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The Effect of *Escherichia coli* Lipopolysaccharide on the Hormones in Rabbits

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ABSTRACT – With a rabbit model, the present study was performed to examine the effect of *Escherichia coli* lipopolysaccharide (*E. coli* LPS) on hormones. Cortisol, epinephrine, and norepinephrine concentrations in LPS-treated groups were high at all sampling periods (from 3 hrs to 72 hrs) as compared to control group ($p<0.05$ or $p<0.01$). The each peak time was at respectively 24 hrs, 3 hrs, and 12 hrs. Insulin and glucagon concentrations in LPS-treated groups elevated up to 12 hrs ($p<0.01$ or $p<0.05$) with a each peak point at 12 hrs or 6 hrs, while those of the rest sampling points (from 24 hrs to 72 hrs) were lower than that of control ($p<0.05$). Increase of cortisol concentration was generally dose-dependent, whereas the changes of the other hormones were irregular patterns. These observations show that *E. coli* LPS lead to releases of stress hormones such as cortisol, epinephrine, and norepinephrine and disturbances of endocrine systems. These LPS-induced hormonal disorders may cause physiologically fatal results.

Key words □ Lipopolysaccharide, Rabbit, Hormone

Systemic injection of bacterial toxin such as lipopolysaccharide (reffered to as 'Endotoxin'), a component of the cell wall of gram-negative bacteria, has been widely used as a model of sepsis and results in a plethora of potentially deleterious cardiovascular and metabolic responses.[1] In recent studies have demonstrated a strong correlation between toxigenic bacteria, their toxins and SIDS (sudden infant death syndrome).[2-4] Several toxin hypotheses have been proposed as causes of SIDS, and SIDS may be associated with bacterial toxin-induced hormonal changes.[1,3-5] This study was undertaken to examine the effect of *Escherichia coli* lypopolysaccharide(LPS) on plasma hormones in the rabbit.

Materials and Methods

Animals and Care

76 male New Zealand White rabbits aged 8-week, weighing 1.7-1.9 kg, were housed in cages. They were maintained for at least one week prior to their used in experimental protocols. Animal room was on a 12-hr light/12-hr dark cycle with about 20°C temperature and 40% humidity.

Experimental protocol

Rabbits were randomly designated into control and lipopolysaccharide (LPS)-treated group. LPS-treated group was classified into subgroups with the doses (0.10 mg/kg and 0.50 mg/kg) and time (3, 6, 12, 24, 48, and 72 hrs) of injected LPS (*E. coli* serotype O55 : B5, Sigma, Co., U.S.A). All groups were characterized in Table 1. The each group consisted of 6 rabbits (n=6). *E. coli* LPS was freshly prepared in 0.9% sterile saline (0.10 mg/0.1 ml) and administered intravenously at a dosage of 0.10 mg/kg (Group A) or 0.50 mg/kg (Group B) via rabbit's ear vein.

Blood collection and Analysis

All rabbits were induced anesthesia using ether and ketamine (10 mg/kg, IM) and their hearts were exposed through abdominal incision at supine position. 30 ml of

Table 1. Classification of LPS-treatment groups according to the dose of endotoxin administration and blood sampling time

Group	Dose (mg/kg)[a]	Time(hrs)[b]					
		3	6	12	24	48	72
Group A	0.10	$G\text{-}A_3$	$G\text{-}A_6$	$G\text{-}A_{12}$	$G\text{-}A_{24}$	$G\text{-}A_{48}$	$G\text{-}A_{72}$
Group B	0.50	$G\text{-}B_3$	$G\text{-}B_6$	$G\text{-}B_{12}$	$G\text{-}B_{24}$	$G\text{-}B_{48}$	$G\text{-}B_{72}$

a : Dose of injected LPS, b : Time of blood collection.

blood was taken by direct puncture of the heart and was separated into plasma for measuring hormones such as cortisol, epinephrine, norepinephrine, insulin and glucagon.

Cortisol was measured by competitive RIA method with testing kit (Coat-A-count cortisol, DPC., U.S.A). Epinephrine and norepinephrine were analyzed by HPLC system with commercial kit (BIO-RAD, U.S.A). Insulin and glucagon concentrations were determined by competitive RIA method with each testing kit (Coat-A-cout insulin, and double antibody glucagon, DPC., U.S.A.).

Data analysis

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 $p \leq 0.05$.

