the population is also expected to be an effective measure to control Mtb infection, especially in countries with a high tuberculosis prevalence. This study was conducted to obtain a solution for tuberculosis treatment that no longer focuses on the use of antibiotics, but uses immuno-stimulants by investigating the effect of zinc supplementation on lung organ damage in tuberculosis-infected mice.

Materials and Methods

This study was conducted based on true experimental design, using a post-test control group design with the subjects were thirty male Wistar (*Rattus norvegicus*) rats aged 2-3 months with initial body weight 200-350 gram, obtained from the PUSVETNA, Surabaya, East Java, Indonesia. Dependent variables in this study are levels of damage in the lung tissue of the rats after infected by *Mycobacterium tuberculosis* and control variables in this study are temperature, humidity, close body, age, physical activity, and stress.

The induction of tuberculosis rats refers to previous studies (Mustika *et al*., 2014; Rodrigues *et al*., 2009; Heng *et al*., 2011; Kumar *et al*., 2014). Mice were infected with *Mycobcterium tuberculosis* through the trachea. The rat was anesthetized before being infected with *Mycobacterium tuberculosis*. Anesthesia is performed by injection with ketamine HCl 50 mg/kg weight and Xylazine 0.2 ml subcutaneously. The mice were fixed supine and incised in the median line of the cervical area. *Mycobacterium tuberculosis* dose was 10^8/ml injected into the trachea using tuberculin needle in vertical position, then the wound sutured. Rats were returned to the cage and then given standard feed.

After the mice were adapted 7 days before treatment, 5 rats were set as a negative/healthy control group (K0), and the remaining 30 mice were infected with *Mycobacterium tuberculosis* H37Rv and waited for 29 days. Rats were divided into 6 groups randomly, each group consists of five rats. K0 was negative control group, K1 mice were injected with Mtb H37 Rv, K2 were positive control group which received INH (6 mg/day) and rifampicin (10 mg/day) P1, received INH, rifampicin and zinc (50 mg/kg.bw/day) P2 were treated, INH, rifampicin and zinc 100 mg/kg.bw/day, and P3 were treated INH, rifampicin and zinc 200 mg/kg.bw/day. Groups K0 and K1 were sacrificed on the 30th day to prove that K1 had been positively infected with TB.

Lung organs were preserved to conduct a histopathological preparations by Hematoxin-Eosin staining to determine the degree of lung tissue damage. Lung organs were fixed with 10% formaldehyde solution in PBS for 2 days then immersed in paraffin and cut as thick as 5µm, carried out along the widest area of each lobe, so that all lung tissue examined was the same area. Observations were made in five fields of view for each lung tissue preparation.

The score used to assess rat lung tissue damage in this study uses the Dormans score (Table 1). Damage assessment based on histopathological parameters: peribronchiolitis, perivasculitis, alveolitis, and granuloma formation. Peribronchiolitis is inflammation of the bronchioli. Perivasculitis is a condition in blood vessels. Alveolitis is inflammation of the alveoli tissue. Granuloma is a cellular aggregate which is a typical sign of tuberculosis infection (Dormans, 2004).

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Table 1. Dormans Score of Lung Damage (Dormans *et al*., 2004)

Score	Description
0	There are no inflammatory cells
1	At a minimum, there are predominantly polymorphonuclear inflammatory cells
2	Mild, mononuclear dominant inflammatory cells are present at thickness <5 µm
3	Medium, there are dominant mononuclear inflammatory cells at a thickness of 5-10 µm
4	Clear, there are mononuclear dominant inflammatory cells at thickness> 10 µm
5	Severe, there are many inflammatory cells and endothelial damage

Ethical Clearance

This experimental design has fulfilled and approved by the Ethics Committee of Faculty of Medicine, Wijaya Kusuma University, Surabaya, Indonesia, by registered number: 21/SLE/FK/UWKS/2020.

