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Brief Communication

Alterations in Blood Electrolyte of Rabbits with Experimental Injection of *Escherichia coli* Endotoxin

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Abstract: We studied the effects of *Escherichia coli* (*E. coli*) endotoxin on blood electrolytes levels in rabbits. Endotoxin (*E. coli* serotype O55 : B5) was injected via rabbits' ear vein : 0.10 mg/kg (Group A) or 0.50 mg/kg (Group B). Blood samples were taken at postendotoxemic 3, 6, 12 and 24 hrs and were analyzed for detections of the levels of blood electrolytes such as Ca^{++}, Mg^{++}, Na^{+}, K^{+} and Cl^{-}. As compared to control group, in endotoxin-treated rabbits Ca^{++} levels elevated at 6 hrs but decreased at 24 hrs, Mg^{++} levels were high at 3, 6 and 12 hrs, Na^{+} and K^{+} levels increased at all sampling times and Cl^{-} levels rose at 3, 12 and 24 hrs ($p<0.05$). Interestingly, endotoxic rabbits having hypermagnesemia (about 4.0 mg/dL) showed severe syndromes such as increased secretion, shock, tachypnea, seizure and/or diarrhea, suggesting that these may be clinical signs of imminent death in rabbits.

These observations testify that bacterial endotoxin leads to dyshomeostasis of blood electrolytes and the physiological imbalances may cause fatal disorders and subsequent death.

Key Words: Endotoxin, Rabbit, Electrolyte

It has been recognized that endotoxin, a lipopolysaccharide component of the cell walls of gram-negative bacteria, plays a pivotal role in the development of the bacterial infected-sepsis syndrome[2]. It was reported that systemic administration of bacterial endotoxin to experimental animals induced nonphysiological conditions with cardiovascular responses and metabolic and hematological disruptions[6,7,9]. However, the effects of endotoxin on blood electroloytes have rarely been known. Therefore, we designed experimentally an endotoxic rabbit model to clarify the effect of endotoxin on blood electrolytes.

54 male rabbits (New Zealand White) with a mean body weight of 1.80±0.50 kg were used for this study. Before experimental use, rabbits were acclimated to our animal facilities for 7 days at 20~22°C and 40% humidity on a 12 hr light-dark cycle. They were maintained on a laboratory chow and tap water ad libitum. Rabbits were randomly designated into control and endotoxicosis group. Endotoxicosis group (endotoxin group) was divided into subgroups according to the doses (0.10 mg/kg or 0.50 mg/kg) and times (3, 6, 12 and 24 hrs) of endotoxin injection. A model of sepsis was used in this study; endotoxicosis using a bolus injection. *Escherichia coli* endotoxin (*E. coli* serotype O55 : B5, Sigma, Co., America) was freshly dissolved in 0.9% sterile saline (0.10 mg/0.1 ml) and administered at 0.10 mg/kg (Group A)

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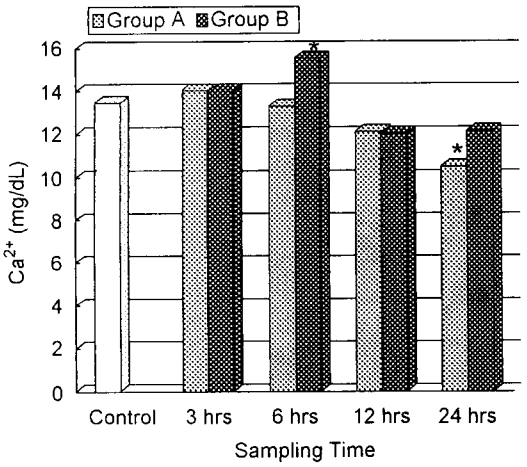

Fig. 1. The effect of intravenously injected *E. coli* endotoxin on rabbit serum Ca^{2+} levels. Group A: endotoxin 0.10 mg/kg-injected rabbits, Group B: endotoxin 0.50 mg/kg-injected rabbits ($^*p<0.05$ compared to control level).