Results

Cortisol

Plasma cortisol levels are shown in Figure 1 for all groups. In LPS-treated groups cortisol concentrations at all sampling periods were significantly high ($p<0.05$ or $p<0.01$) compared to control group (0.37 ± 0.01 μg/dl). As the increases of Group B were higher than those of Group A, it was a dose dependent manner. Its peak level was reached at 24 hrs of Group B (6.30 ± 0.02 μg/dl).

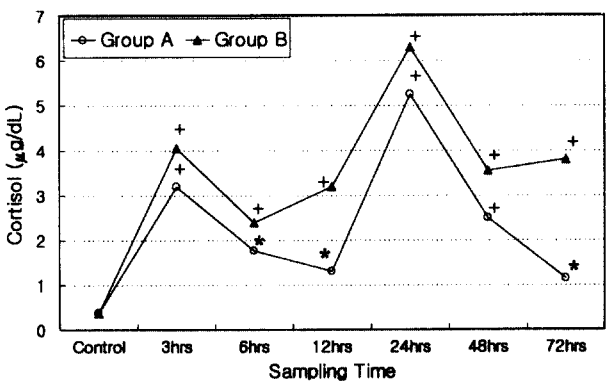

Fig. 1. The effect of intravenously injected *E. coli* lipopolysaccharide (LPS) on rabbit plasma cortisol levels.
Group A: LPS 0.10 mg/kg-injected rabbits, Group B: LPS 0.50 mg/kg-injected rabbits (* $p<0.05$, + $p<0.01$ compared to control level).

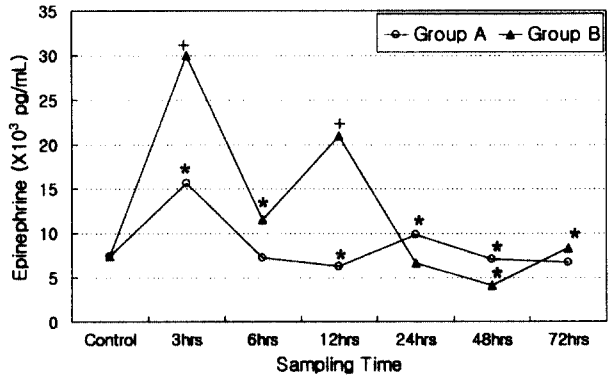

Fig. 2. The effect of intravenously injected *E. coli* lipopolysaccharide (LPS) on rabbit plasma epinephrine levels.
Group A: LPS 0.10 mg/kg-injected rabbits, Group B: LPS 0.50 mg/kg-injected rabbits (* $p<0.05$, + $p<0.01$ compared to control level).

Epinephrine

As showed in Figure 2, epinephrine concentrations in 3, 24, and 48 hrs of Group A and 3, 6, 12, and 72 hrs of Group B were higher than those of control group (7,427 ± 1,002 pg/ml), while the rests were similar or lower. G-B_3 was a peak point (30,020 ± 4,523 pg/ml).

Norepinephrine

Figure 3 shows the changes of the norepinephrine levels. Plasma norepinephrine concentrations in all LPS-treated groups increased at all sampling periods as compared to control group (4,185 ± 1,130 pg/ml, $p<0.05$ or $p<0.01$). Its peak point was on G-B_{12} (127,276 ± 13,570 pg/ml).

Insulin

As showed in Figure 4, insulin levels in Group A elevated up to 12 hrs, a peak point (40.10 ± 7.58 μIu/ml), and afterward fallen below control value (11.60 ± 0.02 μIu/ml). Those of Group B also increased up to 12 hrs but less than Group A, and changes of the rest values declined like Group A.

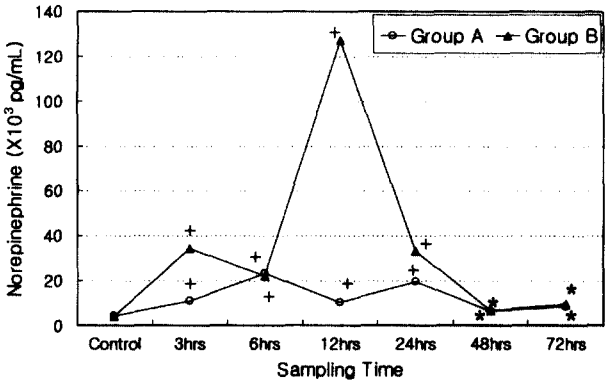

Fig. 3. The effect of intravenously injected *E. coli* lipopolysaccharide (LPS) on rabbit plasma norepinephrine levels.
Group A: LPS 0.10 mg/kg-injected rabbits, Group B: LPS 0.50 mg/kg-injected rabbits (* $p<0.05$, + $p<0.01$ compared to control level).

