ELECTRON MICROSCOPIC SCANNING PROFILE OF BALB/c MICE INTESTINAL VILLI AFTER PER ORAL LPS (LIPOPOLYSACCHARIDE) DURING PROBIOTIC INDUCTION

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ABSTRACT

Diarrhea is one of the most common diseases in children, the disease is generally caused by a toxin from bacterial enteropathogens (LPS). To overcome this has been attempted prevention by administering probiotics orally, but the effect of probiotics on the prevention of gastrointestinal disorders due to toxins from bacterial enteropathogens has not been clear. This study used experimental animals mice BALB/c, which consists of 3 groups, each group consisted of 4 mice. Group-1 weren’t given LPS-1 and probiotics, group-2 were given LPS on day 15, while the 3rd group were given the probiotic for 21 days and given LPS given on day 15. When compared to intestinal villi diameter between groups, showed a significant difference (p<0.05); groups-1 with group-2-3 showed a significant difference (p<0.05). Group-1 with group-3, showed a significant difference (p<0.05). Ultrastructure observation of small bowel mucosa using scanning electron microscopic (SEM), showed that image of the intestinal mucosa of group-1-2 is very different. Group-2 with group-3 is very different, but the picture of intestinal mucosal ultrastructure between group-1 with group-3 is almost the same. The conclusion, the gut mucosal surface ultrastructure in experimental animals that have been given probiotics and induced by LPS is similar to the intestinal mucosa of normal animals, while the diameter of villi growth intestine showed a significant difference. Diameter measurements to determine the growth of intestinal villi, it seems less sensitive, therefore it needs further research oriented molecular system that can be used as an indicator in the determination of intestinal mucosal repair.

Keywords: bacterial enteropathogens, probiotics, intestinal mucosa, scanning electron microscopic

INTRODUCTION

Diarrhea is one of the most common disease in children, this disease each year has increased, as reported by Ratnawati in 2009, where the incidence of diarrhea in children in 2000 around 301 per 1000 population, whereas incidence rates in 2003 approximately 374 per 1000 population. It is known that many factors that trigger the occurrence of diarrhea in children, including inability to adapt to a baby’s intestines or food ingredient can be caused by viruses, bacteria, parasites, toxins, etc. (Sinuhaji 1992), with many of these factors, then one of the causes gastrointestinal disturbances highlighted the occurrence of diarrhea in infants trigger is an infection caused by bacterial enteropathogens. When the bacteria enter the gastrointestinal tract, the bacteria in the gut will be releasing an endotoxin, known as LPS (Lipopolysaccharide). LPS is then bound by a protein on the surface of the intestine, where this protein is produced by hepatocytes, called LBP (Lipopolysaccharide binding protein), the bond between the LPS with LBP, LPS will lead to more easily form a complex with CD14. This CD14-LPS complexes induces a series of conductivity occurs subsequent intracellular signal will trigger the activity of the nuclear factor κB (NFκB), a transcription factor that causes the expression several proinflammatory cytokines such as IL-6, IL-8, TNF-α (Wang 2001).

The existence of these proinflammatory cytokines (especially IL-6 and TNF-α) will trigger the process leukopoitik in bone marrow, while IL-8 acts as a chemoattract neutrophil factor (NCF), and become involved in migration of neutrophils from bone marrow into the lesion in this case to intestine (Kuby 2000). Neutrophil migration into intestinal tissue is then release a proteolytic enzyme that is MMP-8 (matrix metaloproteinnase-8) are intended to damage the cause of inflammatory agents (LPS), but these enzymes also result in damage to collagen is collagen type-1 in the gastrointestinal tract (Darnell 1990). The influence of collagen damages the intestinal mucosa, resulting in damage to the intestinal mucosa. Mucosal damage resulted in an interruption occurs absorption and eventually can cause diarrhea, which endanger the safety for the infant/child. To overcome this needs a lot of rethinking how to prevent it. One of which can be used to prevent gastrointestinal disorders due to toxins from bacterial enteropathogens is by giving probiotics orally, but the effect of probiotics on the prevention of
gastrointestinal disorders result from bacterial toxins enteropathogen, until now there has been clear.

