Farklı hormonların ve hücre hatlarının bir Üropatojen E. coli susunun üremesi üzerine etkileri

The alterations on growth of a Uropathogenic E. coli with the effects of both different hormones and cell lines

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ÖZ

GİRİŞ veAMAÇ: Bir infeksiyon sürecinde patojenlerin önemli biyolojik özelliklerinin konak faktörleri tarafından etkilendiği bilinmektedir. Bu çalışmada, çeşitli memeli hormonlarının farklı hücre hatları varlığında bir üropatojen E. coli (UPEC) susunun üremesi üzerine olası etkileri araştırılmıştır.

YÖNTEM veGEREÇLER: Hücre hatlarının (HEK293, A549, L929, Ishikawa) kültürü yapılmıştır. Fizyolojik seviyelerine göre her bir hormonun iki farklı konsantrasyonu (20 ve 200µIU insulin; 0.0017 ve 0.04µg/mL norepinefrin; 0.1 ve 0.4ng/mL östradiol; 2 ve 20ng/mL progesteron; 6 ve 60pg/mL melatonin) deneylerde kullanılmıştır. Bakteri ve hormonlar her bir hücre kültürüne inoküle edilmiştir. Üremeler, üç saatlik inkübasyon sonucunda 600nm’de absorbans ölçümü ile belirlenmiştir.

BULGULAR: UPEC susunun üremesi yüksek düzey östradiol, yüksek düzey norepinefrin ve her iki düzey melatonin varlığında anlamlı derecede azalmıştır (p<0.05). Bununla birlikte farklı hormon konsantrasyonları varlığında üreme üzerine etkili bulunan hücre hatları HEK293 ve Ishikawa olmuştur. Ishikawa hücre hatının progesteron hariç diğer tüm hormonlar ile birlikte UPEC-suğunun üremesini artırılmıştır (p<0.05). HEK293+insulin ve HEK293+östradiol birlikte üremeyi baskılarken, HEK293+melatonin ve HEK293+progesteron üremeyi anlamlı düzeyde artırılmıştır (p<0.05).

TARTIŞMA veSONUÇ: Memeli hormonlar ve farklı konak koşulları birer çevre faktörü olarak mikroorganizmaların davranışlarını ve dolayısı ile infeksiyon hastalıklarının patojenitesini belirlerler.

Anahtar Kelimeler: UPEC, üreme, hormonlar, hücre kültürü, alemler arası iletişim

ABSTRACT

INTRODUCTION: It is well known that, biologically important processes of pathogens can be affected from host factors during infectious processes. We aimed to investigate the possible effects of various mammalian hormones in different cell lines on growth of a uropathogenic E. coli (UPEC) strain.

METHODS: Cell monolayers (HEK293, A549, L929, Ishikawa) were cultivated. Two different concentrations of each hormone were analyzed (20 and 200µIU insulin; 0.0017 and 0.04µg/mL norepinephrine; 0.1 and 0.4ng/mL estradiol; 2 and 20ng/mL progesterone; 6 and 60pg/mL melatonin) according to their physiological levels. Bacterium and hormones were inoculated onto each cell cultures. After three hours incubation growths were determined by optical density measurement at 600 nm.

RESULTS: Growth of UPEC has been decreased significantly in the presence of high level estradiol, high level norepinephrine and all levels melatonin (p< 0.05). Besides, HEK293 and Ishikawa cell lines were found to be the most effective on growth of pathogen in the presence of different hormones. The coexistence of Ishikawa and all types of hormones except progesterone were shown to be enhanced the growth of UPEC (p< 0.05). The coexistence of HEK293+insulin and HEK293+estradiol reduced the growth of UPEC; whereas HEK293+melatonin and HEK293+progesteron enhanced the growth significantly (p< 0.05).

DISCUSSION AND CONCLUSION: Mammalian hormones and different host conditions determine the pathogenicity of infectious diseases as environmental factors because which could affect microorganisms’ behaviors.

