ORIGINAL ARTICLE

Melatonin and Polymyxin B Administration, Alone or in Combination, Prevent the Elevation of Random Blood Glucose and Lactate Levels in Wistar Rats Endotoxicosis Model

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ABSTRACT

Background: Sepsis induces hyperglycemia due to insulin resistance and hyperlactatemia due to anaerobic glycolysis pathway caused by tissue hypoxia. Melatonin is an antioxidant that may prevent increased glucose and increased lactate levels, however its role in sepsis need further investigation.
Aim: To observe the effects of melatonin with and without Polymyxin B on random blood glucose and lactate levels in Wistar rats endotoxicosis model.
Methods: This was animal experimental study with randomized control design. There were twenty four male Wistar rats that were intraperitoneally injected with 2 mg lipopolysaccharide (LPS) per 200 grams rats and randomly divided into four groups, i.e. control group receiving placebo, melatonin only group, Polymyxin B only group, and combination group (receiving melatonin and Polymyxin B). The effects of oral melatonin and Polymyxin B treatments on random blood glucose and lactate levels were investigated from the retro-orbital blood vessel sampling in baseline (B), 6 hours after intraperitoneally LPS injection (T0), 1 hour (T1) and 2 hours after melatonin administration (T2). The means of glucose and lactate levels were analyzed with Student's t-test using computer program.
Results: There were no significant differences in random blood glucose and lactate levels between control group, melatonin only group, Polymyxin B only group and combination group in B, T0, and T1. In T2, there were significantly lower levels of random blood glucose in melatonin only group (132.83 ± 10.7 mg/dL), in Polymyxin B only group (131.06 ± 11.53 mg/dL), and in combination group (130.03 ± 11.06 mg/dL) in comparison with control group (171.67 ± 15.88 mg/dL) ($p<0.05$). In T2, there were significantly lower levels of lactate in Polymyxin B only group (11.6 ± 1.5 mmol/L), and in combination group (10.0 ± 1.1 mmol/L) in comparison with control group (16.9 ± 1.2 mmol/L) ($p<0.05$). There was also lower levels of lactate in melatonin only group (11.6 ± 6.6 mmol/L) compared to control group, although it was not statistically significant ($p>0.05$).
Conclusion: Melatonin administration as well Polymyxin B, alone or in combination, could prevent the elevation of random blood glucose and lactate levels in Wistar rats endotoxicosis model. Therefore, if confirmed by further research, melatonin might have a role in management of endotoxicosis.
Keywords: endotoxicosis, random blood glucose, lactate, melatonin, lipopolysaccharide

INTRODUCTION

Sepsis is a significant health problem since it is one of the frequent causes of mortality in the non-coronary intensive care unit (ICU)[1,2,3]. The incidences of sepsis and septic shock were high in the United States, which were 10,319,418 reported cases from 750 million hospitalizations during 22 year study period[2]. The mortality rate of sepsis was 215,000 deaths or 9.3% of all deaths in the United States in 1995[4]. The study group in Management of Severe Sepsis in Asian Intensive Care Units (MOSAIC) showed that in-hospital mortality rate due to sepsis in 16 Asian countries in 2009 was 44.5%[5]. Severe sepsis in southeast Asia from 2013 to 2015 was 194(28%) of 731 children and 546(68%) of 804 adults, and was associated with increased mortality[6].

Sepsis affected on high annual economic burden.[4] Several factors associated with sepsis in developing countries were non-hygienic environment, low socio-economic status, and other concomitants diseases, such as malnutrition, diabetes mellitus, immunosuppression, and cancer.[1]

Sepsis was thought to induce circulating cytokines storm causing metabolism disturbance, multiple organ failure and mortality[7,8]. The faster we can provide a prompt diagnosis and management of severe sepsis, the better the prognosis[9],[10]. Biomarkers are recent approaches to assess the diagnosis and prognosis in sepsis[11]. These biomarkers are *tumor necrosis factor-α* (TNF-α), *interleukin*-1 (IL-1), *interleukin-6* (IL-6), *interleukin-8* (IL-8), *C-reactive protein* (CRP), and *procalcitonin*[9,11]. The increased expression of proinflammatory cytokines, such as interleukins and TNF-α, was thought to associate with activated nuclear factor kappa-B (NF-κB)[7,12]. NF-κB was induced by Toll-like receptor (TLR) engagement[12]. These biomarkers yet have some limitations, such as high cost, less available in rural area, and lag time of typically 2–3 hours[9,13]. Thus, it is needed an easier examination that were more feasible[10, 13] and inexpensive[14].

Patients with sepsis are often showing hyperglycaemia or basal blood glucose levels more than 140mg/dL, athough without diabetes[15]. Hyperglycemia in patients with sepsis was hypothesized to be caused by proinflammatory cytokines, insulin resistance, and the increased release of counter-regulatory stress hormones, such as glucagon, cortisol, growth hormones, catecholamines (epinephrine, norepinephrine)[15,16,17]. These

pathways might cause an increased glucose production by gluconeogenesis and glyconeolysis[15], an inhibition in the release of insulin hormone, and decreased utilization[16]. These make random blood glucose as an important examination in sepsis[17].

In severe sepsis or septic shock, hyperlactatemia is usually occured. Sepsis causes an increase in lactate through the anaerobic glycolysis pathway caused by tissue hypoxia. Lactate is the final product of glucose metabolism that is normally produced 1400 mmol/L daily. Lactate is used in evaluating the severity and treatment in sepsis[18].