Statistical analysis

The results of the histopathological examination on lung tissue from each treatment group were assessed by scores from Dorman to assess the lung damage. The scores from Dorman were based on histopathological parameters as follows: Perialveolitis, peribronchiolitis, perivasculitis and granuloma. Data analysis were analyzed using SPSS v16.0 by Kruskal Walis test followed by PosHoc-Mann Whitney test, value was given as mean rank ± SD, considered significant if p-value <0.05 (P ≤0.05).

Results and Discussion

Histological observations on lung tissue, based on Dorman scores, showed that for each type of damage, there were several categories of lung tissue damage that appeared to be reduced in the group given treatment in the form of Zinc supplementation compared with controls (Figure 1 & 2).

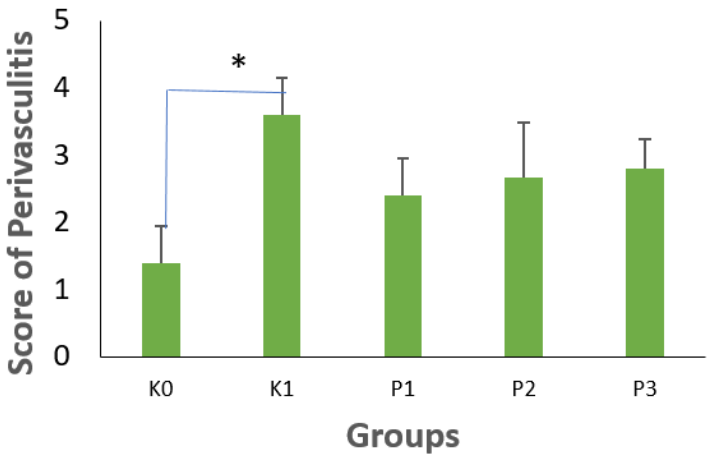

Figure 1. K0 was negative control group, K1 mice were injected with Mtb H37 Rv, K2 were received INH (6 mg/day) + rifampicin (10 mg/day) P1 were received INH + rifampicin + zinc (50 mg/kg. BW/day) P2 were received, INH + rifampicin + zinc 100 mg/kg.BW/day + P3 were treated INH + rifampicin + zinc 200 mg/kg.BW/day+

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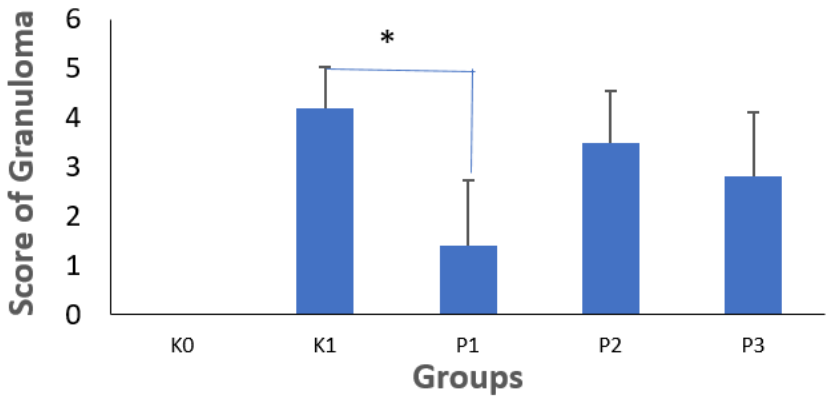

Figure 2. K0 was negative control group, K1 mice were injected with Mtb H37 Rv, K2 were received INH (6 mg/day) + rifampicin (10 mg/day) P1 were received INH + rifampicin + zinc (50 mg/kg. BW/day) P2 were received, INH + rifampicin + zinc 100 mg/kg.BW/day + P3 were treated INH + rifampicin + zinc 200 mg/kg.BW/day+

Peribronchiolitis was found to have a higher rank in the control group (K0, K1, K2) compared to the Mtb group treated with the addition of Zinc supplements (P1, P2, P3). Meanwhile, perivascultis was found to be increased in the Mtb group treated with the addition of Zinc supplements, compared to the control group. The highest mean rank was shown by the condition of granulomas in the Mtb group treated with the addition of Zinc supplements, while the lowest was found in granulomas in the control group. Based on the Kruskal-Wallis Mean Rank Test (Table 2), it is known that there is a relationship between treatment groups with the type of damage that occurs in lung tissue. The control group was known to have a significant difference in the degree of lung organ damage compared to each type of damage observed. P2 with the lowest dose of Zinc supplementation, 50 mg/kg.bw/day has the most significant results compared to other higher doses. Thus, in this study it is known that the optimal dose of Zinc supplementation to be given as additional nutrition in Mtb rats is 50 mg/kg.bw/day.