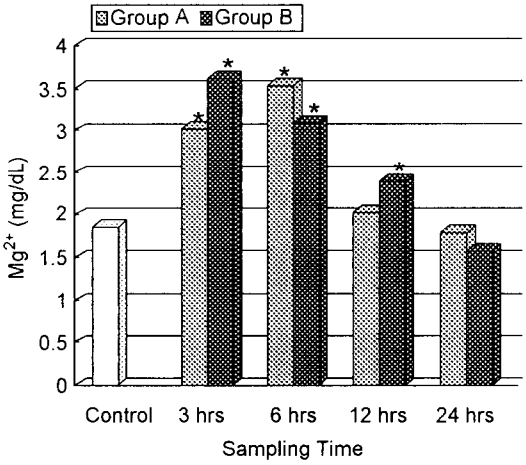

Fig. 2. The effect of intravenously injected *E. coli* endotoxin on rabbit serum Mg^{2+} levels. Group A: endotoxin 0.10 mg/kg-injected rabbits, Group B: endotoxin 0.50 mg/kg-injected rabbits ($^*p<0.05$ compared to control level).

or 0.50 mg/kg (Group B) to the rabbits as a single, bolus injection via the ear vein. Control rabbits received an appropriate volume of sterile 0.9% saline. All rabbits were induced anesthesia using ether and ketamine (10 mg/kg) and their hearts were exposed through abdominal incision at supine position. Thirty ml of blood was di-

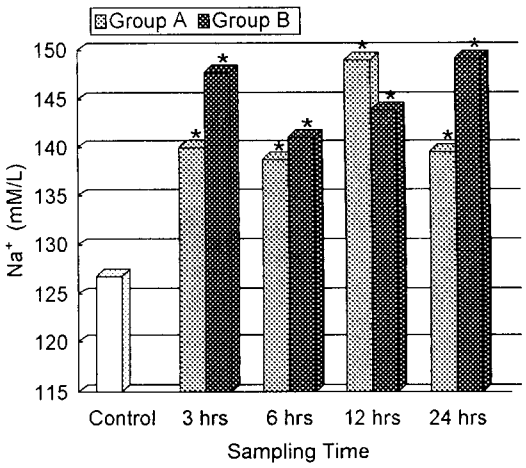

Fig. 3. The effect of intravenously injected *E. coli* endotoxin on rabbit serum Na^+ levels. Group A: endotoxin 0.10 mg/kg-injected rabbits, Group B: endotoxin 0.50 mg/kg-injected rabbits ($^*p<0.05$ compared to control level).

rectly collected from the heart and was separated into serum for measuring blood electrolytes such as Ca^{++}, Mg^{++}, Na^+, K^+ and Cl^-. The electrolytes were analyzed by Autohumalyzer 900S (Automated Clinical Chemistry Analyzer Co., Germany) with appropriate testing kit (CHOONG WAE, Co., Korea).

All data were expressed as means ± SE (standard error). Statistical analysis was accomplished by using MANOVA (multiple analysis of variance) with SAS package (version 6.03). If the significant values represented a probability (p) of ≤ 0.05, comparison across treatment groups (at the same time period) were performed as a contrast test. Statistical significance was accepted with $p \leq 0.05$.

As shown in Fig. 1, Ca^{2+} levels in 24 hrs of Group A (10.62 ± 1.03 mg/dl) decreased but increased in 6 hrs of Group B (15.51 ± 0.64 mg/dl, $p<0.05$), while the rest of endotoxin groups was not significant compared to that of control (13.46 ± 1.39 mg/dl). Fig. 2 shows serum Mg^{2+} levels in all groups. Mg^{2+} concentrations in 3 and 6 hrs of Group A and 3, 6 and 12 hrs of Group B were higher than that of control group (1.86 ± 0.13 mg/dl, $p<0.05$). As shown

