POTENCY OF Lactobacillus reuteri ON REDUCED INFLAMMATION IN SEPTIC ENCEPHALOPATHY

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ABSTRACT

Sepsis is the leading cause of death in critically ill patients. Septic encephalopathy is a severe brain dysfunction caused by systemic inflammation in the absence of direct brain infection. Intestinal apoptosis plays a central role in the pathophysiology of sepsis and is associated with a poor outcome in sepsis. In experimental animals model of sepsis, improve intestinal inflammation lowers survival due to sepsis. Lactobacillus reuteri is recently used as immunonutrition in critically ill patients is still disputed. This study was aimed to evaluate the effect of L. reuteri on intestinal inflammation grade in mice model of sepsis. Male Balb/C mice weighing 15-23 g were used in the study. Mice models of sepsis were induced by an intraperitoneally (i.p). injection 4 mg/mice of cecal inoculum each day for 7 days. The mice were divided into three groups: control (n=6), sepsis (n =6) and sepsis plus L. reuteri (n=6). Mice were sacrificed 24 hours after the final cecal inoculation to determine intestinal inflammation grade. We used L. reuteri as probiotic. Mann-Whitney test for intestinal inflammation grade at p < 0.05 was used to determine significant differences of each group. Control group mice showed intestinal inflammation of 94.4% on grade 0; while sepsis mice group (cecal inoculum exposed) demonstrated higher grade of intestinal inflammation of 55.6% on grade 3; and sepsis plus L. reuteri mice group showed higher than control group but lower compared to sepsis without L. reuteri group, namely 50% on grade 0. It can be concluded that L. reuteri can reduced inflammation in mice model of sepsis including septic encephalopathy.

Key words: sepsis, probiotic, cecal inoculum

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INTRODUCTION

Sepsis is still a major cause of death in a number of intensive care unit (ICU), whose incidence is increased. Sepsis patients potential to develop towards the organ rudiment acute brain (acute Cerebral dysfunction) that its incidence to 70%. (Wilson and Young 2003). Pathophysiology sepsis is a result of complex interactions between the infection of pathogenic bacteria, inflammation and the coagulation the characterized as imbalance between proinflammatory cytokines with cytokine anti-inflammation. Sepsis occurred in the suppression of immune system, in addition to the increase in deaths occurred epithelial cell lines and digested lymphocyte (Chang et al. 2007), so that the destruction occurred and dysfunction mucosa channel that lead to digestive loss mucosa defense, increased permeability mucosa and translocation products from the bacteria into the blood circulation , which is then further activation will cause a systemic response Systemic inflammatory Response Syndrome (SIRS), especially in the lungs, liver, kidney, intestine and other organs including the central nervous system. (Arun et al. 2001) This condition can trigger the occurrence of septic encephalopathy (SE) as a result of systemic inflammation though not established the existence of brain infection directly and obtained the characteristic clinical signs of a slowing of mental processes, which decreases awareness, disorientation, delirium and coma occur. So that will increase morbidity and mortality. (Sprung et al. 1990).

Many business experts have been made in tackling the sepsis that developed into septic shock and multiple organ failure (MOF), but still not successful in both patients and severe sepsis still tends to end with death. Management sepsis is based not only eliminates pathogenic bacteria but also supports the normal flora of the host, so that the immunonutrition also quite useful. Probiotic (Lactobacillus reuteri) potent better in preventing infection channel digested, now used as immunonutrition tested on patients who critically, including sepsis as immunomodulation. (Calder 2003) However, the benefits of the use of L. reuteri as immunonutrition in the form of a formal medical field is not fully understood because the material has not been known and actively working mechanism (Brown and
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Valiere 2004), including its use in patients who are critically debated.

Author's preliminary research indicates that Probiotic able to decrease the level of dissolved inflammation channel model mice allergy (Diding et al. 2008). This study aims to determine the influence of test preclinical *L. reuteri* to the degree inflammation channel mice dissolved in Balb / C model of sepsis.

