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Original article

Effects of acute lipopolysaccharide-induced toxemia model on some neglected blood parameters

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Abstract

The presence of lipopolysaccharide (LPS) in blood induces an inflammatory response which leads to multiple organ dysfunction and numerous metabolic disorders. Uncontrolled, improper or late intervention may lead to tissue hypoxia, anaerobic glycolysis and a disturbance in the acid -base balance. The effects of LPS-induced toxemia on biological and immunological markers were well studied. However, parameters such as base excess, ions, and acid-base balance were not fully investigated. Therefore, the objective of this study was to examine these blood parameters collectively in LPS-induced inflammatory toxemia in rat's model. After induction of toxemia by injecting LPS at a rate of 5 mg/kg body weight intravenously, blood was collected from the tail vein of twenty rats and immediately analyzed. After 24 hours, the animals were sacrificed and the blood was collected from the caudal vena cava. The results revealed that the levels of pH, bicarbonate, partial pressure of oxygen, oxygen saturation, Alveolar oxygen, hemoglobin, hematocrit, magnesium (Mg^{2+}), and calcium (Ca^{2+}) were significantly decreased. On the other side, the levels of *Base* excess blood, Base excess extracellular fluid, partial pressure of carbon dioxide, lactate, Ca^{2+}/Mg^{2+}, potassium, and chloride were significantly increased compared to those found pre toxemia induction. However, sodium level showed no significant change. In conclusion, Acute LPS-toxemia model disturbs acid-base balance, blood gases, and ions. These parameters can be used to monitor human and animal toxemic inflammatory response induced by bacterial LPS conditions to assist in the management of the diagnosed cases.

Key words: Toxemia, lipopolysaccharide, base excess, pH, animal model

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Introduction

The presence of bacterial lipopolysaccharide (LPS) in blood circulation results in fever, multiple organ dysfunctions, and several metabolic disturbances due to an inflammatory reaction and ultimately elevation of cytokines concentrations (Remick et al. 1987). Despite advances in the critical care of human medicine, toxemia and septic shock due to infection play a considerable role in the morbidity and mortality of patients in intensive care units (Martin et al. 1997). According to several published studies, there is a similarity between humans and animals endotoxemia. High levels of morbidity and mortality may also be expected in veterinary medicine (Vela et al. 2006).

Uncontrolled, improper or a failure to control toxemia may lead to tissue hypoxia, anaerobic glycolysis and a disturbance in the acid-base balance; therefore, a demand for reliable biochemical markers are essential to assist in diagnosis and treatment of toxemic patients (Johnson et al. 2004). Analysis of blood gas can help in the assessment of patient's gas exchange, ventilator control and acid–base balance which were reported to be disturbed in LPS-induced toxicity (Verme and Paul 2010). The blood gases analysis is requested in several conditions including systemic toxemia and sepsis in order to evaluate the lung gas exchange ability (Malinoski et al. 2005). Blood parameters most frequently examined in an emergency clinical situation are partial oxygen concentration (pO_2), oxygen saturation (SO_2), pH, partial carbon dioxide concentration (pCO_2), bicarbonate (HCO_3^-), and lactate (Allen 2005). The elevated level of lactate in septic patients is an indicator of increased likelihood of death (Trzeciak et al. 2007). However, lactate may not be routinely checked in medical diagnosis (Emmanuel et al. 2012).

Electrolyte balance is one of the key issues in maintaining homeostasis in the body, and it also plays important roles in protecting cellular function, tissue perfusion and acid-base balance (Lee et al. 2000, Shiber and Mattu 2002). Most of the important and prevailing electrolyte imbalances are hypo- and hyper-states of sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), and (Mg^{2+}) magnesium as reported by Bockenkamp and Vyas (2003). Measurement of electrolyte imbalance is necessary for emergency unit patients which may remain unrecognized and leads to death. Therefore, timely recognition is an important way to ensure electrolyte-imbalance treatment (Mahowald and Himmelstein 1981).