Zinc is one of the trace elements with various biological activities, including antioxidant activity, cellular metabolism, and as a component of protein involved in cell structure. Zinc also plays an important role in immune function, protein and DNA synthesis, cell division, and energy metabolism and growth (Bonaventura *et al.,* 2015; Yasuno *et al.,* 2011). Research by Karyadi *et al.* (2002) proved the effect of supplementation of vitamin A and zinc on sputum conversion and and changes in lung lesions of pulmonary tuberculosis patients.

Increased levels of zinc in the airway lumen, through the mechanism of release from airway epithelial cells or from the bloodstream triggers the entry of zinc into macrophages or other immune cells. This contributes to the anti-inflammatory function of these cells. A number of genes related to defense have also been identified to be responsive to zinc including cytokine receptors and genes related to Th1 immune responses (Prasad., 2014).

Zinc deficiency causes interference with interferon-fungsi function. These cytokines encourage activation of macrophages, nitric oxide production and proliferation of cytotoxic T-cells that cause phagocytosis and destruction of microbial pathogens (Hojyo and Fukada, 2016). Decreased zinc levels result in decreased proliferation and differentiation of B and T lymphocytes. In zinc deficiency, thymic atrophy occurs with a decrease in double positive lymphocytes and a decrease in mature T cells. Zinc deficiency also causes a decrease in antigen-specific B and IgG1 cell populations.

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This is due to a decrease in zinc levels causing a decrease in B cell maturation regulation through the Z1P10 pathway. In B cell formation, cytokines induce JAK-STAT activation which is converted into intracellular Zn signaling through the Z1P10 pathway. This triggers the maturation of B cells through inhibition of caspase activation (Hojyo and Fukada, 2016).

Damage that occurs in the lungs, one of which is caused by granuloma and perivasculitis production increased in patients with Mtb. It has been reported that granuloma and perivasculitis is also detected during infections caused by intracellular bacteria such as *Mycobacterium tuberculosis, L. monocytogenes and S. typhimurium*. (Jin and Dong, 2013) .

An excessive increase in granuloma and perivasculitis can cause severe tissue damage. Therefore, in the case of handling *Mycobacterium tuberculosis* as intracellular bacteria that can live in phagocytic cells, the role of granuloma and perivasculitis is needed early during pathogen infection because indirectly these cytokines can function as protectors, but when chronic conditions cause excessive increase in granuloma and perivasculitis can cause immunopathology. The significant increase in granuloma and perivasculitis in MDR-TB conditions increases the severity of tissue damage associated with the low effectiveness of this second-line drug used in treatment, and this is associated with a high antigen load.

Zinc supplementation in this study shows that there is a reduction in lung organ damage, which can be caused by a decrease in oxidative stress, and suppression of proinflammatory cytokine levels due to immune system modulation that results in clearing of the Mtb bacteria to improve in the host body. Previous studies have shown that zinc levels decrease in tuberculosis patients compared to healthy subjects. Administration of zinc in human research subjects aged 20-50 years decreases the concentration of MDA (Malondialdehyde), 4-hydroxy alkenal (HAE) and 8-hydroxy deoxyguanine in plasma. Zinc is also used by the immune system to damage bacteria, including tubercle bacilli (Stensland *et al.,* 2015).

Conclusion

Zinc supplementation in this study shows that there is a reduction in lung organ damage and having a positive impact on increasing the perivasculitis and granuloma. Zinc supplementation by its optimal dose of 50 mg/kg.bw/day given as additional nutrition in Mtb rats.