Keywords: UPEC, Growth, Hormones, Cell culture, Interkingdom-communication

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INTRODUCTION

Microorganisms sense and respond to their environmental conditions for regulating growth, virulence and antibiotic susceptibility. Investigations focusing on interactions between microorganisms and their hosts are interesting and caused to born a new research field referred as “microbial endocrinology” (1-7). Many of previous studies have suggested that host hormones (as one of these environmental factors) modulate microorganisms’ pathogenesis (1-7). Moreover communication is an important process occurs bidirectional between both microorganisms (known as quorum sensing-QS) and their hosts (inter kingdom communication) (1, 8). Host hormones are shown to be effective as signaling molecules in these related communication pathways (1, 6-8).

It is well known that host cells release some factors act as immune regulatory, thus bacteria regulate their biofilm formation and/or motility to grow effectively (9, 10). The detection of cell– bacteria interactions have great importance for evaluating effects of host conditions on growth of bacteria and based on this idea, we analyzed the alterations on bacteria growth in the coexistence of different mammalian cell lines and host hormones.

Norepinephrine and melatonin are effective in nervous system and regulates gut functions, estradiol and progesterone have effects in reproductive system. Insulin is also very important in human endocrine system which provides sugar using (11-14).

Urinary tract infections are among the most frequent infections encountered in many countries. The most of community acquired urinary tract infections are caused by Escherichia coli. Uropathogenic strains of E. coli (UPEC) are characterized by the expression of distinctive virulence factors which can be grouped as adhesins, toxins, siderophores, etc. (15, 16, 17).

In the present study we aimed to imitate in vivo conditions with using both host hormones [(Norepinephrine (NE), Estradiol (Est), Insulin (Ins), Melatonin (Mlt) and Progesterone (Prg)] and host cell lines (human embryonic kidney cell line, human endometrial adenocarcinoma cell line, human lung carcinoma cell line and mouse fibroblast cell line) for detecting of growth alterations in a UPEC C7 strain.

MATERIALS AND METHODS

Strain

E. coli C7 strain was used in this study which kindly provided by Prof. Dr. Shingo Yamamoto (Hyogo College of Medicine, Japan). Bacteria were kept at -80°C until experiments. This strain was previously analyzed and shown to carry various virulence genes encoding S/F1 fimbria, uropathogenic specific protein and cytotoxic necrotizing factor.

Hormones

Low and high concentrations of each hormones [Ins (20 μIU/mL and 200 μU/mL), NE (0.0017 and 0.04 µg/mL), Est (0.1 and 0.4 ng/mL), Prg (2 and 20 ng/mL) and Mlt (6 and 60 pg/mL)] were used in the experiments. All hormone concentrations were added according to their psychological levels of blood in a healthy individual.

Cell cultures

Human embryonic kidney cell line (HEK293 ATCC CRL-1573), human endometrial adenocarcinoma cell line (Ishikawa 99040201-Sigma-Aldrich), human lung carcinoma cell line (A549) and mouse fibroblast cell line (L929) were used in the experiments. Cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Sigma, 5546) supplemented with 1% 2mM L-glutamine (Biological Industries, B103-020-1B), 10% fetal bovine serum (FBS) (Biowest, S1810-500) and 1% penicillin/streptomycin (50 IU/ml penicillin and 50μg/ml streptomycin; Biological Industries, 03-031-1B) and were prepared in 96-well plates the day before experiments.

Detection of the Alterations on Growth of UPEC in the presence of Hormones and Host Cells

107 CFU/mL overnight culture of UPEC was prepared. Before the inoculations of bacterium and hormones, cell culture medium DMEM (10% FBS,
%1 pen/strep) was aspirated and changed with DMEM (10% FBS, without pen/strep). Growth alterations were determined via optical density measurement in a spectrophotometer followed by three hours of incubation.