Effects of LPS-induced inflammation on biological and immunological markers such as interleukin-1, interleukin-6, tumor necrosis factor-α (TNF-α), procalcitonin, inflammatory mediator prostaglandin E_2, and cyclooxygenase-2, were well recognized by several researchers (Weinberg 2000, Johnson et al. 2006). However, blood parameters such as base excess, blood gases, ions, lactate, and hematocrit have not yet studied, collectively. Therefore, the purpose of this work is to examine the effects of LPS-induced systemic toxemia on these blood parameters altogether. The rat model was chosen to evaluate the effect of LPS (Salomao et al. 2012).

Materials and Methods

All experimental protocols employed in this study were agreed by the committee on the care of laboratory animal resources, Chonbuk National University, and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication no. 85-23, revised 1996).

Animals

Twenty male Sprague-Dawley rats (220–250 g, Samtako Biokorea, Daejeon, Korea) were used. They were kept in cages maintained at 23±2°C and 50±5% humidity in a 12 h light/dark cycle with free access to water and feed.

Induction of toxemia

After one week of acclimation, LPS endotoxin of *E. coli* was used to induce acute toxemic reactions. The LPS-induced toxemia in rats was made by injecting 5 mg/kg BW of LPS (E. coli O127: B8; Sigma, St. Louis, MO, USA) in 1 mL normal saline intravenously.

Measurement of Blood Ions, Metabolites, and Enzymes

Blood was collected from tail vein just before toxemia was induced and from caudal vena cava after 24 h of toxemia induction. The rats were anesthetized by intraperitoneal injection of tiletaminezolazepam (Zolitel 40 mg/kg). Nova Stat Profile 8 CRT (NOVA Biomedical Corp, Waltham, MA, USA) was used for measuring pH, Base excess blood (BE-b), Base excess extracellular fluid (BE-ecf), pCO_2, pO_2, SO_2, Alveolar O_2, HCO_3^-, lactate, hematocrit (Hct), hemoglobin (Hb), and the concentrations of electrolytes including Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-.

