Expression of Immunoglobulin a (IgA) in Wistar Rats Fed and Infected by Salmonella Typhimurium: Immunohistochemical Studies

Norma Farizah Fahmi(✉) and Rizka Efi Mawli

STIKes Ngudia Husada Madura, Gebang, Indonesia
rezaiei.cha@gmail.com

Abstract. Typhoid fever caused by Salmonella enterica serotype Typhi continues to be a public health problem in developing countries and is a cause of illness and death. The emergence of the problem of antibiotic resistance has also worsened cases of typhoid fever. Probiotic bacteria or lactic acid bacteria (LAB) can be used as an alternative because they provide several benefits for the host, including protection from pathogenic bacteria to increase levels of Immunoglobulin A. This study used LAB species Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus salivarius, Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, and Lactococcus lactis found in commercial probiotics. The mixture of isolates was given to white rats (Rattus norvegicus) that had been infected with Salmonella typhi with a dose variation of 0.5 ml, 1 ml, and 2 ml, and the expression of immunoglobulin A (IgA) was observed. The results showed that commercial probiotic isolates could maintain the expression of Immunoglobulin A. in mice infected with Salmonella typhi with the best dose of 2 mL.

Keywords: Immunoglobin A · Salmonella typhi · Probiotics

1 Introduction

Fever typhoid is an acute fever disease and frequently threatens transmitted soul through the fecal-oral route by the bacteria of Salmonella enterica serotype Typhi (Alba et al., 2016; Mogasale et al., 2016). However, it can also be caused by Salmonella paratyphi A, Salmonella paratyphi B, and Salmonella paratyphi C (Rahmasari & Lestari, 2018).

In developing countries, fever typhoid endemic occurs, ranging from 25 to 1,000 cases / per 100,000 people per year. In highly endemic areas, fever typhoid most often happens to children 1–5 years old (Khanam et al., 2013). The individual can be infected through water consumption or contaminated food, also after contact with patients. Pathogens do not infect animals, and hence, their transmission is only from man to man (Alba et al., 2016). Fever is part of response phase I to various stimulation infections, wounds or trauma, such as case lethargy and reduced appetite that can cause dehydration, hard sleep, hypozincemia, acute phase protein synthesis and others (Ardiaria, 2019).

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Typhoid fever keeps going and becomes a problem for health in people in developing countries because it can cause disease and death. The worst effect is the emergence of resistant antibiotics. Therefore, controlling infection through a non-antibiotic approach is urgently needed. The use of Lactobacillus for controlling typhoid fever is a promising approach because it could give protection through various mechanisms (Abdel- Daim et al., 2013). Lactobacillus and Bifidobacterium have been categorized as probiotic bacteria (Martinez et al., 2014).

Probiotic bacteria give an amount of benefit to the host, including protection from bacterial pathogens. These bacteria protect the host through several mechanisms, including competing for nutrition and niches and producing antimicrobial substances (Andino et al., 2014), balancing and restoring gut microbiota, protecting from pathogens, immunomodulation, and maintaining intestinal barrier integrity (Piqué et al., 2019). Besides, probiotic bacteria could disturb the gene expression of bacterial pathogens, so pathogens could not colonize and cause disease (Andino et al., 2014). The latest studies revealed that the LAB probiotic strain was able to increase host defense in the intestine and increase the rate of Immunoglobulin A (IgA) (Kawashima et al., 2018).

The main mechanism of protection against pathogenic antigens of mucosa immunity is mediated by cells producing secretory IgA and IgA that can neutralize and prevent the entry of harmful antigens into the host. Local immune response stimulation effectively prevents disease by microorganisms that enter the host body through the oral route (Julia et al., 2013). Research conducted by Andrews et al., (2019) identifies plasma IgA response as an excellent biomarker under acute typhoid stage in the South Asian area (Andrews et al., 2019). IgA production is required for maintaining intestinal homeostasis in stable conditions and hindering the attack of the pathogen (Kawashima et al., 2018).

This study aims to know whether commercial probiotics in the powder containing Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus salivarius, Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, and Lactococcus lactis can maintain expression of Immunoglobulin A in infected mice with Salmonella typhi.

2 Method

Research Design

This study is a pure experimental laboratory (true experimental) with a posttest-only control group design.

Study Participants

The subject in this study is 25 white rat males with the R. norvegicus Wistar strain for 5 treatment groups, 12 weeks old with a weight of 200–250 g with healthy condition from Biochemistry Laboratory, Faculty of Medicine, Airlangga University.

Instrument and Data Collection

This study used a pure experimental laboratory (true experimental) with a posttest-only control group design. This study used 5 groups of rats that were positive control group (only infected with Salmonella typhimurium), negative control group (only given

probiotics), treatment group 1 (given probiotic of 0.5 ml for 7 days and infected with Salmonella typhimurium), treatment group 2 (given 1 ml of probiotic for 7 days and infected with Salmonella typhimurium), group treatment 3 (given probiotic of 2 ml for 7 days and infected with Salmonella typhimurium). Salmonella typhimurium bacteria were obtained from the Microbiology Laboratory, Faculty of Pharmacy, Widya Mandala Surabaya Catholic University.