Melatonin (N-acetyl-5-methoxytryptamine) is a versatile endogen neurohormone or indolamine hormone molecule secreted by the pineal gland.[19] Melatonin plays a role in regulating sleep cycle or circadian rhythm, immunomodulation, immunoregulation, mitochondrial protection function, reproduction, mood, as well as a potent antioxidant, anti-inflammatory, sedative, analgesic, and chronobiotic effects.[19, 20] The benefit of melatonin has been reviewed in several clinical applications such as critical care, perioperative management, and pain medicine.[19] Melatonin treatment is effective in ischemic or reperfusion state[19]. Melatonin-injected animals also retained the glucose and lactate rhythymicity when compared to saline-injected animals. Melatonin influenced both glucose metabolism and the production of lactate.[21]

Polymyxin B is a cationic peptide antibiotic that can neutralize endotoxin. Polymyxin B with the endotoxin neutralizing ability might be used as a non-toxic therapeutic agent to encounter endotoxicosis[22]. Harm et al showed in vitro inactivation of endotoxins by polymyxin B.[23] Amongst several approaches in combating endotoxic shock, peptide mediated neutralization of LPS seems to be the most promising[22]. Inactivation of endotoxins by polymyxin B infusion may be used to resolve the necessity for endotoxin elimination in treatment of sepsis[23].

Previous studies showed that melatonin and 6-hydroxy-melatonin might be used as sepsis therapy[19,24-26], due to their roles as antioxidant[24-26] and anti-inflammatory.[24] This study aimed to examine the effects of melatonin with and without Polymyxin B on random blood glucose and lactate levels in the Wistar rats model of endotoxicosis, since hyperglycemia and hyperlactatemia might be used as simple parameters for insulin resistance and high inflammatory state in sepsis[16].

METHODS

This was an animal experimental study with randomized control group design. The inclusion criterias were male Wistar rats aged 2-3 months, body weight 150-300 grams, no physical abnormality and looked active during adaptation periode. In this study, an appropriate endotoxicosis model was created in rats through intraperitoneal injection of 2 mg lipopolysaccharide (LPS) suspension per 200 grams rats. The effects of oral melatonin and Polymyxin B treatments on random blood glucose and lactate levels were investigated. Ethical clearance for animal conduct have been recieved from ethical committee.

The study samples were twenty four (24) male wistar rats which consist of six (6) male wistar rats for each group. All rats were adapted for seven days and fed with standard food. On the day-8, all samples were intraperitoneally injected with 2 mg LPS per 200 grams rats and then divided into four groups through simple random sampling. All rats were labeled by hidden number, so the blood sample taken could be numbered by the same hidden numbers.

Group 1 or control group was orally administered with placebo only (aquadest) (n=6). Group 2 was group with orally administered 4 mg melatonin treatment only per 200 grams rats via oral sonde (n=6), group 3 was group with Polymyxin B treatment only (n=6), and group 4 was group with combination of melatonin and Polymyxin B with treatment (n=6).

Blood samplings were taken via retro-orbital vein to measure the levels of random blood glucose and lactate in one hour before LPS injection (baseline) (B), six hours after LPS injection (T0), one hour after melatonin administration (T1) and two hours after melatonin administration (T2).

Random blood glucose levels was measured with point of care testing (POCT) by glucose test meter (Accu-Chek Kit, Roche Diagnostics GmbH, Germany). Lactate levels was measured with lactate test meter (Accutrend Plus Kit, Roche Diagnostics GmbH, Germany). Endotoxins (LPS) were from Pseudomonas aeruginosa and Escherichia coli (L2630 Sigma, Sigma-Aldrich, Vienna, Austria).[23] Polymyxin B (X-Gen Pharmaceuticals, Inc. Big Flats, NY) intravenous injection dose was 100 ng/ml as previously reported in in vitro inactivation of endotoxins by Polymyxin B.[23] Melatonin treatment was using melatonin powder (Melatonin M5250, Sigma-Aldrich, Darmstadt, Germany).

Those data were tested for normality with Saphiro-Wilk test. Random blood glucose and lactate samples were compared within group using a one-way variance analysis (ANOVA). The means were compared using a parametric Student's t-test, including paired t-test and independent t-test. They will be tested with non-parametric Mann-Whitney Rank Test, if their distribution were abnormal. Data were analyzed with computer program. The p-value of less than 0.05 is considered to be statistically significant.

RESULTS

Effect of Melatonin in Random Blood Glucose Levels: There were no significant differences in the levels of random blood glucose between the control group and the melatonin treatment only group (115 ± 10.17 vs 114 ± 11.21, p=0.867), between the control group and the Polymyxin B treatment only group (115 ± 10.17 vs 115 ± 15.10, p=0.898), between control and group with combination therapy in baseline (B) (115 ± 10.17 vs 112 ± 11.17, p=0.837). There were no significant differences between group with melatonin treatment only and Polymyxin B treatment only (114 ± 11.21 vs 115 ± 15.10, p=0.872), between melatonin treatment only and group with combination therapy in baseline (B) (114 ± 11.21 vs 112 ± 11.17, p=0.862). There was no significant difference between group with Polymyxin B treatment only and group with combination therapy in baseline (B) (115 ± 15.10 vs 112 ± 11.17, p=0.864) (table 1, figure 1).

There were no differences in random blood glucose levels between in six hours after LPS injection (T0) in comparison with baseline (B) in each groups (133.1 ± 19.97 vs 115 ± 10.17; 133.5 ± 17.09 vs 114 ± 11.21; 132.9 ± 11.03 vs 115 ± 15.10; 131.9 ± 12.59 vs 112 ± 11.17 (p>0.05)). These showed that the levels of random blood glucose were higher in the T0 in comparison with baseline, although they were not significant (table 1, figure 1).