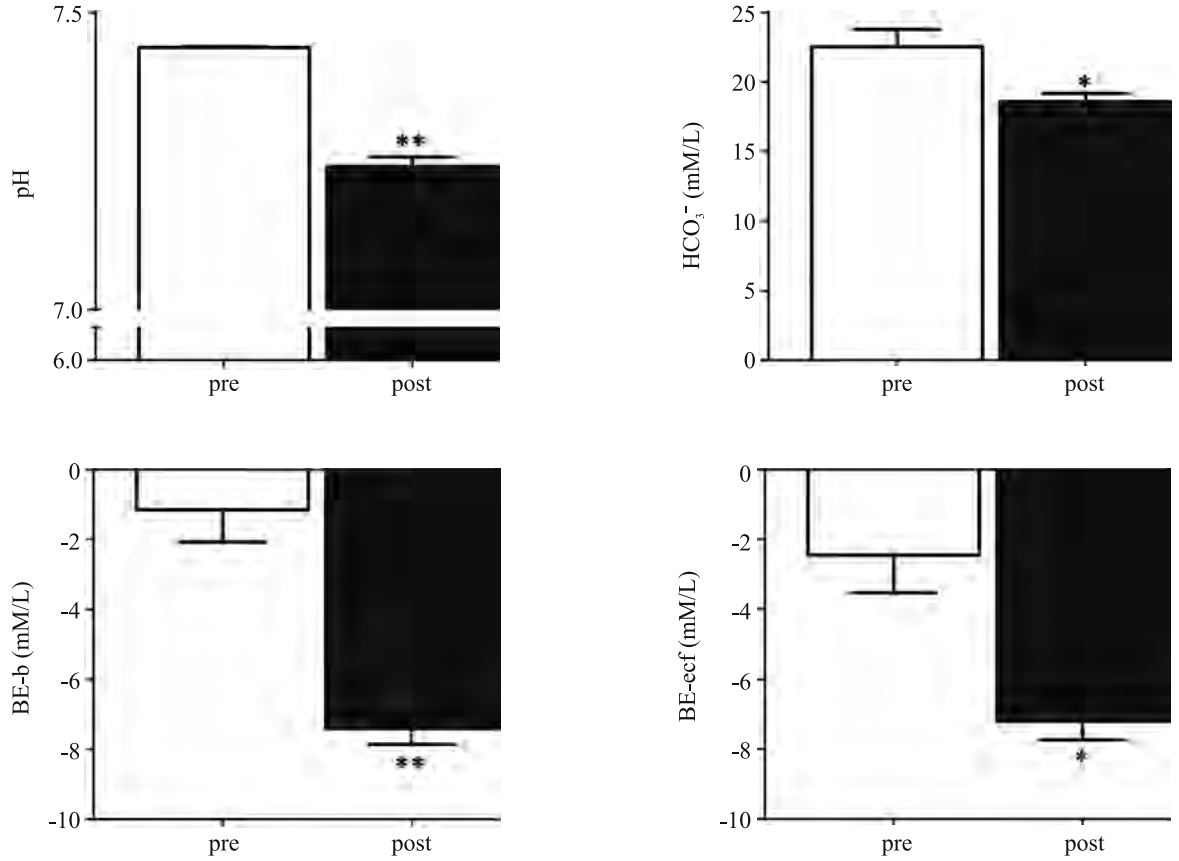

Fig. 1. Effects of LPS-induced toxemia on pH, Bicarbonate (HCO_3), Base Excess-blood (BE-b), and Base Excess-extracellular fluid (BE-ecf). The data were showed as Statistical analysis was carried out using paired Student's t-test *: $p<0.05$ and **: $p<0.01$ versus pre sepsis. The data are reported as the mean±SEM ($n=20$).

Statistical Analysis

The Prism 5.03 software (GraphPad Software Inc., San Diego, CA, USA) was used for the statistical analysis of the data and making graphs. Results were expressed as mean±standard error of the mean (SEM). The unpaired Student's *t*-test was used for comparison between rats before and after induction of toxemia. The level of significance was set at $p<0.05$.

Results

Effects of LPS-induced toxemia on pH, bicarbonate, and base excess

As shown in Fig. 1, LPS produced a significant reduction in the levels of pH ($p<0.01$) and HCO_3^- ($p<0.05$) compared with those found before toxemia induction. In addition, the levels of BE-b ($p<0.01$) and BE-ecf ($p<0.05$) were significantly increased compared to those found pre toxemia induction.

Effects of LPS-induced toxemia on blood gas composition

The levels of pO_2, SO_2, and Alveolar O_2 showed a significant decrease in post toxemia compared with those found in pre toxemia; *p-values* were 0.01, 0.001, and 0.001 respectively; but a significant increase was seen in the levels of pCO_2 compared to those found in pre toxemia induction; *p-value* was 0.01 as demonstrated in Fig. 2.

Effects of LPS-induced toxemia on blood ions

As reported in Fig 3, LPS induced a significant decrease in the levels of Mg^{2+} ($p<0.001$) and Ca^{2+} level ($p<0.001$) in post toxemia compared with those found in pre toxemia group. Moreover, the levels of Ca^{2+}/Mg^{2+} ratio, K^+, and Cl^- were significantly reduced in post toxemia rats compared to those found pre toxemia induction; *p-values* were 0.05, 0.01, and 0.001 respectively. Na^+ showed no significant change.