Data Analysis

Results were analyzed using mean, median, mode, standard deviation, and coefficient variation using the One-way ANOVA test or Kruskal Wallis compared among control and treatment groups 1: control and treatment groups 2: and control and treatment groups 3.

3 Results

In this study, the researchers conducted the Widal test to know the success of Salmonella Typhimurium infection. The result of the Widal test is shown in Table 1.

Based on Table 1, it can be concluded that the positive control and treatment group were infected with Salmonella typhi with a titer of 1/80. In contrast, the negative group was not infected because it was only given probiotics without being infected.

The data of median, minimum, and maximum expression of immunoglobulin A in the cytoplasmic mucous in Wistar rats' intestines infected with Salmonella typhimurium (%) is shown in Table 2.

Based on the data in Table 2, it is known that cytoplasmic mucous of lgA expression in rat's intestine show the highest score in treatment group 3 with a median value of 90 and a maximum is 90. Meanwhile, the lowest score is in the negative control group, with a median value of 40 and a maximum of 50 (Fig. 1).

The results proved the different expressions of immunoglobulin A between treatment groups. Then normality test was conducted. A normality test was conducted to know the results of the expression of immunoglobulin A by immunohistochemistry. The data

Table 1. The Results of the Widal Test in the Control and Treatment Groups

Group	Widal Test Results
Control (+)	
Control (-)	1/80
treatment 1	1/80
treatment 2	1/80
treatment 3	1/80
Control (-)	1/80
treatment 1	1/80
treatment 2	1/80
treatment 3	1/80

Table 2. Data of median, minimum, and maximum IgA expression

Group	median	Minimum	Maximum
Control (+)	20	0	80
Control (-)	40	0	50
Treatment 1	80	70	80
Treatment 2	80	10	90
Treatment 3	90	30	90

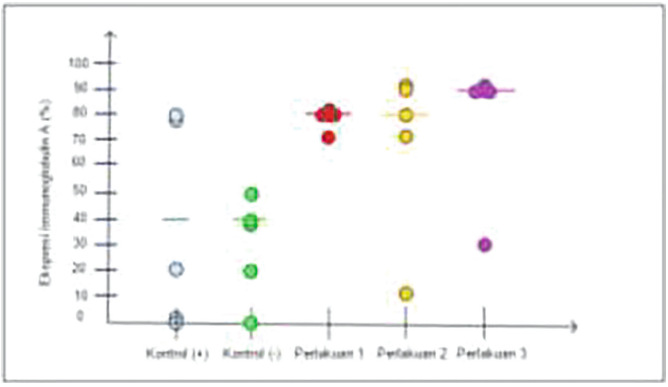

Fig. 1. Box-plot Expression of IgA in the cytoplasmic mucous of the intestine

were normally distributed so that it could be determined the data analysis model that must be used in data analysis. Normality test results can be seen in Table 3.

Based on Table 3 of normality test data, the probability (p) score of the positive control group was 0.200, the negative control group was 0.191, the treatment group 1 was 0.001, treatment 2 was 0.094, and treatment 3 was 0.001. Based on the data from the 3 groups, it can be concluded that the data were normally distributed (positive control, negative control, and treatment 2). Meanwhile, the data for treatment 1 and treatment 2 were not normal. Then, for the next test, the researchers used a nonparametric statistical test in the form of the Kruskal Wallis Test.

Based on Table 4, the significance value was 0.032. It means there were significant differences in the results of immunoglobulin A expression among the five groups.

Figure 2 shows the IgA expression using the IHC method. K + dazzled positive in 25% cytoplasm of lymphocytes cell and 80% in the cytoplasm of mucous cells of the intestine with strong intensity; K- dazzled positive at 40% cytoplasm of lymphocyte cells with strong intensity and 50% in the cytoplasm of mucous cells of the intestine with strong intensity; P1 dazzled positive in 20% cytoplasm of lymphocyte cell with strong intensity and 80% in the cytoplasm of mucous cells of the intestine with strong intensity; P2 dazzled positive at 50% cytoplasm of lymphocyte cell with strong intensity and 80% in the cytoplasm of mucous cells in the intestine with strong intensity; P3

Table 3. Normality Test Results

Group	Kolmogorov-Smirnov p-value	Shapiro-Wilk p-value
Positive Control	0.200	0.057
Negative Control	0.191	0.44
treatment 1	0.001	0.00
treatment 2	0.094	0.03
treatment 3	0.001	0.00