In six hours after LPS injection (T0), there were no significant differences in the levels of random blood glucose between control group and melatonin only group (133.1 ± 19.97 vs 133.5 ± 17.09, p=0.589), between control group and Polymyxin B only group (133.1 ± 19.97 vs 132.9 ± 11.03, p=0.567), and between control group and group with combination therapy (133.1 ± 19.97 vs 131.9 ± 12.59, p=0.559). There were no significant differences of random blood glucose levels between group with melatonin only and group with Polymyxin B only (133.5 ± 17.09 vs 132.9 ± 11.03, p=0.578), between group with melatonin only and group with combination therapy in T0 (133.5 ± 17.09 vs 131.9 ± 12.59, p=0.596). There was no significant difference between group with Polymyxin B only and group with combination therapy (132.9 ± 11.03 vs 131.9 ± 12.59, p=0.698) (table 1, figure 1).

In 1 hour after melatonin administration (T1), there were higher levels of random blood glucose in control group in comparison with melatonin only group (163.33 ± 13.84 vs 149.17 ± 39.23, p=0.424), between control and Polymyxin B only group (163.33 ± 13.84 vs 141.92 ± 12.03, p=0.392), and between control and group with combination therapy (163.33 ± 13.84 vs 140.05 ± 12.51, p=0.364), although they were not significant. There were no significant differences of random blood glucose levels between melatonin only group and Polymyxin B only group (149.17 ± 39.23 vs 141.92 ± 12.03, p=0.582), between group with melatonin only and group with combination therapy in T1 (149.17 ± 39.23 vs 140.05 ± 12.51, p=0.576). There was no significant difference between group with Polymyxin B only and group with combination therapy (141.92 ± 12.03 vs 140.05 ± 12.51, p=0.577) (table 1, figure 1).

In 2 hours after melatonin administration (T2), there were significant differences in the levels of random blood glucose between the control to the melatonin only group (171.67 ± 15.88 vs 132.83 ± 10.7, p=0.042), between control to the Polymyxin B only group (171.67 ± 15.88 vs 131.06 ± 11.53, p=0.041), and between control to the combination group (171.67 ± 15.88 vs 130.03 ± 11.06, p=0.038). There were no significant differences of random blood glucose levels between group with melatonin only and Polymyxin B only group (132.83 ± 10.7 vs 131.06 ± 11.53, p=0.651), between group with melatonin only and group with combination therapy in T2 (132.83 ± 10.7 vs 130.03 ± 11.06, p=0.612). There was no significant difference between group with Polymyxin B treatment only and group with combination therapy (131.06 ± 11.53 vs 130.03 ± 11.06, p=0.647) (table 1, figure 1).

There were significant increases in random blood glucose levels in the control group after T2 in comparison to T0 (171.67 ± 15.88 vs 133.1 ± 19.97) (p=0.028) and after T2 in comparison to T1 (171.67 ± 15.88 vs 163.33 ± 13.84) (p=0.045), as well as between T1 and T0 (163.33 ± 13.84 vs 133.1 ± 19.97) (p=0.028) with paired t-test. Whereas in the group with melatonin only, there were no significant increases in random blood glucose levels after T1 (149.17 ± 39.23 vs 133.5 ± 17.09, p=0.500) and T2 (132.83 ± 10.7 vs 133.5 ± 17.09, p=0.979) in comparison to T0, as well as after T2 in comparison to T1 (132.83 ± 10.7 vs 149.17 ± 39.23, p=0.538) (table 1, figure 1). These showed that the random blood glucose increased during the progression of sepsis in control group, while it was not significantly increased in group with melatonin only. Melatonin alone might prevent the increase of random blood glucose levels after LPS injection.

There were no significant increases in random blood glucose levels in the group with Polymyxin B only between T2 and T0 (131.06 ± 11.53 vs 132.9 ± 11.03, p=0.587), between T2 and T1 (131.06 ± 11.53 vs 141.92 ± 12.03, p=0.528), and between T1 and T0 (141.92 ± 12.03 vs 132.9 ± 11.03, p=0.598) with paired t-test (table 1, figure 1). There were no significant increases in random blood glucose levels in the group with combination therapy between T2 and T0 (130.03 ± 11.06 vs 131.9 ± 12.59, p=0.595), between T2 and T1 (130.03 ± 11.06 vs 140.05 ± 12.51, p=0.548), and between T1 and T0 (140.05 ± 12.51 vs 131.9 ± 12.59, p=0.573) with paired t-test (table 1, figure 1). These showed that the random blood glucose increased during the progression of sepsis in control group, while they were not significantly increased in group with Polymyxin B only and in group with combination therapy. Polymyxin B and melatonin might prevent the increase of random blood glucose levels after LPS injection.

There were larger difference between T0-T1 (ΔT0-T1) in control group in comparison to melatonin only (30.33 ± 9.75 vs 15.67 ± 6.30, p=0.098), Polymyxin B only (30.33 ± 9.75 vs 9.02 ± 1.0, p=0.046), and combination group (30.33 ± 9.75 vs 8.15 ± 0.1, p=0.038). There were larger difference between T0-T2 (ΔT0-T2) in control group in comparison to melatonin only (38.67 ± 5.66vs 1.67 ± 1.14, p=0.026), Polymyxin B only (38.67 ± 5.66 vs 1.84 ± 0.5, p=0.032), and combination group (38.67 ± 5.66 vs 1.87 ± 1.53, p=0.029) (table 1).