**MATERIAL AND METHOD**

18 test animals such as chicken mice Balb / C males, with body weight ± 15-23 grams, and the age of 4-6 weeks. Mice Balb / C obtained from the Animal Experiment Unit Development Gadjah Mada University, Yogyakarta. Food used mice feed mice BR I. *Lactobacillus reuteri* is used L. reuteri ATCC 55730 ....... 108 CFU produced by Farmasierra Manufacturing SL Madrid, Spain. Try animal adapted for a week. Then try animals were divided into three groups, each group of 6 chickens. Group I were given no treatment (as control), group II is mice model sepsis, and group III mice model of sepsis is that given Probiotic.

To create a model of sepsis in the animals try to use cecal inoculum injection (4 mg / mice) of intraperitoneal (ip). (Brahmbhatt et al. 2005; Chopra and Sharma 2007). Cecal inoculum made new every day from mice donor suspended sacrificed with 200 mg cecal material in 5 mL dextrose water 5% (D5W) sterile. (Ren et al. 2002). In the day-to-7 all mice sacrificed, the network was taken 1.5 cm along the intestine, and then soaked in a solution buffered 10% formalin for 10 hours, after it was created paraffin block. Next performed a snippet of serial paraffin blocks are made to slide, each slide 3. Slides staining using hematoxylin-and eosin (HE) to determine the picture dissolved histological channel identified with the light microscope. Determining the degree inflammation channel dissolved, with the grading is done. (Chang et al. 2007) Grade 0: no inflammation cell infiltration (normal network), Grade 1: inflammation cell infiltration up to the layer epithelial from mucosa intestine, Grade 2: inflammation cell infiltration up to the layer epithelial mucosa and less infiltration to submucosal layer, Grade 3: cell infiltration to inflammation submucosal layer, and Grade 4: inflammation cell infiltration up to the layer muscular / transmural.

Data are analyzed statistically using the statistical test Kruskal-Wallis. If there are significant differences then proceed with the Mann-Whitney test to see its inflammation using the program SPSS for Windows Release 11.5 and p <0.05 chosen as the minimal significance level.

**RESULTS**

Polymicrobial infection with the cecal inoculum may trigger the occurrence of sepsis in the animal test. This can be seen the change that is meaningful in the sepsis group compared mice control. Mice group sepsis showed signs of pylorecton, pericolic discharge, appear listless, decrease appetite and drinking, and diarrhea. From the results of the analysis of post-mortem cavum peritoneum and ascites seen infarct peritoneum, and the great damage and attachment in a number of organs including liver, lien, kidney and brain.

On this research group obtained 94.44% control sample with the picture histological the normal or grade 0 and 5.56% with grade 1 samples. Sepsis groups shows the sample with 11.11% grade 2; 55.56% with grade 3 sample and the sample with 33.33% grade 4. While sepsis group given *L. reuteri* showed 50% of the sample with the picture histological the normal or grade 0; 33.33% samples with grade 1 and 16.67% with grade 2 sample. Results degrees inflammation channel digested from each group shown in table 1 and picture 1.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Control</th>
<th></th>
<th>Sepsis</th>
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<th>Sepsis + <em>L. reuteri</em></th>
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<td></td>
<td>Number</td>
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<td>0</td>
<td>6</td>
<td>33.3</td>
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Table 1. Channel degrees Inflammation dissolved in the respective treatment groups
**DISCUSSION**

Sepsis is a clinical syndrome as a manifestation of the process inflammation immunologic which occurred because of the response body (immunity) against excessive stimulus product microorganisms such as bacteria gram negative and positive, fungi, viruses, and parasites with or without the bacteria found in blood. (Guntur 2008; James et al. 2005)

Cecal inoculum delivery of materials, can cause intestinal inflammation mucosa up to 55.56% among grade 3 (table 1). This can cause intestinal hypoperfusion form of interference microcirculation mucosa intestine, dysfunction intestinal barrier with increased intestinal permeability, bacterial invasion and pathogenic its toxin into the systemic circulation (Jürgen et al. 2006) and release cytokine inflammation which is a sign of a reaction inflammation. (Jürgen et al. 2006; O'Connor 2007). This will trigger encephalopathy including the occurrence of sepsis into the clear, because in the sepsis is the cause of this systemic inflammation where the infection does not get the brain directly and obtained the characteristic clinical signs of a slowing of mental processes, which decreases awareness, disorientation, delirium and coma occur. And, most importantly that septic encephalopathy (SE) is an early sign of sepsis and associated with increased mortality and morbidity (Alexander et al. 2008).