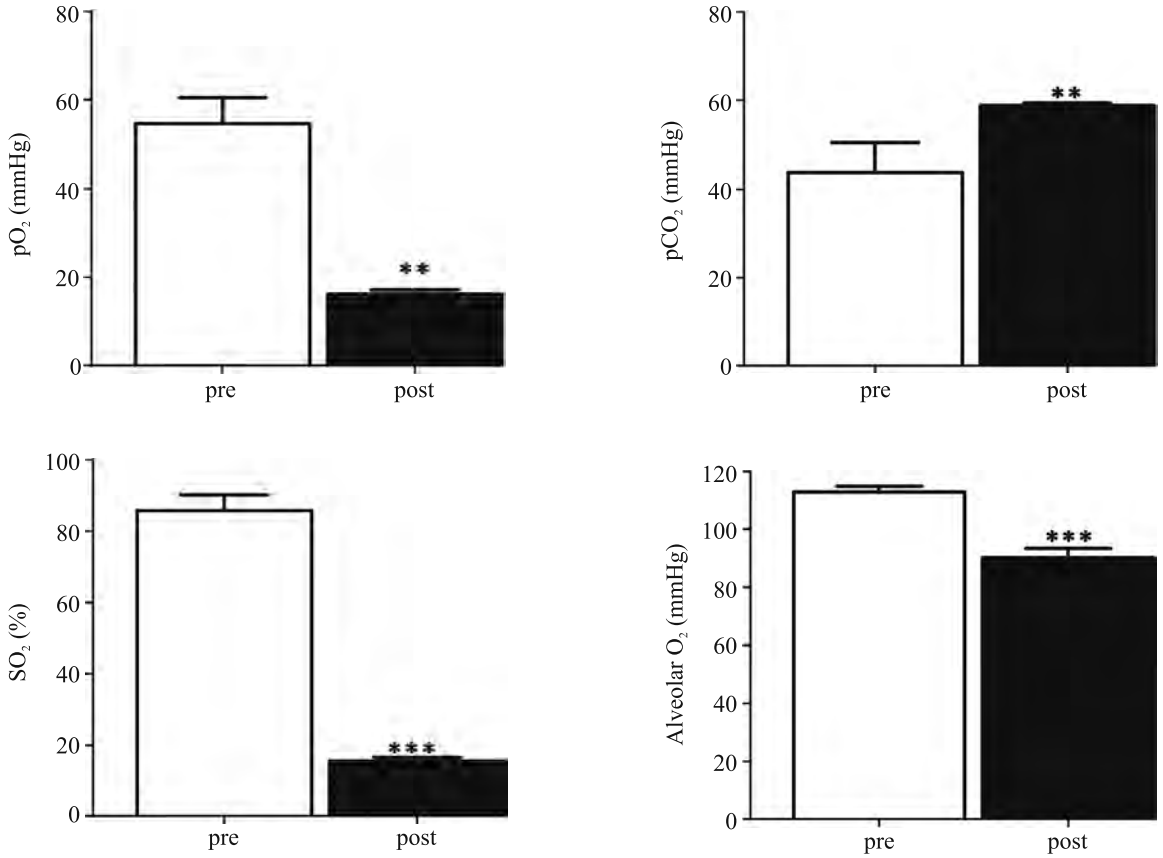

Fig. 2. Effects of LPS-induced toxemia on blood gas composition. partial pressures of oxygen (pO_2), partial pressures of carbon dioxide (pCO_2), oxygen saturation (SO_2), and Alveolar O_2The data were showed as Statistical analysis was carried out using paired Student's t-test **: $p<0.01$ and ***: $p<0.001$ versus pre toxemia. The data are reported as the mean±SEM ($n=20$).

Effects of LPS-induced toxemia on hemoglobin, Hematocrit, and Lactate

As presented in fig 4, LPS produced a significant decrease in the levels of Hb ($p<0.05$) and Hct ($p<0.05$) in endotoxemic rats compared with those found in pre toxemia. Furthermore, lactate level was significantly elevated ($p<0.01$) in post toxemia group compared to pre toxemia induction.

Discussion

Recently biomedical and immunological markers and techniques have been developed, still, there is no diagnostic tool which allow a rapid and reliable discrimination between systemic inflammatory toxemia and sepsis. However, measurement of blood biomarkers may help in the diagnosis and treatment of endotoxemia (Bloos and Reinhart 2014).

The importance of blood pH springs from its substantial role in the regulation of all functions at the level of cells, tissue and organs. Alteration in HCO_3^- concentration, for instance, have been suggested to be responsible for metabolic acidosis or alkalosis (Foch-Andersen 1995, Kellum 1999, Severinghaus 1999, Siggard-Andersen and Worthley 1999). LPS was shown by Eric and his co-researchers (2015) to induce lactic acidosis and acid-base balance disturbance. Their study reported that the levels of pH, HCO_3^-, base access, and lactate were decreased in LPS-induced sepsis. This result was consistent with our study's result in Figs. 1 and 2. Taken this together, it can be concluded that LPS induce respiratory and metabolic acidosis by lowering the levels of pH, pO_2, and bicarbonate but, increasing the level of lactate.

High level of pCO_2 could be attributed to dysfunction of the endothelial cell layer has been identified to play a pivotal role in the pathophysiology of lung injury or acute respiratory distress syndrome as mentioned by Tobin (2001).