Effect of Melatonin in Lactate Levels: There were no significant differences in lactate levels between control group and melatonin treatment only group (1.5±0.7 vs 1.4±0.2, p=0.677), between control and Polymyxin B treatment only group (1.5±0.7 vs 1.5±0.5, p=0.783), between control and group with combination therapy in baseline (B) (1.5±0.7 vs 1.2±0.7, p=0.653). There were no significant differences in lactate levels between melatonin treatment only group and Polymyxin B only group (1.4±0.2 vs 1.5±0.5, p=0.675), between melatonin treatment only group and group with combination therapy (1.4 ± 0.2 vs 1.2 ± 0.7, p=0.612). There was no significant different in lactate levels between group with Polymyxin B treatment only and group with combination therapy in baseline (B) (1.5±0.5 vs 1.2±0.7, p=0.657) with independent t-test (table 2, figure 2).

There were differences in lactate levels between six hours after LPS injection (T0) in comparison with baseline (B) in each groups (13.8 ± 1.6 vs 1.5 ± 0.7; 12.2 ± 2.5 vs 1.4 ± 0.2; 11.2 ± 1.3 vs 1.5 ± 0.5; 11.1 ± 2.6 vs 1.2 ± 0.7) (p<0.05). These showed that LPS had significant effects on the increased of lactate levels in the T0 compared to baseline (table 2, figure 2).

In six hours after LPS injection (T0), there were no significant difference in lactate levels between control group and melatonin treatment only group (13.8 ± 1.6 vs 12.2 ± 2.5, *p*=0.199), between control group and Polymyxin B group (13.8 ± 1.6 vs 11.2 ± 1.3, *p*=0.182), and between control group and group with combination therapy (13.8 ± 1.6 vs 11.1 ± 2.6, *p*=0.187). There were no significant differences in lactate levels between melatonin treatment only group and Polymyxin B only group (12.2 ± 2.5 vs 11.2 ± 1.3, *p*=0.275), and between melatonin only group and group with combination therapy in T0 (12.2 ± 2.5 vs 11.1 ± 2.6, *p*=0.296). There was no significant different between group with Polymyxin B treatment only and group with combination therapy (11.2 ± 1.3 vs 11.1 ± 2.6, *p*=0.198) with independent *t*-test (table 2, figure 2).

In 1 hour after melatonin administration (T1), there were no significant differences in lactate levels between control group and melatonin only group (13.6±2.8 vs 12.2±5.5, *p*=0.671), between control group and Polymyxin B group (13.6±2.8 vs 11.9±2.3, *p*=0.657), and between control group and group with combination therapy (13.6 ± 2.8 vs 10.0±2.1, *p*=0.635). There were no significant differences between melatonin only group and Polymyxin B treatment only (12.2±5.5 vs 11.9±2.3, *p*=0.295), and between melatonin group and group with combination therapy in T1 (12.2±5.5 vs 10.0 ± 2.1, *p*=0.276). There was no significant different between group with Polymyxin B treatment only and group with combination therapy (11.9 ± 2.3 vs 10.0 ± 2.1, *p*=0.177) with independent *t*-test (table 2, figure 2).

In 2 hours after melatonin administration (T2), there were higher level of lactate in control group compared to melatonin only group (16.9±1.2 vs 11.6±6.6, *p*=0.153), in control group compared to Polymyxin B only group (16.9±1.2 vs 11.6±1.5, *p*=0.043), and in control group compared to group with combination therapy (16.9±1.2 vs 10.0±1.1, *p*=0.032). There were no significant differences of lactate levels between melatonin only group and Polymyxin B only group (11.6±6.6 vs 11.6±1.5, *p*=0.425), and between melatonin only group and group with combination therapy in T2 (11.6± 6.6 vs 10.0±1.1, *p*=0.312). There was no significant different between group with Polymyxin B treatment only and group with combination therapy (11.6±1.5 vs 10.0±1.1, *p*=0.172) (table 2, figure 2).

There were significant increases in lactate levels at control group in T2 in comparison to T0 (16.9±1.2 vs 13.8±1.6) (*p*=0.001) and in T2 in comparison to T1 (16.9±1.2 vs 13.6±2.8) (*p*=0.005) with paired *t*-test, however there was no significant increase between T1 and T0 (13.6±2.8 vs 13.8±1.6) (*p*=0.964). Whereas in the group with melatonin only, there were no significant increases in lactate levels after T1 (12.2±5.5 vs 12.2±2.5, *p*=0.165) and T2 (11.6±6.6 vs 12.2±2.5, *p*=0.134) in comparison to T0, as well as after T2 in comparison to T1 (11.6±6.6 vs 12.2±5.5, *p*=0.768) (table 2, figure 2). These showed that the lactate level increased during the progression of sepsis in control group, while the lactate level was not significantly increased in group with melatonin only. Melatonin alone might prevent the increase of lactate levels after LPS injection.

There were no significant increases in lactate levels in the group with Polymyxin B only between T2 and T0 (11.6±1.5 vs 11.2±1.3) (*p*=0.197), between T2 and T1 (11.6±1.5 vs 11.9±2.3) (*p*=0.182), and between T1 and T0 (11.9±2.3 vs 11.2±1.3) (*p*=0.896) with paired *t*-test (table 2, figure 2). There was no significant increases in lactate levels in the group with combination therapy between T2 and T0 (10.0±1.1 vs 11.1±2.6) (*p*=0.378), between T2 and T1 (10.0 ± 1.1 vs 10.0 ± 2.1) (*p*=0.748), and between T1 and T0 (10.0±2.1 vs 11.1 ± 2.6) (*p*=0.123) with paired *t*-test (table 2, figure 2). These showed that the lactate level increased during the progression of sepsis in control group, while the lactate levels were not increased in group with Polymyxin B only and in group with combination therapy. Polymyxin B and melatonin might prevent the increase of lactate levels after LPS injection.