Although the pathogenesis of SE circumstances such as sepsis is not directly triggered by pathogenic toxin, in the SE this encephalopathy can develop as a result of the cause of SIRS is that its infection is not found. Data from the experiment and clinic seen a number of factors such as cytokine pro-inflammation local, weak cerebral microcirculation serebral, imparity neurotransmitter and negative influence of organ failure peripheral contribute to an SE. Moreover, there is a settling of inflammation may cytotoxicity increase stress and oxidative further SE may cause a more severe, than that also play a role in the occurrence of deviation or slowdown neuronal function. (Wilson and Young 2003)

Patients who have pathological conditions on the central nervous system have previously may higher risk of going for the SE, and similar things can also be seen in an animal model of sepsis (Pickering et al. 2005).

Sepsis and the severe shock septic still holds an important role in morbidity and mortality. Increased sepsis episode is influenced by several factors, among others, increasing the population ages, the number of immunocompromised patients, the use of life-sustaining technology and the pattern of drug resistance antimicrobial. So that sepsis is still an important clinical problem, although there has been progress in therapy, such as the use of activated protein-C, and the use of low dose glucocorticoid (Xiao et al. 2006) but still not successful in both severe sepsis and patients still tend to end with death.

Sepsis treatment is based not only on the elimination pathogenic bacteria but also supports the normal flora of the host. One of the many efforts that later tested and examined to overcome problems with sepsis is the preparation Probiotic. Probiotic is a single culture or a mix of microorganisms living nonpathogenic (Lactobacillus, Bifido-bacterium, and Acidophillus) who, when given to humans or animals can be useful in health care to its host, because the press may growth of pathogenic bacteria in the intestinal human / animal so will improve the balance of the channel digested (Calder 2003).

Sepsis occurred in the patients immune system suppression and increased mortality occurred epithelial gastrointestinal cells, so that the destruction occurred and the channel dysfunction mucosa digestion. One of the mechanisms that support the destruction of dissolved mucosa channel induced endotoxin that apoptosis is increased. Increased apoptosis digestive channel that often occur in sepsis and death of cells mucosa excessive may support the atrophy, destruction and interference function mucosa defense alimentary tract. (Alscher et al. 2001)
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On this research shows that sepsis group given *L. reuteri* showed the degree of improvement inflammation a meaningful comparison group without sepsis given *L. reuteri* (Table 2). This is in accordance with the opinion that the work Probiotic stabilizes gut barrier mucosa and production induced lga epitop against pathogenic bacteria or its components such as endotoxin or exotoxin in the intestinal lumen and mucosa. (Galdeano et al. 2007) Probiotic reduces local and systemic inflammation on epithelial cells through NF-translocation retardation B cells in the nucleus. NF-1B is a factor transcript synthesis cytokine for the expression of genes in the set produces cytokine mediators proinflammation such as tumor necrosis factor α (TNF-α) and interleukins (IL-1 & IL-6). (Donglai et al. 2004), so proinflammatory cytokines production will be reduced or obstructed. In addition Probiotic increase imunologic intestinal barrier function and formation of mucosa (Donglai et al. 2004; Sya et al. 2007), restore the permeability intestinal towards the surface normal. (Matsumoto et al. 2005). With the ability of *L. reuteri*, the *L. reuteri* can be used as adjuvant therapy in the diseases are critical, including sepsis. So that will be able to reduce SIRS, with a decrease in the degree inflammation is also expected to be able to reduce the occurrence of SE that will ultimately reduce mortality due to sepsis. Because cytokine-cytokine pro-inflammation and lipopolysaccharide (LPS) will trigger the expression of CD40, vascular adhesion molecule-1 or intercellular adhesion molecule-1, and E-selectin on the cell endothelial-vessel blood vessel in the brain that will be able to cause change and interference behavior, through cell-cell microglial important role in mediating changes in the behavior of systemic infection (Dantzer 2004).

**CONCLUSION**

*Lactobacillus reuteri* can reduce the degree mice model inflammation in sepsis, it is expected will also be able to reduce the occurrence of septic encephalopathy.

**REFERENCES**


