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Original paper

Curcumin Effects in Inducing Vitamin D Receptor (VDR) and Inhibiting of *Salmonella typhi* Growth *in vivo*

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Abstract

Background and aim: Curcumin has an antimicrobial effect, presumably due to its ability to bind vitamin D receptors (VDR). This study aimed to investigate the curcumin effects in inducing VDR and inhibiting of *Salmonella typhi* growth *in vivo.*

Materials and methods: Mice were divided into: group I (curcumin 200 mg/kg/bw), group II (curcumin 400 mg/kg b.w), positive control and negative control. The intervention was carried out for 5 days. After the fifth day, mice were maintained for 3 weeks to determine the amount of colony growth in the post-intervention period and examination of level VDR in serum.

Results: In group with curcumin 200 mg/kg/bw there was a decreased in average number of colonies of 23.60 CFU/ml ($p<0.001$). The decline in the average number of colonies was also present in the intervention group with curcumin 400 mg/kg/bw at 17.20 CFU/ml ($p<0.000$). Serum levels of VDR significantly increased five days after treatment with curcumin (200 and 400 mg/kg bw) $p<0.018$ and $p<0.002$, respectively).

Conclusion: Curcumin (200 and 400 mg/kg/bw) significantly increases serum levels of vitamin D receptors and inhibit the growth of S. Typhi colony. Antimicrobial component curcumin as a potential agent in adjuvant therapy for increasing immunity as well as a therapeutic alternative other than antibiotics in treating typhoid fever.

Keywords: *Curcumin, VDR, Salmonella, Colony count*

Introduction

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Typhoid fever is an acute systemic infection caused by *Salmonella Typhi* (*S. Typhi*). Typhoid fever is characterized by a prolonged heat wave followed by bacteremia and bacterial invasion of *Salmonella typhi* well as multiplication into mononuclear phagocytic cells of the liver, spleen, lymph nodes and intestinal Peyer's patches [1], Typhoid fever occurs worldwide, especially in developing countries with poor sanitation. Eighty percent of cases of typhoid in the world comes from Bangladesh, China, India, Indonesia, Laos, Nepal, Pakistan. Typhoid fever each year infects 21.6 million people (3.6 / 1,000 population) with a mortality rate of 200,000 / year. The incidence of typhoid fever is high (> 100 cases per 10,000 population per year) are recorded in central Asia, south Asia, south east Asia, Africa, Latin America, and Oceania (except Australia and New Zealand) and that includes low (<10 cases per 10,000 population per year) in the rest of the world [2], [3].

The first antibiotic to treat typhoid fever is chloramphenicol, used in 1948 and then became the treatment of choice to three decades in addition to ampicillin and trimetoprimsulfametoksazol [4][5]. The first report on *S. Typhi* resistance to chloramphenicol in 1974 [6]. Twenty years later reportedly *S. Typhi* resistance to chloramphenicol, ampicillin, and trimetoprimsulfametoksazol, otherwise known as MDR (multiple drug resistance) of *S. Typhi* [7], Currently *S.Thypi* increased resistance to second-line therapy is the 3rd generation cephalosporin and quinolone class have also been widely reported [8] - [11]. Drug resistance in typhoid fever is a serious thing, because it takes a fairly expensive replacement drug for the treatment of typhoid. A serious effort is required by the medical service to get a correct diagnosis so that treatment or vaccination can be used to control the spread of drug resistance this typhoid [12].

Curcumin has anti-inflammatory, anti-cancer, and anti-microbial. Anti-microbial mechanisms of curcumin correlated with its ability to bind to the vitamin D receptor (VDR) as a potential ligand. This condition increases the expression of cathelicidin antimicrobial peptide (CAMP) and eradicate the bacteria. Additionally, curcumin can enhance the mRNA expression of CAMP; so as to increase the levels of cathelicidin in the network [13], Cathelicidin is a small peptide that has a structural similarity with other antimicrobial proteins, such as defensins. Cathelicidin has the ability to eradicate Gram positive and Gram-negative bacteria and also some fungi and parasites. Cathelicidin infiltrating the bacterial membrane to alter the integrity of the membrane, but some bacteria are known to have intrinsic resistance to cathelicidin. Bacteria such as *Enterococcus faecalis*, *Streptococcus pyogenes*, and *Proteus mirabilis*, can synthesize certain protease enzymes which can destroy cathelicidin [14].