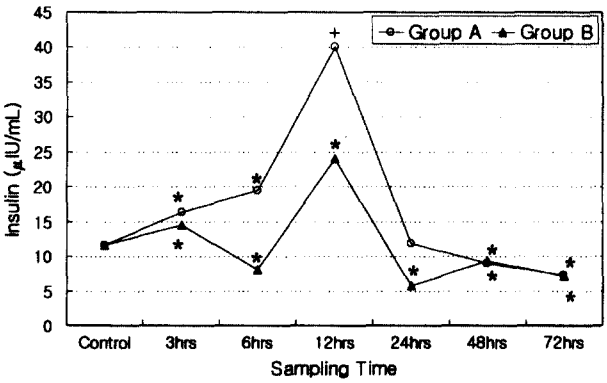

Fig. 4. The effect of intravenously injected *E. coli* lipopolysaccharide (LPS) on rabbit plasma insulin levels.
Group A: LPS 0.10 mg/kg-injected rabbits, Group B: LPS 0.50 mg/kg-injected rabbits (* $p<0.05$, + $p<0.01$ compared to control level).

Glucagon

Figure 5 displays changes of glucagon levels in all rabbits received LPS. Glucagon concentrations in Group B elevated up to 12 hrs ($p<0.05$ or $p<0.01$) with a peak level (1,350 ± 153.50 pg/ml) on G-B_6, whereas, from 24 hrs to 72 hrs, the levels decreased as compared to control (463 ± 36.75 pg/ml). Only glucagon at 6 hrs in Group A (691 ± 56.80 pg/ml) was significantly high but the other sampling points were slightly lower than those of control ($p<0.05$ or $p>0.05$).

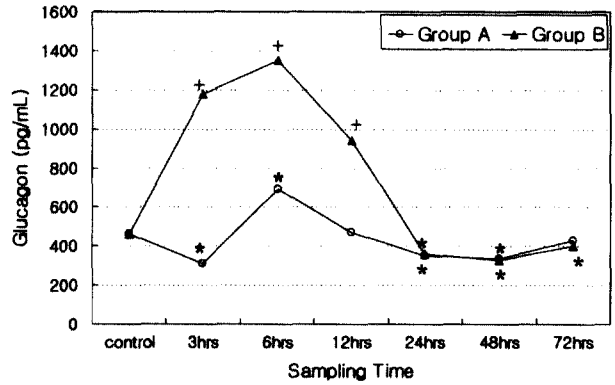

Fig. 5. The effect of intravenously injected *E. coli* lipopolysaccharide (LPS) on rabbit plasma glucagon levels.
Group A: LPS 0.10 mg/kg-injected rabbits, Group B: LPS 0.50 mg/kg-injected rabbits (* $p<0.05$, + $p<0.01$ compared to control level).

Discussion

The aim of this study was focused on testing the hypothesis that *E. coli* LPS causes hormonal disorders. The observations of the present study demonstrate well such a hypothesis with the severe changes of several hormones. The elevations of cortisol levels during 72 hrs suggest that stressful conditions by LPS were prolonged. Even though it has been known that cortisol secretion often increases greatly in stressful situations, it is still unclear why this is of significant benefit to the animal. One guess is that the glucocorticoids (i.e., cortisol) cause rapid mobilization of amino acids and fats from their cellular stores, making these available both for energy and for synthesis of other compounds, including glucose, needed by the different tissues of the body.[6]

In endotoxic shock endotoxin causes the body to reduce cardiac output, causing sympathetico-adrenal stimulation which in turn causes increased catecholamine secretion.[5] Catecholamines are released to stimulate heart rate, blood pressure and respiration, to dilate the respiratory passages and to promote glycogen breakdown to increase glucose in the blood in an attempt to rescuscitate the failing organs.[7,8]

Following catecholamine release, vasconstriction and diminished perfusion of organs with alpha receptors

occur causing shock lesions and death.[1,9] On the basis of these theories, increased catecholamine (epinephrine and norepinephrine) levels in this study may imply physiologically critical conditions such as LPS-induced shock or impending death.