Patient alveolar gas exchange can be evaluated by blood gas analysis and acid-base balance (Verma and Paul 2010). Since lung plays an important role in gas exchange, it's function can be assessed by analyzing blood gas, in this study the lung was severely affected as shown by pH and gas disturbances as p in Fig. 1 and 2.

Electrolyte abnormalities can provoke life-threat-

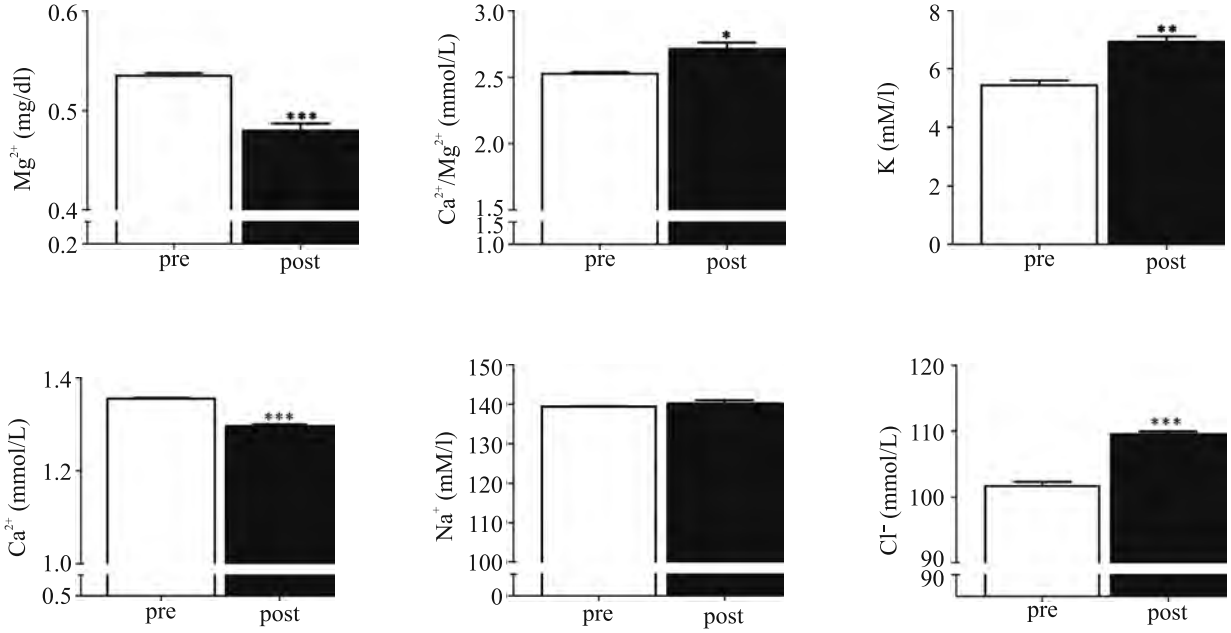

Fig. 3. Effects of LPS-induced toxemia on blood ions. Magnesium (Mg^{2+}), Calcium (Ca^{2+}), Mg^{2+}/Ca^{2+} ratio, Sodium (Na+), Potassium(K^+), and Chloride(Cl^-). The data were showed as Statistical analysis was carried out using paired Student's t-test *: p<0.05; **: p<0.01; and ***: p<0.001 versus pre toxemia. The data are reported as the mean±SEM (n=20).