Given the high morbidity rate of typhoid and the growing cases of relapse and resistance careers or *S. Typhi* bacteria, then attempt to combine the antimicrobial agent and herbal remedies have become the topic of the latest trends. Adjuvant therapy can be applied by patients with typhoid fever, which can reduce the risk of antibiotic resistance, especially in the case of relapse. So this study to prove the effect of curcumin on the growth of bacterial colonization of *S. Typhi* mediated by the production of Vitamin D receptors.

Materials and Methods

This research is true experimental pre-post test design with to see the effectiveness curcumin induces in Vitamin D receptor and suppresses the growth of bacteria in the peritoneal fluid of *S. Typhi* strain male mice balb / c.

Curcumin

Curcumin in this research was purcashed from Merck (Curcumin for synthesis, with chemical formula [4- (OH) -3- (CH_3O) C_6H_3CH = $CHCO]_2CH_2$, This IS ALSO known as 1,7-Bis (4-

hydroxy-3-Methoxyphenyl) -1.6-heptadiene-3,5-dione, turmeric yellow, diferuloylmethane.The curcumin dose per day given was 200 mg / kg bw and 400 mg / kg bw Curcumin was given 3 days after induction by *S. Typhi*. Curcumin was dissolved in distilled water and given through the nasogastric sonde, once a day for 5 days.

Experimental animals

Balb / c mice (aged 8-12 weeks, weighing 30-40 grams; n = 25) were maintained in theMolecular Biology and Immunology Laboratory, Microbiology Department Faculty of Medicine, Hasanuddin University (Makassar, Indonesia). The mice were acclimatized for 8 days, then divided into five groups (n = 5). All groups were intraperitoneally with S. Typhi strains induced thy1 (3 ml x 103 ml / CFU). All mices were designated into five groups; CM200 (S.typhi group with curcumin induced a dose of 200 mg / kg bw), CM400 (S.typhi group with curcumin induced a dose of 400 mg / kg bw), postive control group (induced S.typhi group with antibiotic levofloxacin dose of 1.95 mg / kg bw) and negative control group (induced S.typhi group with placebo).

Levofloxacin

Levofloxacin was obtained from Kimia Farma, Pharmaceutical, Indonesia. Dose 750 mg of Levofloxacin given to mice was obtained from the multiplication with a conversion factor of 0.0026. Based on the result, the positive control group was given a dose of Levofloxacin 1.95 mg / day. Antibiotics was dissolved in distilled water and given through the nasogastric sonde, once a day for five days.

Vitamin D Receptor examination

Blood samples from all groups for determination of serum vitamin D receptor were collected. The examination was done using mouse VDR ELISA Kit (Sandwich ELISA), purchased from LSBio.

Sampling of peritoneal fluid and bacterial colony examination

Mice were fixed in the supine position, the abdomen is cleaned with alcohol 70% and as much as 0.8-1 mL saline was injected into the peritoneal cavity. Mice were then allowed to stand for 1 minute as he rocked slowly. Peritoneal fluid removed from the peritoneal cavity of mice supine position, then fluid aspirated by as much as 0.5 mL syringe. Peritoneal fluid retrieval is performed three times, that is when the fourth day after the mice induced germ S. Typhi (4^{th} day), the last day administration of the intervention (10^{th} day) and 3 weeks after the intervention (30^{th} day).

Examination of bacterial colonization by using the pour plate method. This method is performed by diluting the peritoneal fluid samples of 0.5 mL in 4.5 mL of saline (0.9% NaCl). Dilution is done three times so that the culture obtained is not too dense or fulfill cup (culture too dense will interfere with observations). Approximately 1 ml of the suspension was poured into a sterile petri dish, followed by pouring the fertilizer medium (nutrient agar) sterile warm (45^0C) then sealed and incubated for 1-2 days at a temperature of 37^0C.

