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Description of Gastric Acidity (pH) with Simple Method in Lipopolysaccharide Induced Mice

(DESKRIPSI DERAJAT KEASAMAN (pH) LAMBUNG DENGAN METODE SEDERHANA PADA MENCIT YANG DIINDUKSI LIPOPOLISAKARIDA)

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ABSTRACT

The gastrointestinal acidity/pH can considerably influence the stability and absorption of oral medications. As a result, understanding the circumstances for drug delivery requires knowledge of gastrointestinal pH. Mice injected with lipopolysaccharide (LPS) are used in the most well-studied animal models of sepsis. However, information on gastrointestinal pH in sepsis mice is still insufficient. This study was conducted to identify gastric pH values in mice induced by lipopolysaccharide. The LPS was injected intraperitoneally as well as 0.9% NaCl as control groups. Treated groups (LPS) consisted of four groups and the control group (0.9% NaCl) consisted of four groups. Ten mice were used in each group. Gastric pH measurement was conducted using pH meter Lutron PH-201. Based on this study, the factors that influenced gastric pH in sepsis animal models were the LPS doses and the time after LPS injection. The results of gastric pH measurement in sepsis mice did not show a decrease in the pH compared to the normal conditions. The dose of LPS significantly influences the gastric pH change.

Keywords: gastric pH; sepsis mice; lipopolysaccharide; LPS

ABSTRAK

Derajat keasaman/pH gastrointestinal dapat sangat memengaruhi stabilitas dan penyerapan obat oral. Sebagai konsekuensinya, memahami keadaan untuk penghantaran obat membutuhkan pengetahuan tentang pH gastrointestinal. Mencit yang disuntik dengan lipopolisakarida (LPS) digunakan sebagai model hewan sepsis yang paling banyak dipelajari. Namun, informasi tentang pH gastrointestinal pada mencit sepsis masih terbatas. Penelitian ini dilakukan berujuan untuk mengetahui nilai pH lambung mencit yang diinduksi lipopolisakarida. Senyawa LPS disuntikkan secara intraperitoneal dan juga 0,9% NaCl sebagai kelompok kontrol. Kelompok perlakuan (LPS) terdiri atas empat kelompok dan kelompok kontrol (0,9% NaCl) terdiri atas empat kelompok. Sebanyak 10 mencit digunakan dalam setiap kelompok. Pengukuran pH lambung dilakukan dengan menggunakan pH meter Lutron PH-201. Berdasarkan penelitian ini, faktor yang memengaruhi

pH lambung pada hewan model sepsis adalah dosis LPS dan waktu setelah diinjeksi LPS. Hasil pengukuran pH lambung mencit sepsis tidak menunjukkan penurunan pH dibandingkan dengan kondisi normal. Dosis LPS secara signifikan memengaruhi perubahan pH lambung.

Kata kunci: pH lambung; sepsis; lipopolisakarida; LPS

INTRODUCTION

It has been postulated that one of the main functions of gastric acid is to prevent ingested microorganisms from reaching the small intestine, where they have the potential to cause disease or to gain access to other parts of the body (Tennant *et al.,* 2008). The normal murine gastric acidity/pH is around 3.0 (fed) and rises to 4.0 following a fast (McConnell*et al.*, 2008).

In sepsis and septic shock, the most prevalent microbial mediator is lipopolysaccharide (LPS). Lipopolysaccharide is a pathogen-associated molecular pattern detected by the pattern recognition receptor toll-like receptor 4 (TLR4). It is a key component of the outer membrane of Gram-negative bacteria (Gabarin *et al*., 2021).

In the context of the sepsis model in mice, there was not any data supporting the gastric pH value in mice after lipopolysaccharide intraperitoneal injection. This brings into question the use of rodents in investigations on gastric pH value after induced LPS intraperitoneal in mice; how the gastric pH in sepsis condition is, whether it acid or base.