Statistical analysis

Statistical analysis was determined by using one-way ANOVA followed by Tukey’s multiple-comparisons test (GraphPad Prism5 program). All measurements were compared to control conditions (DMEM). The samples were tested in duplicate and each experiment was performed thrice. Multiple comparisons were made at a level of P < 0.05.

RESULTS

The effects on growth of UPEC were detected in different statistical analysis. We evaluated effects of only hormones and effects of both host cells and hormones separately.

It has been shown that, the growth of UPEC has been decreased significantly (p< 0.05) in the presence of high level Est, high level NE. All concentrations of Mlt are also shown to be reduced the growth of UPEC significantly (p< 0.05) (Figure 1). The alterations on growth of UPEC in the presence of other hormones were found to be statistically not significant.

The growth of UPEC was shown to be decreased significantly with the coexistence of HEK293 cell line and low level Est, high level Est and low level NE. Besides, all concentrations of Prg and Mlt were increased the growth of UPEC and these differences were found to be statistically significant (p< 0.05) (Figure 2). All concentrations of Ins and high level NE were found to be not affected the growth of UPEC.

The coexistence of Ishikawa cell line and all concentrations of Ins, NE and Est except low level NE were found to increase the growth of UPEC; in addition high level Mlt was also increased the growth of UPEC in the coexistence of Ishikawa cell line (p<0.05) (Figure 2). Neither all levels of Prg nor low level Mlt and high level NE significantly affected the growth of UPEC.

It was shown that, in the coexistence of hormones and lung carcinoma cell line, the growth of UPEC was altered in opposite ways. When A549 cell line were treated with high level Ins and high level Prg the growth was enhanced significantly (p< 0.05) but in contrast, the presence of low level Est was shown to reduce the growth of UPEC significantly (p< 0.05) (Figure 2). The other concentrations of tested hormones were found to be not altered growth of UPEC.

The presence of all five different hormones in the L929 cell line were shown to be not altered the growth of UPEC (p> 0.05) (Figure2).

Significant alterations on growth of UPEC C7 in coexistence of hormones hand cell lines were shown in table 1. As it was seen in the table, HEK293 and Ishikawa cell lines were found to be the most effective on growth of pathogen in the presence of different hormones. The coexistence of Ishikawa and all types of hormones except progesterone were shown to be enhanced the growth of UPEC. The coexistence of HEK293+Ins and HEK293+Est reduced the growth of UPEC; whereas HEK293+Mlt and HEK293+Prg enhanced the growth significantly (Table 1).
Figure 2. Growth alterations of UPEC in the coexistence of various cell lines (a-d) and different host hormones. a, Growth of C7 with the coexistence of HEK293 cell line and hormone; b, Growth of C7 with the coexistence of Ishikawa cell line and hormones; c, Growth of C7 with the coexistence of A549 cell line and hormones; d, Growth of C7 with the coexistence of L929 cell line and hormones.

Table 1. Alterations on growth of UPEC C7 in the coexistence of hormones and cell lines

| Cell Lines | Norepinephrine 0.0017µg/mL | Insulin 0.04µg/mL | Estradiol 200µIU/mL | Melatonin 6pg/mL | Progesterone 20ng/mL |
|------------|-----------------------------|-------------------|--------------------|------------------|----------------------|
| HEK293     | NS                           | NS                | NS                 | NS               | NS                   |
| Ishikawa   | ↑                            | NS                | ↑                  | ↑                | NS                   |
| A549       | NS                           | ↑                 | ↑                  | ↑                | NS                   |
| L929       | NS                           | NS                | NS                 | NS               | NS                   |

NS: Statistically not significant
↑: Significantly increasing of growth
↓: Significantly decreasing of growth

DISCUSSION

It has been suggested that, host conditions including hormones, are suggested to serve as microbial growth inducer or reducer therefore hormones could change microorganisms’ behaviours and contribute the pathogenicity of infectious diseases (1-8). Moreover it was shown that, hormones can affect differently microorganisms’ virulence, antibiotic susceptibilities and expression levels of various genes depend on culture conditions. In recent years, many studies have demonstrated that various microorganisms are able to sense and respond to hormones released by the host via quorum sensing.
In this respect, we aimed to imitate in vivo conditions in cell culture to detect the alterations on growth of a UPEC strain in the presence of only hormones and both host cells and hormones.

