Reaction to Escherichia Coli Endotoxin in Adult Rats Neonatally Exposed to Different Endotoxins: Timing of Puberty and Levels of Gonadotropins

Neonatal Dönemde Farklı Endotoksinlere Maruz Kalan Siçanların Erişkinlik Döneminde Escherichia Coli Endotoksinine Yanıtları: Pubertaya Erişim Ve Plazma Gonadotropin Düzeyleri

Abstract

Aim: Neonatal bacterial infections appear to program immune system in a sexually dimorphic way and are associated with perturbed neuronal development. Bacterial cell wall components or endotoxins cause similar effects, and therefore, are used as experimental models. Aim of the current study was to assess the effects of neonatal exposure to different bacterial endotoxins on timing of puberty and to investigate the reaction to Escherichia (E.) coli endotoxin on plasma gonadotropin levels during adulthood.

Material and Method: Female (n=28) and male (n=28) rat pups were injected intraperitoneally with sterile saline solution (n=7), 100 ug/kg Escherichia coli (O111:B4) endotoxin (n=7), Salmonella enterica typhimurium endotoxin (n=7) or Klebsiella pneumoniae endotoxin (n=7) on neonatal day 7. Timing of puberty was assessed daily by observing vaginal opening. All animals were injected intraperitoneally with a different E. coli serotype (O26:B6) at 100 ug/kg on postnatal day 80 and four hours post-injection, blood samples and gonads were collected. Blood was used for gonadotropin (LH and FSH) analyzes. Females and males were compared by Kruskall Wallis H test. Multiple comparisons were made by Mann Whitney U test with Bonferroni correction. An alpha level of P<0.05 was accepted as significant.

Results: No effects of neonatal and adulthood endotoxin exposures were observed on body weight (p>0.05) No effects of neonatal endotoxin exposures were observed on timing of puberty (p>0.05). Neonatal and adulthood endotoxin exposures did not affect FSH and LH levels and gonad weights (p>0.05)

Conclusion: It might be concluded that effects of single neonatal exposure to different endotoxins are corrected before the commencement of the puberty, and hence, pubertal development is not affected.

Key words: Neonatal period; Different LPS; Puberty; Gonadotropins Levels

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Anahtar Kelimeler: Neonatal dönem; Farklı LPS; Puberty; Gonadotropin düzeyleri
INTRODUCTION

People are constantly exposed to toxins caused by the molecules related to microorganisms (1). Bacteria-induced endotoxins are present everywhere around us (2, 3). It can be found in the houses, healthcare organizations, agricultural lands, and wastes in different rates from nanograms to micrograms (3). Actually, there are microorganisms producing endotoxin within the microbiota that forms our digestive system (4). We are exposed to such endotoxins depending on change in the intestinal permeability (5). As a result, the number of endotoxins in the bloodstream increases and they can cause low-grade chronic inflammation, and may play a role in the development of many serious chronic diseases (obesity, atherosclerosis, neurological diseases) (5, 6).

Bacterial infections cause a strong response in the immune system of the organism and thus, create inflammatory stress. The effect mode of the immune stress on reproductive functions is exactly understood and it is thought that it may occur via the endocrine, paracrine, and neuronal systems (7). Especially the infections exposed in the neonatal period affect the reproductive system (8). It is reported that exposure to lipopolysaccharide (LPS), which is a bacterial endotoxin obtained from the gram-negative bacterial cell wall, in the neonatal period affects the Hypothalamic- Pituitary Adrenal axis and causes the disruption of the ovarian cycle (9, 10). On the other hand, exposure to endotoxin in adulthood also affects the reproductive system (8).

The aim of the present study was to investigate the effects of different bacterial endotoxins on access to puberty in the neonatal period and to examine the effects of a different Escherichia (E.) coli endotoxin on plasma gonadotropins in adulthood.

MATERIAL AND METHODS

Animals and Experiment Protocols

Pregnant Sprague-Dawley rats (INU-DEHUM, Turkey) were housed under controlled conditions (12L:12D; 22±2 °C) and supplied with ad libitum food and water. Female (n=28) and male (n=28) rat pups were injected intraperitoneally with sterile saline solution (n=7), 100 ug/kg Escherichia coli (O111:B4) endotoxin (n=7), Salmonella enterica typhimurium endotoxin (n=7) or Klebsiella pneumoniae endotoxin (n=7) on neonatal day 7. Timing of puberty was assessed daily by observing vaginal opening (11). All animals were injected intraperitoneally with a different E. coli serotype (O26:B6) at 100 ug/kg doses on postnatal day 80 and four hours’ post-injection, blood samples and gonads were collected. Blood was used for gonadotropin (LH and FSH) analyzes. This study was approved Animal Research Ethics Committee of Inonu University Medical Faculty (Protocol # 2010/54) (Figure 1).

Figure 1. Experimental Protocol
LH and FSH enzymeimmunoassays
LH and FSH were analyzed according to Pappa et al. (12) with some modifications. Briefly, 96-well immunoplates (Nunc, Roskilde, Denmark) were coated with rat LH or rat FSH. Serum samples or standards were preincubated with primary antibodies and were then transferred into coated plates for competition with antigens on the solid phase. Plates were washed and the secondary antibody conjugated to streptavidin peroxidase was added into each well and color was developed by using tetramethylbenzidine as the substrate. Plates were read at 450 nm using a plate reader (Biotek, Synergy HT, USA). Rat LH, rat FSH and primary antibodies (rabbit anti-rat LH and rabbit anti-rat FSH) were obtained from Dr. A.F. Parlow (NIDDK, NIH, USA) and secondary antibodies (goat anti-rabbit IgG) conjugated to streptavidin peroxidase was purchased from Sigma (Sigma-Aldrich, Taufkirchen, Germany). Sensitivity of the assays was 1 ng/ml for LH and 2 ng/ml for FSH. Inter- and intra-assay coefficients of variations were below 8 % for both LH and FSH.