Statistical analysis

Data are Expressed as the mean ± SE. To assess differences in levels of VDR and bacterial colony count between groups, paired T test was employed using the SPSS 23 software. Considered as statistical significance was $P < 0.05$.

Ethics statement

This study was approved by the Health Medical Research Ethics Committee at the Faculty of Medicine, Hasanuddin University (Makassar, Indonesia) with registration number 901 / H4.8.4.5.31 / PP36-KOMETIK / 2018 on October 31, 2018.

Results

To determine the effects of curcumin on the amount of bacterial colonization *S. typhi* used paired T test to assess the dynamics of change in the number of colonies in respect of changes in the observation time for each group. Summary results can be seen in table 1.

Table 1. Differences between the dynamics of bacterial colonies enumerated before the intervention group (4th day) after intervention (10th day) and 3 weeks after the intervention (30th day).

Groups	Calculation of Bacterial Colonization S.typhi (CFU / ml)					
	4th day	10th day	*P value*	10th day	30th day	*P value*
CM200	26.80± 3.70	3:20± 3:11	0001	3:20± 3:11	0:20±0:48	0113
CM400	17:20± 2:28	0:00± 0:00	0000	0:00± 0:00	0.80± 1:30	0242
positive control	22:00± 6:16	1:20± 1.79	0003	1:20± 1.79	0:00± 0:00	0208
negative control	21.80± 6419	6:00± 2,282	0013	6:00± 2,282	1:20± 0.583	0014

* Values are mean± SD n = 5, P-value of <0.05 is significant

In table 1, it can be seen there are differences between the mean number of bacteria colonies significantly in all groups between before intervention after intervention. In the intervention group with curcumin 200 mg / kg bw, decreased the average number of colonies of 23.60 CFU / ml (*p value* 0.001). The decline in the average number of colonies are also present in the intervention group with curcumin 400 mg / kg bw at 17:20 CFU / ml (*p value* 0.000). However on the 30th day of observation, curcumin group did not provide differences between the mean number of colonies to be meaningful (Figure 1).

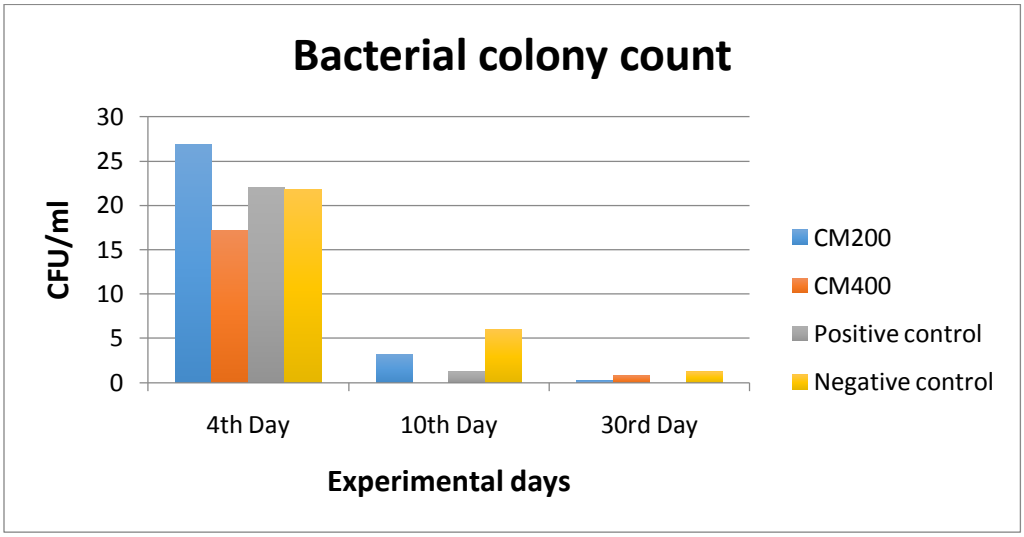

Fig. 1. Dynamics of changes in the number of bacterial colonization of *S. Typhi* by the group during the observation period.