Based on the danger of damage to the intestinal mucosa that lead to diarrhea, then preventive measures are of great importance. If this is not given serious attention, the death rate from diarrhea will have increased sharply. Therefore, this study is not possible in humans, the researchers used animal model of mice induced by probiotics orally, during the administration of probiotic mice were then induced by LPS orally. The LPS is used to damage the intestinal mucosa is LPS from E. coli serotype 055: B5. While probiotics are used to prevent damage to the intestinal mucosa, is the kind of mix/multi species. To know the effect of probiotics on the prevention or repair of intestinal mucosa due to the LPS, the researchers conducted observations in animal intestine using a Scanning Electron Microscope.

MATERIALS AND METHODS

RESULTS

This research is an experimental research design with controle group post test only design, using experimental animals mice BALB/c adult male aged 10 weeks, which consisted of three groups. Group I: without a probiotic or LPS. Group II were given LPS on day 15. While group III were given the probiotic for 21 days, and on day 15, were given LPS. The probiotic used in this study are probiotic species mix/multi species composition consisting of: Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus bulgaricus, Bifidobacteria breve, Bifidobacteria infantis and Streptococcus thermophilus with 10^9/kgBW/day, given oral. While the LPS used to damage the intestinal mucosa is negative gran endotoxin from bacteria, namely E. coli serotype 055: B5 with a dose of 250 μg/kg. Later in the day-to-21 three groups of experimental animals are sacrificed, then the intestine was taken and examined by using Scanning Electron Microscope (SEM).

Figure 1. Views surface intestine of experimental animals group-1 (control group) without given a probiotic or LPS. Photo Sudiana IK, SEM x 350
Figure 2. Views surface intestine of experimental animals group -2 (without the give and Probiotics, but on the 15th day were given LPS). Photo Sudiana IK, SEM x 350

Figure 3. Views surface intestine of experimental animals group-3 (given the probiotics, for 21 days but on day-15 were given LPS). Photo Sudiana IK, SEM x 350
DISCUSSION

LPS is a toxin that can damage the intestinal mucosa, this toxin is produced by enteropathogens (gram negative bacteria) that often infect humans thus causing diarrhea. One effort to prevent or repair damage to intestinal mucosa caused by infection with enteropathogens is giving probiotics, because probiotics can normalize the microflora in the intestine and prevent the invasion of enteropathogens into the intestinal tissue (David 2003). In addition, probiotics can interact with intestinal epithelial signal transduction that triggers the next intra-epithelial and epithelial release proinflammatory cytokines such as interleukin-8 (IL-8), in which IL-8 trigger neutrophil migration into the intestinal lumen and eventually will phagocytosing enteropathogens neutrophils (Karen 2006). To prove this, the researchers conducted research on the ability of probiotics in preventing damage to the intestinal mucosa due to LPS that is released by bacterial enteropathogens.

In this study, researchers used animal model of mice BALB/c adult male (age 10 weeks), adult male was chosen because it seeks to homogenize samples associated with maturation and hormonal factors, because these factors really play a role in endurance. The procedure of this study, experimental animals were divided randomly into three groups: the group-1 as a control, group-2 gut mucosa was damaged by the LPS, the timing of LPS performed on day 15. While the intestinal mucosa in group-3 protected prior to the granting of probiotics for 21 days, but to know the ability of probiotics to protect intestinal mucosa damage or accelerate repair of intestinal mucosa of LPS, then this 3-group were given LPS on day 15. Next to the three groups of experimental animals on day 21, sacrificed and the intestine was isolated then made observations on the intestinal mucosa using the scanning electron microscope (SEM). Observations were made 6 days after administration of LPS. It is expected that the regeneration of intestinal mucosa can already be observed. The indicator used to determine the regeneration of intestinal mucosa is to measure the diameter of the formation of intestinal villi. Furthermore, the measurement results obtained by statistical test by using ANOVA test.