Table 1. Random Blood Glucose Levels in Control Group, Melatonin Only Group, Polymyxin B Only Group, and Combination Group

Random Blood Glucose Levels (mg/dl)	**Control Group (C) (n=6)**	**Melatonin Only Group (M) (n=6)**	***p*** **(between C and M)**	**Polymyxin B Only Group (P) (n=6)**	**Combination Group (M+P) (n=6)**	***p*** **(between P and M+P)**	***p*** **(between M and M+P)**
Baseline (B)	115 ± 10.17; 115 (104 – 125)	114 ± 11.21; 114 (102 – 125)	0.867[b]	115 ± 15.10; 115 (100 – 130)	112 ± 11.17; 112 (100 – 123)	0.864[b]	0.862[b]
Six hours after LPS injection (T0)	133.1 ± 19.97; 133 (113 – 153)	133.5 ± 17.09; 133 (116 – 150)	0.589[b]	132.9 ± 11.03; 132 (121 – 144)	131.9 ± 12.59; 131 (119 – 144)	0.698[b]	0.596[b]
One hour after melatonin administration (T1)	163.33 ± 13.84; 163 (149 – 177)	149.17 ± 39.23; 149 (110 – 188)	0.424[b]	141.92 ± 12.03; 141 (129 – 154)	140.05 ± 12.51; 140 (127 – 152)	0.577[b]	0.576[b]
Two hours after melatonin administration (T2)	171.67 ± 15.88; 171 (156 – 187)	132.83 ± 10.7; 132 (122 – 143)	0.042[b]	131.06 ± 11.53; 131 (119 – 142)	130.03 ± 11.06; 130 (119 – 141)	0.647[b]	0.612[b]
p (T0 - T1)	0.028*[a]	0.500[a]	-	0.598[a]	0.573[a]	-	
p (T1 - T2)	0.045*[a]	0.538[a]	-	0.528[a]	0.548[a]	-	
p (T0 - T2)	0.028*[a]	0.979[a]	-	0.587[a]	0.595[a]	-	
Δ T0 – T1	30.33 ± 9.75	15.67 ± 6.30	0.098[c]	9.02 ± 1.0	8.15 ± 0.1	0.117[b]	
Δ T0 – T2	38.67 ± 5.66	1.67 ± 1.14	0.026*[c]	1.84 ± 0.5	1.87 ± 1.53	0.427[b]	
Δ T1 – T2	8.34 ± 2.04	16.34 ± 11.47	0.832[c]	10.86 ± 0.5	10.02 ± 1.45	0.476[b]	

Value was in mean ± SD; median (min-max)

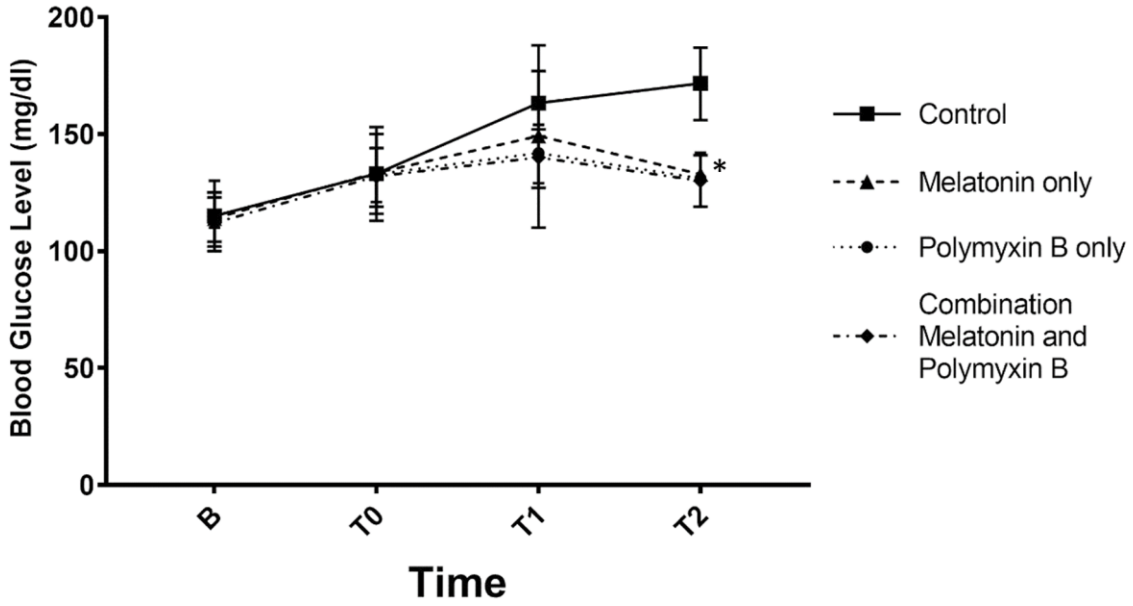
*Significant : $p < 0,05$
[a] *Paired t-Test*
[b] *Independent t-Test*
[c] *Mann-Whitney*

Table 2. Lactate Levels in Control Group, Melatonin Only Group, Polymyxin B Only Group, and Combination Group