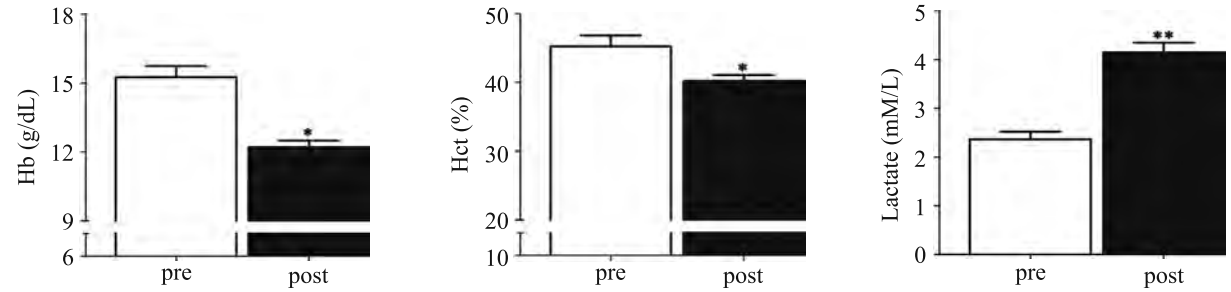

Fig. 4. Effects of LPS-induced toxemia on hemoglobin (Hb), hematocrit (Hct), and Lactate. The data were showed as Statistical analysis was carried out using paired Student's t-test *: $p<0.05$ and **: $p<0.01$ versus pre toxemia. The data are reported as the mean±SEM.

ening events. In such situations, an inaccurate assessment of electrolyte abnormalities can risk life. Hence, a rapid assessment of electrolytes may help people in an accurate intervention (Cox 2001).

In an LPS-induced toxemia study by Eric et al. (2015) concluded that electrolytes were not changed from their respective physiological values. This is in contrary to the result presented by this experiment. However, our result was strengthened with a study of Reinhart and Desbiens (1985) reported that hypomagnesemia has been associated with sepsis. Above all ions, magnesium had the highest prevalence of the abnormal values. This study supports our result in Fig. 3. The critical role of Mg^{2+} in toxemia could be attributed to its immune system effects which are important in the pathogenesis of inflammation and systemic toxemia. Furthermore, Mg^{2+} has a fundamental role in the regulation of cardiovascular homeostasis, a published data by Watanabe and his co-workers showed significantly reduced cardiac tolerance and hypoxia in animals with Mg^{2+} deficiency (Watanabe et al. 2011), this report agreed with our result in Fig. 3. The level of Na^+ has not changed significantly inconsistent with a report by Dembovska et al. (2008) who found that no significant difference in the levels of calcium, sodium, and potassium in an LPS-induced inflammation.

Hyperkalemia observed in our study could be attributed to the impaired ability of the kidney to secrete potassium due to LPS toxicity (Musso et al. 2006). A change in Ca^{2+}/Mg^{2+} ratio can lead to abnormal functioning of the cell (Isabelle et al. 2014), this may be due to the effect of LPS in ion channel through transient receptor potential cation channel, subfamily M, member 7. Kidney injury is one of the consequences of a critically ill-septic patient which had thought be associated with hyperchloremia as observed by Poukkanen

et al. (2013). This report coordinated with our result in Fig. 3.

In the acute phase of toxemia, several potential mechanisms may change the Hb concentration. On the one hand, endothelial activation may lead to increased vascular permeability and fluid sequestration to the interstitium, resulted in hemoconcentration (Steppan et al. 2011) this is partially agreed with our result (Fig. 4) which further was confirmed by the decreased oxygen level as shown in Fig. 2.

Systemic toxemia, a commonly encountered cases of inpatients in an intensive care unit, often leads to multi-organ dysfunction and the kidney is the major victim organ (Rangel-Frausto et al 1995). The present study showed a significantly high level of lactate as shown in Fig. 4, supported by a concurrent study performed by Lara and co-workers (Lara et al. 2016) investigated the renal function in a toxemic rat model, they found that serum levels of lactate and creatinine were significantly elevated compared to that of normal rats. Increased red blood cells (RBC) rigidity was found to be due to reduced deformability just a few hours after toxemia induction in rat and pig models (Bateman et al. 2001). Durocher et al. (1975) reported that reduction in surface charge of RBC can lead to RBC destruction. Furthermore, sepsis can induce RBC surface charge loss which ultimately leads to RBC aggregation as suggested by (Bateman et al. 2017). These two studies confirmed our result that a decreased level of Hct in toxemia model as shown in Fig. 4 might be attributed to a decrease in the size of RBCs from deformability and aggregation.

Conclusion

LPS-induced toxemia model in rats revealed severe consequences in the studied blood parameters which are rarely investigated in health institutions. Monitoring of blood acid-base balance, gases, Base excess, ions, and lactate in toxemia may contribute to the adjuvant diagnosis and treatment associated with the main therapy of patients.

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