To determine the effects of curcumin on serum levels of Vitamin D receptors are used paired T test to assess the dynamics of change in the number of colonies in respect of changes in the observation time for each group. Summary results can be seen in table 2.

Table 2. Differences in the levels of Vitamin D Receptor dynamics between groups at baseline, prior to the intervention (4th day) after intervention (10th day) and 3 weeks after the intervention (30th day).

Groups	Levels of Vitamin D Receptor (VDR) (ng / ml)								
	Baseline	**4th day**	***P value***	**4th day**	**10th day**	***P value***	**10th day**	**30th day**	***P value***
CM200	4:31± 1.78	8:34± 1.79	0016	8:34± 1.79	9:22± 2:04	0018	9:22± 2:04	9.88± 1.99	0023
CM400	4.69± 2:16	7:25± 1:01	0020	7:25± 1:01	10.95± 1.77	0002	10.95± 1.77	12:09± 1.66	0003
positive control	2.94± 1:37	6:55± 1:44	0023	6:55± 1:44	15:25± 1:47	0002	15:25± 1:47	16.63± 1:48	0002
Negative control	4.71± 1.89	7.68± 1.92	0000	7.68± 1.92	7:53± 1.60	0815	7:53± 1.60	9:25± 1:55	0033

* Values are mean± SD n = 5, P-value of <0.05 is significant

In table and figure 2 it can be seen that differences between serum levels of Vitamin D receptors are significant in all groups before and after intervention and on the 30th day of observation.

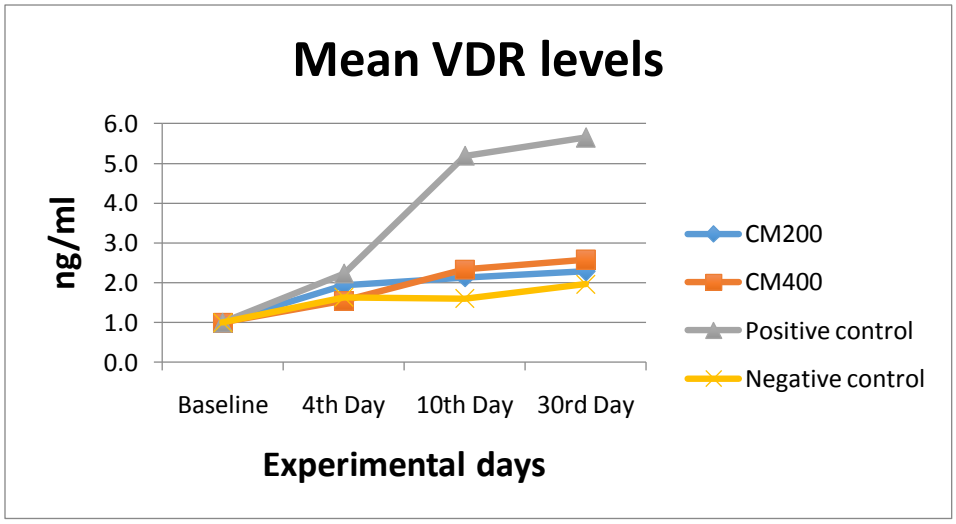

Fig. 2. Dynamics of changes in levels of VDR by the group during the observation period.

Discussion

After entering the body, the bacteria *S. Typhi* will head the digestive tract, especially the ileum, and penetrate blood vessels to spread systemically through the circulation [15], [16]. Most bacteria are killed by stomach acid, so that the bacteria so that a large amount needed to reach the intestine and can provide clinical manifestations [16].