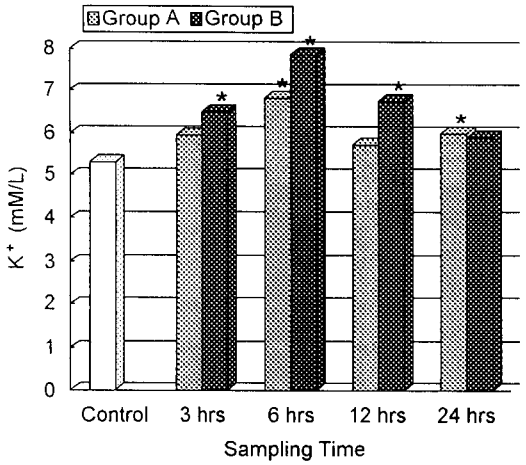

Fig. 4. The effect of intravenously injected *E. coli* endotoxin on rabbit serum K^+ levels. Group A: endotoxin 0.10 mg/kg-injected rabbits, Group B: endotoxin 0.50 mg/kg-injected rabbits ($^*p<0.05$ compared to control level).

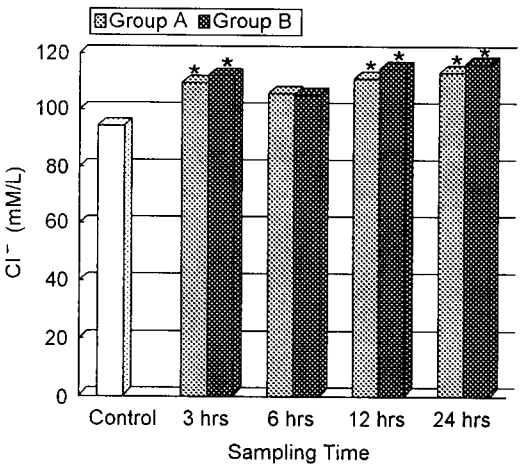

Fig. 5. The effect of intravenously injected *E. coli* endotoxin on rabbit serum Cl^- levels. Group A: endotoxin 0.10 mg/kg-injected rabbits, Group B: endotoxin 0.50 mg/kg-injected rabbits ($^*p<0.05$ compared to control level).

Fig. 3, Na^+ levels of all endotoxin groups were significantly higher than that of control (126.83 $\pm$13.38 mM/L). Fig. 4 exhibits the effects of endotoxin on the K^+ levels. K^+ levels in 6 and 24 hrs of Group A and 3, 6 and 12 hrs of Group B increased ($p<0.05$), whereas the other levels were not significant in comparison to control (5.32$\pm$0.17 mM/L). Fig. 5 illustrates Cl^- levels in all groups. The Cl^- levels of endotoxin-injected groups but 6hrs of Group B were significantly higher than that of control (94.33$\pm$2.21 mM/L).

Calcium (Ca^{2+}) is a major cation which have physiologically important roles and most of the ions exist in bone tissues. Ca^{2+} ions are involved in enzyme activations, blood coagulations, muscular contractions and nerve-stimulating pathways. In this study we observed a increase of Ca^{2+} level at 6 hrs of Group B (endotoxin 0.50 mg/kg) while those in the other endotoxin-injected groups slightly decreased.

Nakamura et al.[10] and Funk et al.[4,5] demonstrated that bacterial endotoxin caused increased release of parathyroid hormone and induced expression of parathyroid hormone-related protein gene. Parathyroid hormone raises Ca^{2+} release from bones, resulting in increase of Ca^{2+} levels in blood. The increase of Ca^{2+} level in our study is probably associated with such mechanisms. On the other hand, decrease in blood Ca^{2+} concentration may be due to parathyroid dysfunction, intestinal absorption, or kidney dysfunction. Renal failure leads to decreased production of active vitamin D, which has a potent effect on increasing calcium absorption from the intestinal tract[1], and subsequent declined intestinal absorption, causing diminishment of Ca^{2+} levels. In the present study severe renal dysfunctions occurred on endotoxin-rabbits (not shown data).

Above mentioned mechanisms may contribute to decrease of Ca^{2+} levels in this study. Elevation or depletion of Ca^{2+} ion in the extracellular fluid causes extremely immediate effects. When the level of calcium in the body fluids rises above normal, the nervous system is depressed, and reflex activities of the central nervous system become sluggish. Also, increased calcium ion concentration shortens the QT interval of the heart. When the extracellular fluid concentration of calcium ions falls below normal, the nervous system becomes progressively more and more excitable because this causes increased ne-

uronal membrane permeability to sodium ions. Hypocalcemia causes tetany. In this study endotoxin-injected rabbits with neurologic sign (i.e., seizure) had relatively low Ca^{2+} levels.