Numerous reports demonstrated that hormones have the potential to affect the outcome of infection. For example, growths of Pseudomonas aeruginosa, Bacteroides melaninogenicus, Campylobacter rectus, E. coli, Staphylococcus aureus, Enterococcus faecalis, Chlamydia trachomatis, C. pneumoniae, Aspergillus clavatus, A. fumigatus, A. niger, Trichophyton mentagrophytes, Candida albicans, Neisseria gonorrhoeae, Acinetobacter baumanii, Burkholderia pseudomallei and Coccidioides immitis have been reported to be altered when exposed to catecholamines, melatonin, gender hormones and insulin (6, 7, 19-25).

In this regard it is obvious that, hormones regulate the growth of microorganisms via different mechanisms. It has been reported that, catecholamines could modulate the releasing of iron from transferrin or they also could act as auto-inducers (quorum sensing) which are necessary for growth and virulence. Although the effect of NE on growth has been found to be depended on concentration of hormone and characteristics of the strain tested we would like to underline that NE mostly enhances the growth of UPECs. In addition, gender hormones could have roles as components of vitamin K (7, 26, 27). Some microorganism uptake progesterone and estradiol which increase their growth rate in the absence of vitamin K. Gender hormones are also able to affect electron transport systems to regulate the growth of bacteria. Moreover we also have detected that, the impact of Est on growth of UPEC was definitely depending on characteristics of strains. Similar to other hormones, melatonin can bind different ions (including iron (III), copper and zinc) thus represses cytoplasmic accessibility for microorganisms which are essential for their metabolic pathways (26-28). Melatonin also known as antioxidant which its’ antimicrobial effects may be associated with effects (28-31).

In our study, host hormones were also investigated for evaluating of influences on host cell’s various characteristics (growth rate, morphology, colony formation, tumorigenicity, etc.) in different cell cultures. Some researchers reported that cells have different properties such as their protein synthesis and differentiation of their cellular structure when treated with host hormones (32, 34).

Host hormones alter growth rate of cells by increase of macromolecule synthesis or affecting cell surface features (35). Lesmeister et al. (2005) suggested that Est was altered innate immune response via Toll-like reseptors (TLRs) in human endometrial epithelial cell lines (36). This result suggested that hormones regulated endometrial environment to cause increase of inflammatory imbalance; which affects their susceptibilities to infections. Estrogen treatment enhanced survival by decreasing the oxidative stress conditions following intraperitoneal LPS challenge in rat model (37). Similarly progesterone was shown to reduce different immune cell functions (macrophages, NK cells and B cells) (23). Kita et al. (1989) suggested that different sexual hormones have different roles in infection process. Est and Prg change the gastric mucosal response to H. pylori infection in ovary rectomized gerbils, by altering of mucosa turnover (38). Prg-treated gerbils were shown to be less suffered from gastritis, and a synthetic Prg derivative reduces of H. pylori viability (23, 39). Another study reported that, Est treatment affected female mice susceptibilities to an intraperitoneal Salmonella Typhimurium infection and, Prg treatment increased resistance to infection (23, 38).

NE is well known as an enteric neurotransmitter, which have roles in defensive mechanisms (including intestinal smooth muscle contraction, sub mucosal blood flow, and chloride and potassium secretion related with intestinal physiological process), in the intestinal system (40). Additionally, some catecholamines are shown to be secreted by T cells, macrophages, and neutrophils (41). In line with this data Kendall and Sperandio (2016) suggested that, pathogens may alter these hormones concentrations not only due to presence of an infection stress, but also which have roles in stimulating of immune responses (40). Thereby
investigations focusing on relationship between some enteric pathogens and NE concluded that, catecholamines (including NE) increased the attachments of enteric pathogens (8, 27). According to Green et al. (2004) the alterations of sympathetic neural outflow may be affected intestinal susceptibility to infection. Another in vivo study conducted by Toscana et al. (2007) showed that, NE leads immune compromising; therefore host became susceptible to all opportunist pathogens (43).