Curcumin is the active compound that is widely known as an anti-inflammatory, anticancer, and recently, antimicrobial. Guo et al research results show that the antimicrobial mechanism of curcumin through its ability to bind to the vitamin D receptor (VDR) as a potential ligand. Curcumin and VDR bonds will trigger the formation of cathelicidin antimicrobial peptide (CAMP) and then it will kill the bacteria. Additionally, curcumin can enhance the mRNA expression of CAMP; so as to increase the levels of cathelicidin in the network [13], Is a small peptide cathelicidin with some structural similarities with other antimicrobial proteins, such as

defensins. Cathelidicin has a broad antibacterial spectrum against Gram-positive and Gram-negative as well as against fungi and parasites. Cathelididin damage bacterial membranes by altering the membrane integrity, although some bacteria are known to have resistance to cathelicidin. Among other types of bacteria Enterococcus faecalis, Streptococcus pyogenes, Salmonella enterica, and Proteus mirabilis, can synthesize specific proteinase to degrade ekspersi cathelicidin[17]. This study proved that curcumin still have a role as an antimicrobial against *S. Typhi*. After therapy for five days with curcumin dose of 200 mg / kg bw (mean difference± SD; 23.60 ± 6:27, p-value 0.001) and 400 mg / kg bw (mean difference± SD; 17:20 ± 2:28 p-value 0.000), Found a decrease in the average number of bacterial colonies on mice meaningful intraperitoneal fluid.

Vitamin D has antiproliferative and antineoplastic affinity, including apoptosis, inhibition of cell cycle proliferation, induction of differentiation, inhibition of invasion and mortality as well as the reduction of angiogenesis. These activities using genomics and nongenomik pathways mediated by receptors and vitamin D receptors (VDR). Vitamin D receptors are expressed in large amounts in the tumor tissue and the infected cells. Recent research has shown VDR and enzymes involved in the metabolism of vitamin D have damage to the VDR signaling pathways. VDR expression by immune cells suggests that vitamin D affects the immune system function. More than 30 different body tissues such as the brain, liver and pancreas, lymphatic, skin, gonads and prostate consists of cells including T and B lymphocytes that express the VDR. Vitamin D receptor binds to the 1,25-hydroxy vitamin D, active form of Vitamin D and the mediated its biological effects [18]. Dendritic cells are the primary targets for immunomodulatory activity of 1,25 (OH) 2 D3, by inhibiting the differentiation and maturation of DC, suppress the expression regulation of MHC-II, costimulatory molecules (CD40, CD80 and CD86) and decreased production of IL-12. In addition, 1,25 (OH) 2 D3 increases the production of IL-10 and promote apoptosis DC. Together, the effect of 1,25 (OH) 2 D3 inhibits activation of T cells that depend on DC [19]. Vitamin D does not have direct anti-microbial effect. The effect is through the modulation of host immune response by inducing secretion of IL-10.

This study proves the role of curcumin in VDR induces in mice infected with *S. Thypi*. After therapy for five days with curcumin dose of 200 mg / kg bw (mean difference± SD; -0.88 ± 5.13, *p-value* 0.018) and 400 mg / kg bw (mean difference± SD; -3.70 ± 1:07, *p-value* 0.002). It reported a significant increase in the mean serum levels of VDR. These results are supported by in vitro studies showing that curcumin also binds with low affinity VDR [20]. VDR activation process then activates the transcription factor genes antimicroba natural peptide, cathelicidin and defensins [21].

Mice that do not have the VDR impaired production of Th1 promotive factor and IL-18, decreased Th1 cell proliferation, and decreased expression of signal transducer and activation of transcription 4 (STAT 4) (a transcription factor Th1 cells). Taken together these circumstances indicate that VDR function is essential for the development of Th1 cells. In mice that did not have VDR decreased proliferative response to stimulation CD3 [19],[22],[23].

Conclusion

Curcumin (200 and 400 mg / kg bw) significantly increased serum levels of vitamin D receptors and inhibit the growth of *S. Typhi* bacteria colony. Curcumin, therefore, can be considered as a potential adjuvant therapy in treatment of typhoid fever.

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Conflict of interests

Authors declare that there is no conflict of interests within this research article and publication.

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