Mice are a popular animal model that is mostly used in translational research to apply to humans due to their anatomical, physiological and genetic similarity to humans (Bryda, 2013). Among animal models of sepsis, mouse models are often used because of the ease of experimentation, small size and the relatively low cost (Lewis*et al.*, 2016; McConnell *et al.,* 2008). The mice gastric pH value in endotoxemia or sepsis is essential as primary data to study any kind of drug delivery system, nutrition delivery system and nano nutrition delivery system in critically ill. The pH of the stomach, in particular condition plays an important role in mediating the dissolution of drugs and consequently, interspecies differences in gastroduodenal pH take on greater importance in animal models used to evaluate the performance of pH-sensitive formulations. This is especially the case for those dosage forms designed to circumvent gastric release, thereby avoiding dose-dumping phenomena. Several methods have been historically used to determine gastric pH in animals, though there remain inconsistencies in reported data. This study was conducted to identify gastric pH values in mice induced by lipopolysaccharide

RESEARCH METHODS

This research has been granted ethical permission from the Ethical Committee of the Faculty of Medicine Universitas Airlangga, with the ethical conduct number: 2.KE.063.04.2019.

Experimental Animals

Inclusion criteria include male BALBc mice, 10-12 weeks old, weighing 25-30 g, obtained from the Center for Laboratory Experimental Animals, Gadjah Mada University, Yogyakarta. The mice underwent acclimation for a week, with adequate food and lighting. Exclusion criteria include mice's general condition seemed weak before the experiment and mice which appeared aggressive. Drop-out criteria include the death of the mice during the trial period.

Experimental Design

This study used a post-test control group design. The study samples consisted of 80 male mice. Lipopolysaccharide was injected to cause sepsis. After seven days of acclimatization, mice were randomly divided into two main groups: The first group consisted of four groups (group A, B, C, and D), it was the group with LPS dose 2.5 mg/kg of body weight. The detailed description is as follows: Group A was one hour after injection of 0.9% NaCl volume same as 2.5 mg/kg LPS; Group B was 24 hours post injection of 0.9% NaCl volume same as 2.5 mg/kg LPS; Group C was one hour after LPS 2.5 mg/kg injection; Group D was 24 hours after LPS 2.5 mg/kg injection. The second group consisted of four groups (group E, F, G, and H), it was the group with LPS dose 10 mg/kg of body weight. The detailed description is as follows: Group E was one hour after injection of 0.9% NaCl volume same as 10 mg/kg LPS; Group F was 24 hours post injection of 0.9% NaCl volume same as 10 mg/kg LPS; Group

G was one hour after LPS 10 mg/kg injection; Group H was 24 hours after LPS 10 mg/kg injection. In addition to dividing by LPS dose, this group of mice was also examined for gastric pH according to time after giving LPS. There was a group that was examined pne hour after treatment with both 0.9% NaCl and LPS, there was a group that was examined 24 hours after treatment. Lipopolysaccharide groups were as treated groups, and 0.9% NaCl groups were as control groups. Ten mice were used in each group. Lipopolysaccharide for this study was purchased from Sigma, extracted by ethanol from *Escherichia coli* 0111:B4. We gave the treated group 2.5 mg/kg bw and 10 mg/kg bw LPS, injected intraperitoneally. The same volume as LPS, 0.9% NaCl was injected intraperitoneally as the treatment in the control groups.

Gastric pH Measurement

The pH meter Lutron PH-201 (Figure 1) was used to measure gastric acidity.pH due to the small, pointed tip and ease of use. An open incision was made in the mice's gastric after anaesthetized by chloroform; furthermore, a pH meter probe was inserted into the gastric for five seconds to measure the gastric pH.

Statistic Analysis

All of the data values were analyzed with SPSS software version 21. Analyzed of variance test was used to determine the differences between treatment groups (LPS) dan control groups (0.9% NaCl). The effect of the LPS doses or the time in gastric pH value was analyzed with stratification analysis.

RESULTS AND DISCUSSION

In the treated group, it was appeared that after one hour of LPS 10 mg/kg injection, the gastric pH was more alkaline than the one hour group given LPS 2.5 mg/kg ($p=0.000$; $\alpha=0.05$). The comparison of gastric pH values in the group one hour after 2.5 mg/kg LPS and 24 hours after 2.5 mg/kg LPS was significantly different ($p=0.001$; $=0.05$), which was more alkaline at 24 hours after 2.5 mg/kg LPS injection. In the group one hour after giving 10 mg/kg LPS compared to the group 24 hours after giving 10 mg/kg LPS, there was no significant difference ($p=0.813$; $\alpha=0.05$).