References

Bonaventura P, Benedetti G, Albarède F, Miossec P. 2015. Zinc and its role in immunity and infammation. Autoimmunity Review, 14, 277–285.

Dormans J., Burger M., Aguilar D., Hernandez-Pando R., Kremer K., Roholl P., Arend S. M., Van Soolingen D. 2004. Correlation of viru-lence, lung pathology, bacterial load and de-layed type hypersensitivity responses afterinfection with different M. tuberculosis geno-types in a BALB/c mouse model. Clin. ExpImmunol., 137(3), 460–468.

Heng Y. S, Seah P. G, Siew J. Y, Tay H. C, Singhal A, Vanessa M, Herve M. (2011). MycobacteriumtuberculosisInfectionIndu cesHypoxic Lung Lessions in The Rat. Tuberculosis, 91, 339-341.

Hojyo, S. & Fukada, T. 2016. Roles of zinc signaling in the immune system. *Journal of immunology research,* 10(1155), 1-22.

Jin W., Dong C. (2013). Review: IL-17 Cytokines in Immunity and Inflammation. Journal Emerging Microbes and Infection, 2013(2), 1-5.

E-ISSN 2355-9926

Kant, S., Gupta, H., & Ahluwalia, S. 2016. Significance of nutrion in pulmonary tuberculosis. *Critical reviews in food science and nutrition,* 66, 955-963.

Karyadi E, West C. E., Schultink W., Nelwan W. H., Gross R., Amin Z., Dolmans W. M. V., Schlebush H., vanderMeer J. W. M. 2002. A double-blind, placebo-controlled study of vitamin A and zinc supplementation in persons with tuberculosis in Indonesia: effects on clinical response and nutritional status. Am J ClinNutr., 75(4), 720-7.

Kumar, V., Abbas, A. K. & Aster, J. C., 2015. Disease of Immune System. Dalam: *Robbins and Cotran Pathologic Basis of Disease.* Philadelphia: Elsevier Saunders, pp. 185-265.

Marreiro D. D. N. et al., 2017. Zinc and oxidative stress : current mechanism. *Antioxidants,* 6(2), 24.

Mustika A., Rahaju A. S, Indrawati R. 2014. Penurunan kerusakan jaringan paru terinfeksi tuberkulosis oleh ekstrak pegagan melalui peningkatan ekspresi *tissue inhibitor of matrix metalloproteinase-1*. Jurnal Veteriner., 15(4), 530-540.

Prasad, A. S., 2014. Zinc is an antioxidant and anti-inflammatory agent : its role on human health. *Frontiers in nutrition,* 1, 14.

Pratomo IP, Burhan E, Tambunan V. 2012. Malnutrisi dan Tuberkulosis. J. Indon. Med. Assoc., 62(2).

Rodrigues M.F, Barsante M.M, Alves C.C.S, Souza M.A. 2009. Apoptosis macrophage during pulmonary mycobacterium bovis infection: correlated with intracellular bacillary load and cytokine levels. Immunol, 128, e691-e69.

Stensland I , Kim J.C , Bowring B , Collins A.M , Mansfield J.P , Pluske J.R. A com-parison of diets supplemented with a feed additive containing organic acids, cinnamaldehyde and a permeabilizing complex, orzincoxide, on post-weaning diarrhoea, selected bacterial populations, blood measures and performance in weaned pigs experimentally infected with enterotoxigenic E. coli. Animals, 2015(5). 1147–68.

Sulis, G., Roggi, A., Matteelli, A. & Raviglione, M. C., 2014. Tuberculosis : Epidemiology and Control. *Mediterraenian Journal of Hematology and Infectious Diseases.*

World Health Organization (WHO). 2016. WHO report 2010: Global Tuberculosis Control.

Yasuno T, Okamoto H, Nagai M, Kimura S, Yamamoto T, Nagano K, Furubayashi T, Yoshikawa Y, Yasui H, Katsumi H, Sakane T, Yamamoto A. 2011. The disposition and intestinal absorption of zinc in rats. European Journal of Pharmaceutical Science, 44(3), 410–415.

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