Endotoxin had striking influence on Mg^{2+} levels in rabbits. Hypermagnesemia (above 3.0 mg/dl) in 3 and 6 hrs of endotoxin groups were consistent with findings reported by Salem and associates[11]. We consider that decreased glomerular filtration rate by endotoxin may accounts for increased Mg^{2+} levels. Magnesium is involved in biochemical reaction. Consequently, the changes of Mg^{2+} levels in blood may induce unphysiological problems. Increased extracellular concentration of Mg^{2+} depresses activity in the nervous system and skeletal muscle contraction. Low magnesium concentration causes increased irritability of the nervous system, peripheral vasodilation, and cardiac arrhythmias. An animal study demonstrated that postendotoxemic hypomagnesemia increased mortality and intravenous supplement of Mg^{2+} attenuated the mortality[11].

However, we observed interesting findings which the hypomagnesemic endotoxin rabbits (0.13～0.25 mg/dl) showed better conditions, whereas the rabbits with hypermagnesemia (about 4.0 mg/dl) had clinically severe syndromes such as increased secretion, tachypnea, shock, seizure, and diarrhea. On the basis of these results, hypermagnesemia may be more deleterious than hypomagnesemia under bacterial or toxic sepsis. Further studies should be carried out to clarify this postulation.

Unlike other literatures, our findings showed elevated Na^+, K^+, and Cl^- levels in endotoxin rabbits. Cullen and associates[3] explained that endotoxin temporarily impairs colonic absorption and leads to consequently decreased electrolytes levels. This discrepancy may be due to decreased urine output with endotoxin-induced acute renal dysfunction. In addition to declined urine output, tachypnea may contribute to elevation of Cl^- level. Endotoxin causes tachypnea with severe pulmonary injury[8]. Also, endotoxin-induced acid-base unbalance, especially metabolic acidosis, accounts for increase of Cl^- level.

Electrolytes unbalances result in physiological disturbances. An excess of sodium ions depresses cardiac function and causes arteriolar dilatation, leading to hypotention and shock. Excess potassium in the extracelluar fluids causes the heart to become extremely dilated and flaccid and slows the heart rate. Very large quantities can also block conduction of the cardiac impulse from the atria to the ventricles through the A-V bundle. Besides, hyperpotassemia causes vasodilation, resulting from the ability of potassium ions to inhibit smooth muscle contraction.

In conclusion, *E. coli* endotoxin causes dyshomeostasis in blood electrolytes and serious physiological disorders, probably leading to grave situations.

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=국문초록=

대장균 내독소에 의한 토끼 혈중 전해질 농도의 변화

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혈액 전해질 성분들에 대한 대장균 내독소의 영향을 조사하기 위해 토끼를 대상으로 한 동물실험을 실시하였다. 대장균 내독소 (혈청형 O55 : B5)를 토끼의 귀정맥을 통해 0.10 mg/kg 혹은 0.50 mg/kg 농도로 주입한 후 3, 6, 12, 24시간대에 채혈하여서 Ca^{++}, Mg^{++}, Na^{+}, K^{+}, Cl^{-} 농도를 측정하였다. 대조군에 비해, 내독소투여 토끼의 Ca^{++} 농도는 6시간대에 증가하였고, Mg^{++} 농도는 3, 6, 12시간대에, Na^{+}과 K^{+}는 모든 채혈시간대에, 그리고 Cl^{-} 농도는 3, 12, 24시간대에 각각 유의하게 높았다 ($p<0.05$). 흥미롭게도, 고 Mg^{+} 혈증 (약 4.0 mg/dL)을 보인 내독소투여 토끼들은 심각한 임상징후들로 인식되는 분비물의 증가, 쇼크, 빈호흡, 경련, 혹은 설사와 같은 증세를 보였다. 본 연구의 결과들은 대장균 내독소가 혈액 전해질 농도의 항상성 혼란을 유도하며 이러한 생리적 불균형은 치명적 상황과 그로 인한 죽음을 야기할 수도 있음을 시사하고 있다.

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