In the control group, it was found that after one hour of 0.9% NaCl 10 mL injection, the gastric pH was more alkaline than the group given 0.9% NaCl 2.5 mL ($p=0.000$; $\alpha=0.05$). It was also discovered a significantly different value of mice gastric pH at 24 hours after giving 0.9% NaCl 2.5 mLc compared to 0.9% NaCl 10 mL ($p=0.000$; $\alpha=0.05$). The comparison between gastric pH values in the group one hour after 0.9% NaCl 2.5 mL and 24 hours after 0.9% NaCl 2.5 mL was significantly different, which more alkaline at 24 hours after 0.9% NaCl injection ($p=0.020$; $\alpha=0.05$). The gastric pH values in group one hour after giving 0.9% NaCl 10 mL were significantly different compared to the group 24 hours after giving 0.9% NaCl 10 mL ($p=0.029$; $=0.05$). The increasing pH in the 0.9% NaCl groups was in accordance with the theory that when fasting or feeding activity of mice was disrupted, it caused an increase in gastric pH.

Endotoxins and purified lipopolysaccharides (LPS) derived from Gram-negative bacteria are potent inhibitors of gastric secretion. This has been shown in the dog (Blickenstaff and Grossman, 1950; Wyllie *et al.,* 1967) and in the rat (Baume *et al.*, 1967; Brodie and Kundrats, 1964; Leenen & van Miert, 1969; Olson *et al.*, 1954). In rat 0.5 mg/kg dose of LPS remarkably inhibits the stimulatory gastric acid secretion via inhibition of H/K-ATPase enzymatic function (Helmer *et al*., 2004). Furthermore, it is also reported that intraperitoneal injection of LPS significantly suppressed gastric emptying in mice (Inada *et al*., 2006)

The present experiment confirmed the effect of a higher dose of LPS than the previous study on mice's gastric pH. Using the LPS sepsis mice model, we demonstrated that the change in gastric pH was affected by the LPS doses. Figure 2 explains that 10 mg/kg LPS significantly changed the gastric pH compared to 2.5 mg/kg LPS, both one hour

Figure 1. The measuring instrument for gastric acidity used in this study was the PH-meter by Lutron PH-20 1

after LPS administration and 24 hours after LPS administration. The underlying cause for these results may be the dysfunction of cells and organs due to sepsis in mice induced with LPS. The LPS non-lethal dose for making systemic inflammation is 5 mg/kg bw (Seemann *et al.*, 2017). However, in this study we used the previous literature about the dose of LPS in order to make severe endotoxemia or sepsis was 10 mg/kg intraperitoneal (Berger *et al.*, 2017; Li *et al*., 2011; Qin *et al*., 2016)

The exciting phenomenon in Figure 2 is the comparison of gastric pH value in 10 mg/kg LPS groups; the gastric pH change was not significantly different between 1st hour groups and 24th hour groups. It strengthens the results of this research that the LPS dose of 10 mg/kg was more alkaline, time travel did not influence the gastric pH change. The critical time of gastric pH change was one hour after 10 mg/kg LPS injection.

Surface pH values in mice are similar to those reported in rats, using a similar methodology (Chu *et al.*, 1999). Research by Chu *et al.* suggest that the rodent stomach uses a set point pH near four as a pivotal value for regulating acid and alkali secretion. The reason why the pH at the surface would be maintained at pH 4 is unknown. Speculation that pH 4 is a protective pH that kills most harmful bacteria, but it is not damaging to surface epithelial cells. It may be noteworthy that *Helicobacter pylori* produces urease, which confers the pathogen's survival in an environment of pH 4 and below (Clyne *et al.*, 1995; Meyer-Rosberg *et al.*, 1996). This research shows that mice gastric pH is around 3-4, both in LPS and NaCl groups. In the LPS groups, there was an increasing trend of gastric pH values becoming alkaline. The explanation for LPS groups related to a gastrointestinal failure caused by sepsis. The alkaline condition of 0.9% NaCl groups may be related to feeding activity (Everds *et al*., 2013; Yamada *et al*., 2015). The previous study informed that mice and humans had contradictive gastric pH phenomenon. The gastric pH in mice increases during the fasting condition. Meanwhile, the gastric pH in humans decreases in the stress condition and fasting (Brenneman *et al.*, 2014; McConnell *et al*., 2008).

