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The Possible Effects of Different Hormones on Growth Rate and Ability of Biofilm Formation in Different Types of Microorganisms

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Abstract

In our study, it has been aimed to reveal the effects of estradiol, insulin, norepinephrine as a host factor on growth rate and biofilm formation of various microorganisms (uropathogenic *Escherichia coli* C7, *Candida albicans* SC5314, *Enterococcus faecalis* ATCC 29212, Methicillin resistant *Staphylococcus auerus* (MRSA) ATCC 43300 and *Pseudomonas aeruginosa* ATCC 27853). For this purpose, microorganisms were grown in different media (TSB and SDB) supplemented with/without various concentrations of hormones. Growth rates were measured in 4th, 6th and 24th hours period by a spectrophotometer. Statistical analysis of growth rates were determined by using two-way ANOVA Bonferroni post-test. Biofilm formations were evaluated via microtiter plate assay. The effects of hormones on biofilm formation were determined via one-way ANOVA Tukey's multiple-comparisons test. All hormone concentrations were shown to reduce the growth of *E. faecalis* statistically at 6 hours incubation. The growth of *C. albicans* was found to be altered (enhanced/reduced) only within the presence of high concentrations of different hormones. Besides there is no statistical significantly difference of any hormones tested on the growth rates of MRSA, *P. aeruginosa* and UPEC ($p > 0.05$). Similar to growth rate results, biofilm formations were shown to be altered with the presence all three hormones tested. However biofilm formations of *P. aeruginosa* and UPEC C7 were found to be not affected with the presence of any hormones tested ($p > 0.05$). It has been emphasized that, mammalian hormones determine the pathogenicity of infectious diseases as an environmental factor because they are known to affect microorganisms' behaviors as well. Our results have proven that, different concentrations of hormones have various effects on growth kinetics and virulence of microorganisms. All these studies showed that, hormones determine the infectious processes directly in human.

Keywords: hormones, growth rate, biofilm, inter-kingdom communication

Резюме

Целта на настоящото изследване е да се разкрие ефектът на естрадиол, инсулин и норепинефрин като фактор на гостоприемника върху скоростта на растежа и образуването на биофилм от различни микроорганизми (уропатогенни щамове - *Escherichia coli* C7, *Candida albicans* SC5314 и *Enterococcus faecalis* ATCC 29212, резистентни на метицилин - *Staphylococcus auerus* (MRSA) ATCC 43300 и *Pseudomonas aeruginosa* ATCC 27853). Микроорганизмите се култивират в среди TSB и SDB, с или без добавка на хормони в различни концентрации. Скоростта на растеж се определя със спектрофотометър след 4-, 6- и 24-часов период. Статистическият анализ на растежната скорост се установява чрез използване на двуфакторен анализ ANOVA с Bonferroni post-тест. Образуваните биофилми се оценяват чрез анализ върху микротитърна плака. Ефектите на хормоните върху

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образуването на биофилм се определя чрез еднофакторен анализ ANOVA и тест за множествени сравнения по метода на Tukey. Установено е, че всички концентрации на изследваните хормони намаляват статистически растежа на E. *faecalis* при 6 часа инкубация. Резултати показват, че растежът на *C. albicans* се променя (увеличава/намалява) само в присъствието на високи концентрации. Освен това, няма статистически значима разлика за ефекта на изследваните хормони върху скоростта на растеж на MRSA, *P. aeruginosa* и UPEC ($p > 0.05$). Подобно на резултатите от скоростта на растеж, формирането на биофилмите се влияе от присъствието на изпитваните три хормона. Обаче, биофилмите от *P. aeruginosa* и UPEC C7 не се влияят от наличието на тестваните хормони ($p > 0.05$). Доказано е, че хормоните на бозайниците определят патогенността на инфекциозните заболявания като фактор на околната среда, тъй като те променят поведението на микроорганизмите. Нашите резултати доказват, че различните концентрации на хормони имат различен ефект върху кинетиката на растежа и вирулентността на микроорганизмите. Всички тези изследвания показват, че хормоните определят инфекциозните процеси директно в човека.