Lactate Levels (mmol/L)	Control Group (C) (n=6)	Melatonin Only Group (M) (n=6)	*p* (between C and M)	Polymyxin B Only Group (P) (n=6)	Combination Group (M+P) (n=6)	*p* (between P and M+P)	*p* (between M and M+P)
Baseline (B)	1.5±0.7; 1.5 (0.8 – 2.2)	1.4±0.2; 1.4 (1.2 – 1.6)	0.677[b]	1.5±0.5;1.5 (1.0 – 2.0)	1.2±0.7; 1.2 (0.5 – 1.9)	0.657[b]	0.612[b]
Six hours after LPS injection (T0)	13.8 ± 1.6; 13.4 (11.9 – 14.9)	12.2 ± 2.5; 12.2 (9.7 – 14.7)	0.199[b]	11.2 ± 1.3; 11.2 (9.9 – 12.5)	11.1 ± 2.6; 11.1 (8.5 – 13.7)	0.198[b]	0.296[b]
One hour after melatonin administration (T1)	13.6 ± 2.8; 13.4 (10.4 – 16.4)	12.2 ± 5.5; 12.2 (6.4 – 18.0)	0.671[b]	11.9 ± 2.3; 11.9 (9.6 – 14.2)	10.0 ± 2.1; 10.0 (7.9 – 12.1)	0.177[b]	0.276[b]
Two hours after melatonin administration (T2)	16.9 ± 1.2; 16.6 (15.1 – 18.1)	11.6 ± 6.6; 11.8 (4.9 – 18.7)	0.153[b]	11.6 ± 1.5; 11.6 (10.1 – 13.1)	10.0 ± 1.1; 10.0 (8.9 – 11.1)	0.172[b]	0.312[b]
p (T0 - T1)	0.964[a]	0.165[a]	-	0.896[a]	0.123[a]	-	
p (T1 - T2)	0.005*[a]	0.768[a]	-	0.182[a]	0.748[a]	-	
p (T0 - T2)	0.001*[a]	0.134[a]	-	0.197[a]	0.378[a]	-	
Δ T0 – T1	-0.07 ± 2.8; 0.7 (-3.1 – 2.7)	2.82 ± 4.8; 2.7 (-2.9 – 8.7)	0.175[b]	1.91 ± 0.9; 1.9 (1.0 – 2.9)	3.88 ± 1.5; 3.9 (2.4 – 5.4)	0.107[b]	
Δ T0 – T2	3.83 ± 1.3; 2.95 (1.8 – 4.9)	2.83 ± 5.1; 0.85 (-1.3 – 13.4)	0.291[c]	4.05 ± 0.6; 4.1 (3.6 – 4.6)	4.02 ± 2.5; 4.0 (1.6 – 6.5)	0.327[c]	

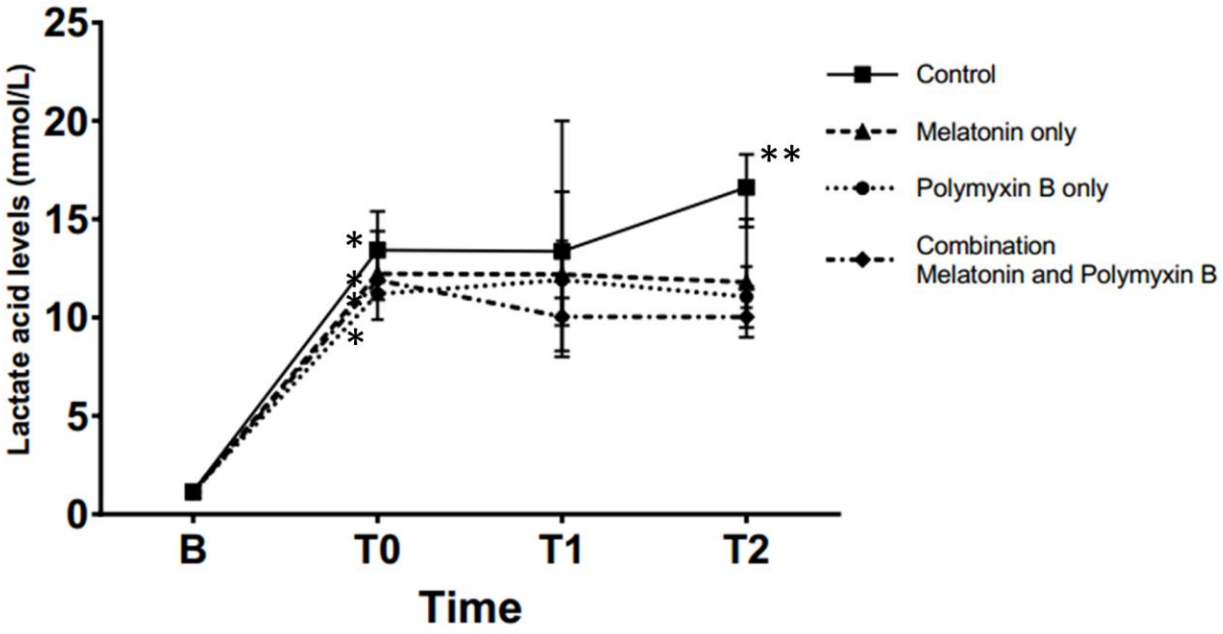
Value was in mean ± SD; median (min-max)
*Significant : $p < 0,05$
[a] *Paired t-Test*
[b] *Independent t-Test*
[c] *Mann-Whitney*

Fig. 1: Random blood glucose levels in control group, melatonin only group, polymyxin B only group, and combination group at baseline (B), six hours after LPS injection (T0), one hour after melatonin administration (T1) and two hours after melatonin administration (T2).