Gastrin is the principal hormonal inducer of gastric acid secretion. The cellular targets for gastrin in the stomach are the acid-secreting parietal cell and histamine-producing enterochromaffin-like (ECL) cell (Jain and Samuelson, 2006). The main manifestations of sepsis include gastrointestinal dysfunction or failure, and gastrointestinal mucosa injury, leading to the translocation of gastrointestinal bacteria and toxins and septic shock (Fink, 1991; Schmidt *et al*., 2013). Furthermore, aggravation of the septic shock condition will further impair the function of the gastrointestinal mucosa, leading to a vicious circle (Wang *et al*., 2017). The gastrointestinal dysfunction in sepsis leads to the theory of gastric parietal cell failure that contributes to the gastric pH value result.

The difficulty of measuring gastric surface pH has limited extensive testing of the model for the regulation of gastric surface pH. This current study used a pH meter by Lutron PH-201. This examination is very rough, using a simple pH meter, but at least can illustrate the gastric pH conditions that did not become more acidic in sepsis mice. This current study in line wuth the previous theory which is proposed by Helmer *et al*. (2004) that LPS inhibits gastric acid secretion caused by the parietal cell's incapacity to go through conformational changes necessary for acid secretion in response to a secretagogue (Helmer *et al*., 2004). The limitation of our study was that we did not examine the vascular flow to the gastrointestinal tract. If we refer to the pathway of sepsis induced LPS, then most likely the mice in our study (induced by 10 mg/kg LPS) experienced gastrointestinal hypoperfusion due to sepsis shock. The possible event in the 2.5 mg/kg LPS was no gastrointestinal hypoperfusion. This was reinforced by the results of statistical analysis that at dose of 10 mg/kg LPS was powerful and caused changes in pH into alkaline compared to 2.5 mg/kg LPS groups (Figure 2).

Our modelled animal in research sepsis

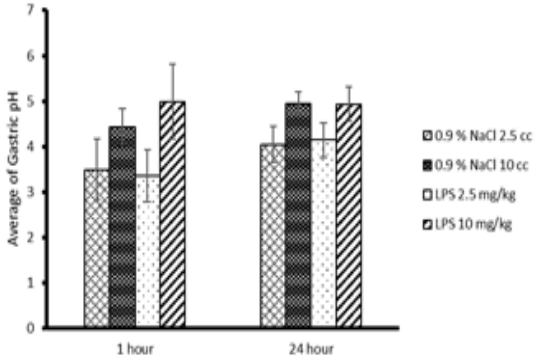

Figure 2. Average of gastric pH in control and treated group. LPS=Lipopolysaccharide