Based on statistical analysis in Table 1, showed that intestinal villi diameter between the control group (X = 1865.0000; SD = 265.32011) with the LPS (X = 0.0000, SD = 0.0000) showed a significant difference of p <0.05. This indicates that LPS given orally to experimental animals will be bound by LPS (Lipopolysacharide). On the surface of intestinal LPS is then bound by a protein called LBP (Lipopolysacharide binding protein), the bond between the LPS with LBP, LPS will lead to more easily form a complex with CD14. This CD14-LPS complexes induces a series of conductivity occurs subsequent intracellular signal will trigger the activity of the nuclear factor xB (NFkB), a transcription factor that causes the expression of several proinflammatory cytokines such as IL-6, IL-8, TNF-α (Wang 2001). The existence of these proinflammatory cytokines (especially IL-6 and TNF-α) will trigger the process leukopoietic in bone marrow, while IL-8 acts as neutrophil chemotactic factor (NCF), and become involved in migration of neutrophils from bone marrow into the lesion in this case to the intestines (Kuby 2000). Neutrophil migration into intestinal tissue is then release a proteolytic enzyme that is MMP-8 (matrix metaloproteinnase-8) are intended to damage the cause of inflammatory agents (LPS). However, these enzymes also result in damage to collagen is collagen type-1 in the gastrointestinal tract (Darnell 1990). Damage to collagen resulted in intestinal mucosal cell damage occurs so that there was absorption disorder which in turn will trigger the occurrence of diarrhea.

Things that need to be discussed is the role of administering probiotics to prevent diarrhea. When viewed the role of probiotics, can normalize the microflora in the intestine and prevent the invasion of enteropathogens into the intestinal tissue (David 2003). In addition, probiotics may also interact with intestinal epithelial signal transduction that triggers the next intra-epithelial and epithelial release proinflammatory cytokines such as interleukin-8 (IL-8), in which IL-8 is very ready to trigger neutrophil migration into the lumen of the intestine when there are enteropathogens that enter the intestinal lumen (Karen 2006). Therefore in this research are used to induce damage to the intestinal mucosa but not bacterial enteropathogens i.e. the product of a bacterial LPS, then this does not result in migration of phagocytes especially neutrophils into intestinal lumen, because the migration of neutrophils into the intestine is to phagocytosing the enteropathogenic tissues (Karen 2006). Probiotics are
given in addition to contribute to normalize the intestinal microflora, as well as prevent the invasion of enteropathogens into the intestinal tissue (David 2003), seems also to act to inhibit or regulate the growth of intestinal mucosa. This can be proved in the intestinal mucosal surface ultrastructure observed by using SEM.

In this research found that the experimental animals which were given LPS alone (Figure 2), compared with a group of experimental animals that have been given the probiotics then given LPS (Figure 3), indicating there is a process improvement intestinal villi growth which is very significant, namely picture ultrastructure surface similar to the control group (in the picture: 1). However, based on statistical tests in Table 1 showed that intestinal villi diameter between groups of experimental animals that were given LPS (X = 0.0000, SD = 0.0000) with a been given the probiotics then given LPS (X = 1455,000; SD = 75.49834) showed a difference which is significant at p < 0.05, from these results showed that in experimental animals that have been given the probiotic gut mucosa more resistant to LPS. While the control group (X = 1865.0000; SD = 265.32011) with groups that have been given the probiotics then given LPS (X = 1455,000; SD = 75.49834) showed a significant difference of p <0.05, from these results can be explained that the process of mucosal repair intestine in experimental animals which had previously been received probiotics which then induced LPS, has not reached the optimal condition. Based on the results of this study can be summarized as follows

CONCLUSION

Based on the observations of the surface ultrastructure of intestinal mucosa using SEM, that administration of probiotics can prevent damage to or regulate the growth of intestinal mucosa, due to the induction by LPS of bacterial enteropathogens. Diameter growth of intestinal villi, in experimental animals that have been given the probiotics then induced by LPS, has not shown that optimal results, ie significantly different from the control group. Diameter measurements to determine the growth of intestinal villi, it seems less sensitive, therefore it needs further research oriented molecular system that can be used as an indicator in the determination of intestinal mucosal repair.

REFERENCES