Insulin and catecholamines have opposite effects on hepatic glucose production and extrahepatic glucose utilization, Thus, under physiological circumstances, insulin levels are usually low when catecholamine levels are elevated and vice versa. However, findings in this study shown that insulin and catecholamine (i.e., epinephrine and norepinephrine) levels are elevated simultaneously in LPS-treated rabbits. This atypical condition promotes both hepatic glucose production (via epinephrine-induced stimulation of glycogenesis) and extrahepatic glucose utilization (via insulin-induced stimulation of glucose uptake in peripheral tissues), leading to a progressive whole body depletion of carbohydrate stores and profound hypoglycemia, followed by circulatory arrest and eventually death.[10-13] Yelich et al.[13] reported that the ability of epinephrine to inhibit hyperinsulinemia is decreased in the endotoxic rat in vivo. Lipopolysaccharide alters intracellular β-cell events that regulate the insulin secretory processes.[12] There were elevated insulin concentrations along with the increased levels of catecholamines (epinephrine and norepinephrine) at 3, 6, and 6 hrs in LPS-treated rabbits. These results support the hypothesis that control ability of catecholamines on insulin secretion is blunted by LPS. Early during the course of endotoxicosis, catecholamine levels characteristically increase, thereby stimulating hepatic glycogenolysis and elevating glucose levels.[8,11] Under normal physiological circumstances, the elevated glucose levels (hyperglycemia) would inhibit glucagon secretion and thus lower plasma glucagon levels. However, this didn't occur at 3, 6, and 12 hrs of Group B. Instead, serum glucagon levels were significantly elevated for 12 hours after LPS injection. Furthermore, LPS-treated rabbits with increased glucagon levels had also higher glucose concentrations (not shown data). This result was consistent with Yelich's reports.[8] Several investigators have been reported that pancreatic glucagon concentrations in plasma were increased in endotoxic shock.[14-17] However, Yelich[18] showed that pancreatic hypersecretion of glucagon didn't occur during endotoxicosis. What is the mechanism for LPS-induced hyperglucagonemia in this study? While the precise mechanism for increased glucagon levels is not known, there are some theories able to explain it. Increased plasma glucagon levels could occur during endotoxicosis by sustaining nominal glucagon secretion under metabolic circumstances that favor minimal glucagon secretions.[12] Glucagon originates from both the pancreas (pancreatic-derived glucagon) and the gastrointestinal tract (gastrointestinal glucagon-like immunoreactivity.[19] Ishida reported that plasma GLI increases to higher levels than pancreatic glucagon (G) during endotoxemia in rabbits.[20] It has been pointed out that the gastrointestinal tract is one of the target organs in shock[21] and is therefore a source of bacteria and their toxins,[22] which are potential contaminants of the circulation. Although it is recognized that a basal level of glucagon may be essential for normal organ function, it has been suggested that elevated plasma concentrations of glucagon may mediate myocardial dysfunction and glucose dyshomeostasis during endotoxin shock.[14] Increased glucagon levels in this study may be due to above mentioned mechanisms.

In conclusion, findings of this study suggest that *E. coli* LPS causes hormonal disturbances and may lead to fatal conditions or SIDS in bacterial infected-patients.

국문요약

대장균의 지다당류가 호르몬에 미치는 영향을 조사하기 위해 토끼를 대상으로 실험을 하였다. 지다당류 투여 그룹의 콜티졸, 에피네프린, 노어에피네프린 농도는 전체 측정시기에 걸쳐(3시간에서 72시간) 대조군보다 유의하게 높았고($p<0.05$ 혹은 $p<0.01$) 이 호르몬들 각각의 최고치는 24시간, 3시간, 12시간대였다. 인슐린 및 글루카곤 농도는 지다당류투여후 12시간까지 대조군보다 높았으며($p<0.05$ 혹은 $p<0.01$) 그 최고치는 각각 12시간과 6시간대였으며, 이

후 24시간에서 72시간까지는 대조치보다 약간 낮았다. 콜티졸 농도의 변화는 용량의존적이었으나 나머지 호르몬의 변화양상은 불규칙하였다. 본 연구의 결과들은 대장균지다당류가 콜티졸, 에피네프린, 노어에피네프린과 같은 스트레스성 호르몬의 분비와 내분비계의 혼란을 유도한다는 점을 보여주고 있다. 이러한 지다당류로 인한 호르몬계의 혼란은 생리학적으로 치명적 결과를 초래할 수도 있다.

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