in mice using 10 mg/kg LPS intraperitoneal injection. We refer to the clinical score of sepsis mice (Shrum *et al*., 2014). Our research has contradictory results with the previous research by Fiddian-Green (1995) that stated that sepsis decreased the gastric pH in humans . It should be considered in which state of sepsis when measuring the gastric pH in humans. Before the introduction of gastric pH value in sepsis, it was widely thought that there were two distinct phases to septic shock. It was noted that patients with septic shock initially went through a hyperdynamic phase (warm shock) characterized by abounding pulse and warm hands, despite concomitant hypotension followed by cold shock, with poor peripheral perfusion, a thready pulse, and cool extremities, which led ultimately to death (Hunter and Doddi, 2010). In our opinion, the hyperdynamic state of sepsis contributes to the change of gastric pH becoming alkaline. In addition, our research also strengthens the research conducted by (Upadhyay *et al.*, 2007) that the intramucosal gastric pH in human sepsis was 7.33 (survival group) and 7.19 (non-survival group). However, in the normal condition, the gastric pH becomes 3-4. This phenomenon has the same trend, the gastric pH in mice and humans with sepsis tends to be more alkaline than in the normal condition. It should be recognized at what stage the sepsis is. The previous study indicated that the indicator of high mortality is if the intramucosal gastric pH reaches zero value, meaning that the sepsis stage is in the hypodinamic state (Yang *et al.*, 2002). In addition, an indirect measurement of actual intramucosal gastric pH provides a sensitive and specific tissue oxygenation index. Based on the previous studies and this study, we hypothesized that the cause increasing of gastric pH was gastric perfusion. It means tissue hypoxia has a contribution to making alkaline gastric pH. Speculation on the results of the gastric pH above may be related to the activity of the gastric parietal cells. The principal stimulants of an acid secretion at the level of the parietal cell are histamine (paracrine), gastrin (hormonal), and acetylcholine (*ACh*; neurocrine). Histamine, released from enterochromaffin-like (*ECL*) cells, binds to H2 receptors that activate adenylate cyclase (*AC*) and generate cAMP. Gastrin, released from G cells, binds to CCK2 receptors that activate phospholipase C to induce the release of cytosolic calcium (Ca^{++}). Gastrin stimulates the parietal cell directly and, more importantly, indirectly by releasing histamine from ECL cells. Acetylcholine (Ach), released from intramural neurons, bind to M3 receptors that are coupled to an increase in intracellular calcium. The intracellular cAMP- and calcium-dependent signalling systems activate downstream protein kinases, ultimately leading to the proton pump's fusion and activation of H+K+-ATPase (Schubert and Peura, 2008). This theory is in normal physiology. The immune and endocrine mediators that are released during sepsis (e.g., tumor necrosis factor [TNF] alpha, interleukin [IL]-1, IL-6, transforming growth factor [TGF] beta, prostaglandin [PG] E2, catecholamines, vasopressin, glucagon, insulin, and glucocorticoids) can produce inappropriate detrimental cellular responses contributing to exacerbation of septic injury (Sayeed, 1996; Sayeed, 2000; Ledderose *et al*., 2016).

The aim of this study was to investigate the gastric pH in the sepsis animal model. The value of gastric pH related to the time-based, acute phase was represented in 1st hour after LPS intraperitoneal injection. The beginning of the chronic phase was represented in 24th hour after LPS intraperitoneal injection. The previous study by Leenan and van Miert (1969) They proposed the gastric pH value become alkaline after LPS injection. The research explained the other pathway LPS induced alkaline gastric pH. The bacterial lipopolysaccharide (LPS) provoked an inhibition of gastric secretion in the rat. The researchers used drugs to block the action of LPS. Reserpine and the catecholamine-synthesis inhibitors a-methyldopa and diethyl-dithiocarbamate blocked this action of LPS, although adrenergic blocking agents or adrenalectomy have no effect on it. Direct stimulation of gastric secretion by carbachol also opposed the LPS effect, whereas central parasympathetic stimulation by 2-deoxy-D-glucose did not. Alterations of gastric secretion usually result from changes in parasympathetic or sympathetic activities (Bass and Patterson, 1967). Lipopolysaccharide produces marked adrenergic stimulation (Gilbert, 1960) and may act on gastric secretion via stimulation of the sympathetic nervous system or release of catecholamines from the adrenal medulla into the circulation; however, LPS could also inhibit the parasympathetic nervous system or directly influence the secretory cells of the gastric mucosa. Hyperthermia can be excluded as the cause of inhibition of gastric secretion by LPS in the rat because in this species LPS does not produce hyperthermia (Filkins and Di Luzio, 1968; van

Miert and Frens, 1968); therefore, the influence of blockade of the sympathetic nervous system and stimulation of the parasympathetic system at different levels were studied on the inhibition of the gastric secretion by LPS (Leenen and van Miert, 1969).

CONCLUSION

The results of gastric pH measurement in LPS induced mice did not decrease in the pH compared to the normal conditions. It means that the dose of LPS significantly influences the gastric pH change in mice.

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