*p<0.05 between control to melatonin only group, between control to polymyxin B only group, and between control to combination group in T2 using independent t-Test.

Fig. 2: Lactate levels in control group, melatonin only group, polymyxin B only group, and combination group in baseline (B), six hours after LPS injection (T0), one hour after melatonin administration (T1) and two hours after melatonin administration (T2)



* p<0.05 between T0 to baseline (B) in each groups using Paired t-Test.
** p<0.05 between control to polymyxin B only group, and between control to combination group in T2 using independent t-Test.

DISCUSSION

The negative effect of sepsis on glucose and lactate metabolism and insulin sensitivity has been documented[15,16]. However, the mechanisms by which these played role were not fully understood[15,16]. Our study successfully created endotoxicosis model in wistar rats that was common in sepsis using LPS injection intraperitoneally. Our study showed that LPS injection had increased the random blood glucose and lactate levels in six hours after LPS injection (T0) in comparison to baseline (B) in all groups. Our study also successfully showed that there was an increased of random blood glucose and lactate levels by LPS injection during the progression of sepsis in control group in our sepsis model. There were significant increases of random blood glucose and lactate levels in control group between T2 in comparison to T1 and T0 after LPS injection.

The introduction of bacterial endotoxins, such as LPS, might directly inisiate insulin resistance[27]. This endotoxin LPS was known as a robust bacterial toxin kept within the bacterial cell and released from the cell surface after destruction of the bacterial cell wall.[28] However subsequent study showed that release of LPS from gram negative bacteria did not require the disintegration of the bacterial cell wall, but, LPS was released as a physiological activity of membrane vesicle trafficking in the form of bacterial outer membrane vesicles, which might also contain other virulence factors and proteins[8,28].

Hyperglycemia in endotoxicosis is associated with high inflammatory state and oxidative stress[8]. Study showed that the elevation of blood glucose levels in patients with sepsis was due to acute immune response and proinflammatory cytokines[8,16]. These proinflammatory cytokines caused insulin resistance by the inhibition of insulin release and the induction of counterregulatory hormone of insulin, such as epinephrine, norepinephrine, and glukagon[15,16]. Sepsis-induced-counterregulatory hormone might cause the reduction of insulin sensitivity.[15-17] The presence of sepsis can be ruled out by high insulin sensitivity in a critically ill patient[13]. Sepsis is ruled out when modeled insulin sensitivity is above SI = 8 x 10^{-5} liter mU^{-1} min^{-1}, while insulin sensitivity below 8 x 10^{-5} liter mU^{-1} min^{-1} may indicate insulin insensitivity due to either sepsis or other underlying conditions[13].

Hyperlactatemia in sepsis is recognized as a marker of tissue hypoxia that indicates the existence of oxygen debt or hypoperfusion leading to an increased lactate acid via anaerobic glycolysis.[3] Sepsis-associated hyperlactatemia is associated with high inflammatory state and oxidative stress[8].

The inflammatory cascade in sepsis is complex. A class of pattern recognition molecules on immune cells, such Toll-like receptors (TLRs), responds to the presence of microbiological products as part of innate immunity.[12] TLRs shows a wide variety of functions, but in sepsis, a main role of TLRs engagement is the induction of proinflammatory mediators and activation of nuclear factor (NF-κB)[12]. NF-κB is integrally involved in a cascade B formerly known as “cytokine storm” associated with increased expression of proinflammatory cytokines, such as IL-1 and TNF. Other receptors, including those for complements, coagulation factors, and leukotrienes, augment and modify the Toll-like receptor-associated response[7].

Stress hyperglycemia makes critically ill patients more vulnerable to adverse outcome and septic complications

due to increased oxidative stress.[15] Previously, hyperglycemic stress was thought to be a protective adaption of the body to current threat, which might increase glucose mobilisation into the non-insulin-cells, thus improving chances for survival. Recently, the state of insulin resistance, glucose intolerance and hyperglycemia is called "stress diabetes" or "diabetes of injury"[15]. Even a moderate hyperglycemia may cause ruinous outcome, since maintenance of normoglycemia has shown to improve survival in patients with critically ill, with or without sepsis[15,29,30].

Our study showed that random blood glucose and lactate levels were lower in group with melatonin alone in comparison to control in two hours after melatonin administration. The mechanisms in how melatonin administration prevented the increased of random blood glucose and lactate levels in our study have not been clearly explained yet. It could be orchestrated by an improved oxidative stress[25,26], an improved immune response and reduced inflammatory cytokines[24], cytoprotective process[32], anti-apoptosis,[33] anti-corticoid function, and preventing mitochondrial dysfunction as reported elsewhere[19,24]. Melatonin modulated the activity of the pineal and pituitary/adrenal axis and the peripheral actions of corticoids and released vasotocin that lowers corticoid levels[19,34]. Bailey CJ et al showed that melatonin could decrease in vitro insulin secretion from the rat pancreas[35].

Melatonin might ameliorate the immunity to viral, bacterial, and parasites infections through several mechanisms[36], such as immunomodulation or antioxidant. Kaya O et al reported that the administration of intraperitoneal melatonin in rats subjected to acute swimming exercise might delay exhaustion by a decrease in lactate acid levels and an increased in plasma zinc levels[37].