Melatonin as another neurotransmitter hormone is identified as a modulator of haemopoiesis and has roles in related with immune cell production and function such as NK, B and T cells (44). In addition to these effects it has anti-apoptotic properties on normal granulocytes and B cells (45). Xu et al. (2018) reported that melatonin is a regulator in regulating TLR-mediated innate immune responses in macrophages similar to Est (46).

A peptide hormone insulin was also identified as having anti-inflammatory effects on both diabetic and nondiabetic human immune cells (47, 48). In addition to these effects, hormones have shown to have effects on development and progression of several malignancies arising in the gonads, urogenital tract, breast, skeletal muscles tissues (49-52).

When we treated Ishikawa cell line with hormones (all concentration of Ins, Est) to imitate in vivo conditions, we mostly found that, they increased of growth of UPEC. However NE and Mlt, effects are found to be concentration dependent in the same cell line. On the other hand, in coexistence of A549 cells (which is another cancer cell line) with high level Ins and level Prg, UPEC growth was found to increased; but low level Est effect was found to be a reducer. In addition when we treated HEK cell line with all concentrations of Est, bacterial growth found to be reduced and it was increased in the presence of Mlt and Prg. These differences were suggested that tested hormones have different activities.

According to our previous results (53-55) it is obvious that, the alterations on growth of microorganisms depends on type of microorganism, growth conditions (the contents of medium used, incubation period, presence of host factors which act as signal molecules such as hormones, antibiotics, different metabolites and their concentrations). In this context Gümus et al (2018) have previously shown that growth of UPEC C7 strain did not affected in the presence of NE low and medium concentrations when tryptic soya broth (TSB) was used; but the growth of UPEC was shown to be reduced significantly in the presence of same concentrations of NE in 4 and 24 hours of exposure in SAPI medium (54, 55). Similarly, the growth of UPEC was shown to be not affected in the presence of low concentrations of Est when tested in TSB, but we have found that the growth of C7 was reduced significantly in the presence of same Est concentration when SAPI medium was used. Therefore for detection the effects of hormones on growth of bacteria host-like media and host cells would lead us to get realistic results.

It is worthy to note that, growth alterations may be due to strains tested. According to previous studies (Gümüş et al. 2018) performed by using TSB, the growth of different microorganisms’ shown to be affected differently as a response to presence of hormones. As an example, the growth of MRSA ATCC 43300 and Pseudomonas aeruginosa ATCC 27853 were not altered in the presence of same concentrations of NE, Ins and E neither in 4, 6 and 24 hours incubation. However the growth of Enterococcus faecalis ATCC 29212 and Candida albicans SC5314 shown to be affected significantly in 6 or 24 hours incubation. The growth of Enterococcus faecalis reduced in 6 hours in the presence of both low and high levels of Ins, E and NE. The growth of Candida albicans reduced in the presence of high level Ins; enhanced in the presence of both low and high levels of E and NE in only 24 hours incubation. These data allow us to conclude that incubation period is also another important factor to detect of microbial growth alterations.
The interactions of host cells and hormones depend on the characteristics of cell lines which could modulate the biological processes of a bacterium. These differences and environmental adaptations would be crucial for environmental survival; even so the hormones that influence the growth of microorganisms are not fully understood, especially in vivo.

As a conclusion, in the present study, different cell lines and signaling molecules (hormones) are used to imitate the host conditions to evaluate the growth ability of a UPEC strain which we thought to plan as one step ahead of in-vivo investigations. The data presented in this study provide a little piece of a big puzzle known as “inter-kingdom signaling pathways” by showing the possible modulations of UPEC-host cells-hormones relationship.

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