Statistical analysis
SPSS 26.0 program was used for statistical analysis. Females and males were compared by Kruskall Wallis H test. Multiple comparisons were made by Mann Whitney U test with Bonferroni correction. Values of p<0.05 were considered significant. All results were expressed as means ±standart error (SE).

RESULTS
Different LPS injection in the neonatal period did not affect timing of puberty (p>0.05) Subsequent to the different LPS injection in the neonatal period, a different LPS injection on the postnatal 80th day did not affect gonadotropin (FSH and LH) levels, gonad weights, and body weights (p>0.05). Findings for male and female rats are summarized in table 1 and figure 2.

|          | Salin (n=7) | E.coli (n=7) | Salmonella (n=7) | Klebsiella (n=7) | P value |
|----------|-------------|--------------|-----------------|-----------------|---------|
| Men      |             |              |                 |                 |         |
| Body Weight (g) | 253 ± 64 | 314 ± 39 | 300 ± 41 | 282 ± 69 | >0.05   |
| Puberty (day)    | 41 ± 4 | 41 ± 4 | 41 ± 3 | 41 ± 4 | >0.05   |
| LH (ng/ml)       | 1.75 ± 0.60 | 1.67 ± 0.50 | 2.65 ± 1.93 | 3.02 ± 2.17 | >0.05   |
| Gonad Weight (g) | 0.93 ± 0.45 | 1.33 ± 0.30 | 1.25 ± 0.47 | 1.49 ± 0.35 | >0.05   |

| Women    |             |              |                 |                 |         |
| Body Weight (g) | 221 ± 10 | 219 ± 20 | 216 ± 22 | 205 ± 24 | >0.05   |
| Puberty (day)    | 40 ± 4 | 43 ± 4 | 43 ± 4 | 43 ± 3 | >0.05   |
| FSH (ng/ml)      | 15.27 ± 10.37 | 11.31 ± 1.32 | 12.16 ± 5.06 | 11.96 ± 1.73 | >0.05   |
| LH (ng/ml)       | 3.86 ± 2.55 | 3.14 ± 1.62 | 3.11 ± 1.17 | 2.33 ± 0.9 | >0.05   |
| Gonad Weight (g) | 0.17 ± 0.03 | 0.27 ± 0.13 | 0.15 ± 0.02 | 0.13 ± 0.02 | >0.05   |
Figure 2. Body and gonad weight, puberty, serum LH and FSH concentrations of the groups
DISCUSSION

Endotoxins are found in the digestive system of humans and animals as endogenous also they are present everywhere in our surrounding. When the findings obtained from the study were examined, different bacterial endotoxin LPS injections had no effect on puberty and the live weight. On the other hand, the administration of an endotoxin LPS that is different from LPS injected in the neonatal period had no effect on gonadotropin (FSH and LH) in the short term. Two LPS injections performed at different times throughout the lifetime did not affect the gonad and the ovarian weight.

Different effects of LPS administration on reproductive system are determined in the neonatal period (8). It is reported that single dose of LPS injection to rat offsprings has accelerated puberty (11, 13). On the other hand, other authors have observed a delayed access to puberty in the rats that received a single LPS injection (10, 14). When the methods of the studies are examined carefully, the reasons for the different effects of LPS administration on puberty in the neonatal period can be as follows: 1. LPS source obtained from different bacteria, 2. LPS obtained from different serotypes of bacteria, 3. The day, dose, and number of the LPS injection. One of the essential purposes of the current study was different endotoxin administration. However, puberty was not affected from different LPS administration in the present study. LPS injection of the rat offsprings could be balanced by increasing their weight gain. In a study, it was reported that a single dose LPS administration caused rapid weight gain in prepubertal period (11). In the present study, in response to the LPS administration, weight gain could be increased and the effect on puberty could be compensated. On the other hand, it is reported that LPS administration in the neonatal period delays puberty (14). However, the day of injection is the postnatal 3rd-5th days. In the present study, the injection was done on the postnatal 7th day and as a single injection. When the studies were examined, it was decided to administer different endotoxins on the postnatal 7th day and as a single dose (100 µg/kg) in the present study by considering different LPS sources and serotypes used. Thus, it can be thought that single dose of endotoxin did not affect the reproductive system or the effects of endotoxin injections in the neonatal period were eliminated until the pubertal period.

In the present study, a single dose of LPS administration in the neonatal period and a different endotoxin administration on the postnatal 80th day had no effect on FSH and LH. It had similar findings with a previous study (11). However, in a study, LPS injection administrated in neonatal period and in adulthood decreased the LH level (15, 16). When the differences between the studies were examined, it may be caused by the bloodletting hours. In the present study, blood was taken following decapitation4 hours after the LPS injection. In future studies, it will be appropriate to attach a catheter, take blood at certain intervals and examine it by considering the circadian rhythm of gonadotropins.

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Conflict of Interest: The authors declare that they have no competing interest.

Ethical approval: Prior to the study, written permission was obtained from the institutions in which the study was conducted, and ethical approval was obtained from the Scientific Research and Publication Ethics Committee of the Malaya Inonu University of Health Sciences in Turkey on Number 2010/54.

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