Normal distribution = $p > 0.05$

Table 4. Kruskal Wallis Test of Immunoglobulin A

Percentage of lgA	
p-value	0.032

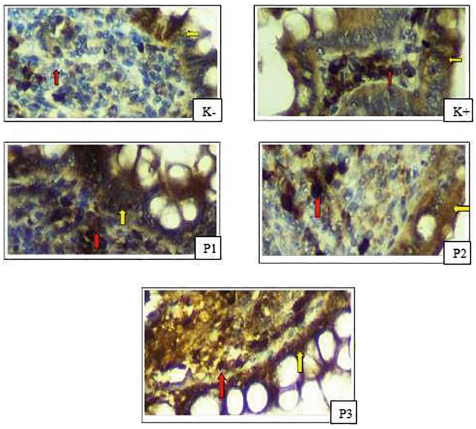

Fig. 2. Overview results of the inspection of IgA expression using the IHC method in the cytoplasm of lymphocytes and mucous cells of the intestine

dazzled positive at 60% cytoplasm of lymphocyte cell with strong intensity and 90% in the cytoplasm of mucous cells with strong intensity. Lymphocyte cells were shown with arrow red, and mucous cells were shown with yellow.

4 Discussion

Based on the results that have been described, the dose of probiotics that can maintain the highest IgA expression after infection of Salmonella type is probiotics with a dose of 2mL with a median of 90% and a maximum of 90%.

The ability to induce an increase in the number of cells produced in the intestinal lamina propria is an important characteristic of probiotic strains (Salva et al., 2010). Several studies have reported the influence mechanism of molecular probiotics, such as the increment of IgA secretion, production of antibacterial substance, an increase of tight junction of intestinal lining against the invasion of bacteria between cells, and competition with pathogenic microorganisms to attach to enterocytes (Azad et al., 2018; Maftei, 2019).

Approximately 80% of plasma cells secrete antibodies in the human body located in the digestive tract. Cells that produce secretory antibodies (especially immunoglobulin A) are actively transported above the surface epithelium into the intestinal lumen and therefore have a unique capacity to interact directly with gut microbes (Jahnsen et al., 2018).

Probiotic Strains significantly influence intestinal lining by stimulating B cells to produce IgA. In in vitro studies with enterocyte cells (HT-29, caco-2, and dendritic cells from PBMC), probiotics can affect APC to produce cytokines, which initiate an adaptive response (Azad et al., 2018). Different strains of Lactobacillus and Bifidobacterium can trigger epithelial cells to express IL-10, TGF-a, and IL-6 and subsequently stimulate IgA production (Azad et al., 2018; Zhang et al., 2018; Mora et al., 2006; Kotani et al., 2014).

Probiotics stimulate intestinal epithelial cells (IECs) (Vlasova et al., 2016) to produce cytokines, such as IL-6, which are capable of pushing the happening exchange of expression or "switching" from IgM to IgA in B cells (Garcia-Castillo et al., 2019). IgA-produced B cells can also be generated through T cells, fine T cell-dependent nor T cell-independent. Response to T cells-dependent usually occurs in the germinal center in the lymphoid network like Peyer's patches and Mesenteric Lymph Nodes. In a structure like that, B cells undergo a number of activation cycles and maturation supported by follicular T cells expressing co-stimulatory molecules and cytokines. Regarding T cells-independent that happens outside the germinal center, B cells are activated by IEC and innate immune cells to produce polyreactive IgA. T- cell independent IgA production was induced by and affected the composition of gut microbiota. Besides, the increased T-independent IgA induction supported by TGF-β, IL-4, IL-2, IL-6, and IL-10 occurs in strains that are immunobiotic (Garcia-Castillo et al., 2019).

The intestinal barrier plays an important role in compartmentalizing bacteria to the lumen via mucus and producing secretory immunoglobulin A (sIgA) (Maldonado Galdeano et al., 2019). Dimeric IgA or Polymeric bond with immunoglobulin receptors of polymeric immunoglobulin receptor (pIgR) is a precursor of the secretory component on the basolateral surface of IECs, and the complex IgA-pIgR transported through transit to the apical surface, where from complex pIgRin by proteolytic splitting to be released to the intestinal lumen as secretory IgA (Tezuka & Ohteki, 2019). Component of IgA secretory functions to tie sIgA to the mucus area so that immunoglobulin A can eliminate mucosal antigens (Maldonado Galdeano et al., 2019),

Probiotics have been made as one prevention and therapy effort to return the composition and function of a healthy gut microbiome (Hemarajata & Versalovic, 2013). Therefore, it is possible that in the future, probiotics could help reduce the risk of serious disease and make prevention and treatment more effective (Hlubeňová et al., 2017).

5 Conclusion

This study uses commercial probiotics from Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus salivarius, Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, and Lactococcus lactis which are capable of maintaining Immunoglobulin A expression in mice infected with Salmonella typhi with best dose as much as 2 mL. Therefore, further research must develop new protocols regarding types of microorganisms, doses, and periods of giving probiotics for certain diseases.

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