Dalton KM showed that melatonin caused an initial suppression of lactate levels and glucose levels[21]. Dalton KM hypothesized that melatonin could decrease hemolymph glucose and lactate acids through the decrease in stress response compared to non-melatonin injected group[21]. Tilden A et al showed that melatonin-injected fiddler crabs demonstrated a delayed hyperglycemic response compared to saline injected crabs that was showing faster hyperglycemic increment[38]. Tilden A et al showed that melatonin influenced both glucose and lactate metabolism that was causing a delayed glucose rise and a lower lactate rise[38]. Exogenous melatonin might be involved in the gluconeogenic pathway, glycogen breakdown and / or fatty acid oxidation that will demonstrate a delayed hyperglycemic response[21]. This delayed hyperglycemic metabolism may also be closely correlated to lactate metabolism.

The role of action duration from melatonin injection were important to get the outcome of random blood glucose lowering effects. In our study, in two hours after melatonin administration (T2), the level of random blood glucose and lactate in group with melatonin only were lower than in control group. These might imply that melatonin in a certain dose was able to inhibit the increase of blood glucose and lactate levels at least in the short term. However, we still not certain whether therapy with melatonin alone might be sufficient to inhibit the increase of blood glucose and lactate levels in the longer term during further sepsis progression, in which several complications might usually appear. Furthermore, we still can not elucidate whether it was needed a higher dose of melatonin to inhibit the increase of blood glucose and lactate levels in the longer term.

In our study, the insignificant decrease of random blood glucose levels in group with melatonin alone in comparison to control group, in one hour after melatonin administration, might indicate that the effectiveness of oral melatonin to show immediate results were not optimal yet that might be caused by the ineffective route of oral drug and insufficient duration of action or dose. However, the random blood glucose levels in the group with melatonin alone in 1 hour was no longer experiencing an up-surge after LPS injection but even decreased, although it was not significant. Melatonin still can prevent the increase of random blood glucose levels both in one and two hours after melatonin administration. Authors did not extent the measurement of blood glucose levels in this study since endotoxic shock have been worsened by more than three hours in the rat endotoxicosis model.

Differ from our study, Dalton KM has used melatonin injection. Dalton KM showed that blood glucose levels in 0.5, 1.0, 1.5 and 5 hours after melatonin injection was lower than non-injected Uca pugilator crabs[21]. Melatonin injection induced initial suppression in blood glucose and lactate levels until 1.5 hours following injection by decreasing the stress response, while the seawater-injected group and the non-injected group showed stress response causing a rise in glucose concentrations[21]. This was consistent with a study by Pierpaoli and Maestroni that reported melatonin's function as an anti-stress hormone[39].

As a powerful antioxidant,[40] melatonin antagonised oxidative stress both in a direct and in an indirect way[20]. It prevented free radical-induced damage and increased the activity of several antioxidant enzymes like glutathione-S transferase, glutathione reductase and catalase.[33] Therefore melatonin is one of the drugs developed as a sepsis therapy[19].

Melatonin effects in preventing the increase in blood glucose levels might also be mediated through its role in controlling the injuries caused by lipopolysaccharide. Sewerynek et al reported that melatonin might decrease oxidative stress induced by LPS, as evidenced by decreased hepatic malondialdehyde (MDA) and 4-hydroxyalkenal (4-HDA)[41]. Wu CC et al have reported that melatonin prevented endotoxinemia induced by LPS and endotoxin-induced circulatory failure in rats by decreasing the production of superoxide in the aorta[42].

Melatonin showed anti-inflammatory effects by inhibiting the expression and release of COX-2, inducible nitrit oxide synthetase (iNOS), NF-kB, TNF-α, and neutrophil infiltration[32,42], and enhancement in IL-2 production[43]. Melatonin showed immunomodulatory properties and modulatory influence on the NOS and cytokine production in inflammatory and oncostatic processes[44].

Crespo E et al showed that intraperitoneal melatonin administration could inhibit expression of iNOS mRNA in liver and lung[45]. Melatonin decreased lipid peroxidation and counteracted the LPS-induced NO levels in liver and lung.

Melatonin prevented LPS-induced endotoxemia and LPS-induced multiple organ dysfunction syndrome in rats[45].

Escames G et al showed that melatonin administration could counteract lipopolysaccharide-induced expression of mitochondrial nitric oxide synthase (mtNOS) and NO production in rats[26], thereby preventing toxicity due to LPS. Melatonin could prevent the mitochondrial failure that occurs during endotoxemia[26].

Our study showed that group with polymyxin B could prevent hyperglycemia, even showed lower blood glucose levels, although it was not significant. We hypothesized that the improved hyperglycemia and improved blood glucose production might be correlated with the improved cytokine secretion. Polymyxin B might be an attractive peptide in combating endotoxic shock through mediating the neutralization of LPS. Harm et al reported an in vitro inactivation of endotoxins by polymyxin B (PMB). The in vitro LPS–PMB complex had lower inflammatory activity in freshly drawn blood samples, which resulted in reduced cytokine secretion. Inactivation of endotoxins by intravenous polymyxin B infusion might be applied to overcome the urgent need for endotoxin elimination in treatment of sepsis[23].

Limitations in our study were that we could not control several factors, such as genetic, silent concomittant diseases, feeding habit, and stress. It is needed to examine the effect of melatonin administration as a prevention therapy in sepsis. We also need further study in the effects of melatonin with multilevel doses and the varying duration of exposure and with larger study subjects.

CONCLUSION

Melatonin administration as well Polymyxin B, alone or in combination, could prevent the elevation of random blood glucose and lactate levels in Wistar rats endotoxicosis model. Therefore, if confirmed by further research, melatonin might have a role in management of endotoxicosis.

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