Introduction

It is well known that, during infection, microorganisms must overcome stress conditions induced by various environmental factors in their host such as hormones and some other chemicals (vitamins, bile salts, sugars, antibiotics, ions, pH etc.). Therefore, biologically important processes (growth, virulence, gene expression etc.) can be affected as a response to these host factors (Kornman *et al*., 1982; Adinolfi *et al*., 1988; Vescovi *et al*., 1996; Plotkin *et al*., 2000; Balague *et al*., 2003; Lyte *et al*., 2010; Delcenseire *et al*., 2012; Hamner *et al*., 2013). Some microorganisms are known to produce mammalian hormones and they are also able to recognize and respond to eukaryotic hormones, which indicate the long-term co-existence and co-evolution of bacteria with their hosts (LeRoith *et al*., 1981; Bassler, 1999; Miller *et al*., 2001; Walters *et al*., 2005). As an example, *E. coli* K12 strain, *Tetrahymena pyriformis*, *Bacillus subtilis*, and *Trichophyton mentagrophytes* can produce insulin, corticotrophin, somatostatin, progesterone, respectively (LeRoith *et al*., 1981; LeRoith *et al*., 1985; Lyte, 2012).

Colonization and the involvement of biofilm formation processes require quorum sensing signals. Host factors, pH, temperature and the amount of nutrients act as a microbial GPS system for communication. It has been reported that mammalian hormones (epinephrine (E), norepinephrine (NE), dopamine, dopa, estrogen, progesterone, serotonin, testosterone, and insulin) can modulate bacterial growth, gene expression and antibiotic susceptibility in several studies (Plotkin *et al*., 2000; Lyte *et al*., 2003; Sperandio *et al*., 2003; Williams, 2007; Hughes *et al*., 2008; Delcenserie *et al*., 2012; Sandrini *et al*., 2014).

In the present study, the effects of human insulin, norepinephrine and estradiol (as host factors) on growth rate and biofilm formation in different microorganisms were investigated.

Materials and Methods

Strains

Different strains used in the present study are Methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, UPEC strain (C7), *Enterococcus faecalis* ATCC 29212 and *Candida albicans* SC5314.

Hormone concentrations

We chose different concentrations of insulin (20μU/mL, 200μU/mL), estradiol (20pg/mL, 150pg/mL, 400pg/mL) and norepinephrine (100pg/mL, 500pg/mL, 1700pg/mL, 7500pg/mL, 40000pg/mL), according to their physiological levels of blood and urine in a healthy human (Aun *et al*., 1975; McCartney *et al*., 1993; Phillips *et al*., 2014; http://stedmansonline.com).

Growth rate assays

To determine the growth rate alterations, microorganisms were grown in tryptic soy broth (TSB-control) and TSB containing different concentrations of hormones. Besides Sabouraud Dekstroz Broth (SDB) was used for detection of *C. albicans* growth rate. All strains were inoculated into 11 different experimental conditions to an initial turbidity of 10^7CFU/mL. (except *C. albicans*: 10^5CFU/mL). All microorganisms were incubated at 37°C. Growth rates were determined by measuring the changes in absorbance at 600 nm in four-, six- and 24-hour periods.

Biofilm formation assays

For detection of biofilm formations, microtiter plate (tissue culture 96-well plate) assay was used. All bacteria were suspended in TSB supplemented with 1% glucose to a final concentration of 10^8CFU/mL with an exception of *C. albicans* which

final concentrations was prepared as 10^6 CFU/mL. Besides SDB was used for Candida assays. Eleven different experimental conditions were prepared according to hormones' concentrations with TSB supplemented with 1% glucose. All bacteria were inoculated into TSB supplemented with 1% glucose + hormones. Organisms were incubated at 37°C. After washing, fixation and staining processes (crystal violet), biofilm formations were determined by optical density measurement in a spectrophotometer (450nm). Unlike bacteria, overnight cultures of *C. albicans* SC5314 strain were prepared in yeast peptone dextrose (YPD) medium. Cell suspension was prepared with RPMI 1640 medium at a concentration of 10^6CFU/mL. Eleven different experimental conditions were prepared according to hormones' concentrations with RPMI 1640 supplemented with 1% glucose. *C. albicans* was inoculated into RPMI 1640 supplemented with 1% glucose + hormones. Organism was incubated at 37°C. After washing, fixation and staining processes (crystal violet), biofilm formations were determined via optical density measurement in a spectrophotometer (450nm).

Statistical analysis

Each assay was performed in duplicate, and the results were expressed as the mean of two independent experiments. Statistical analysis of growth rate alterations was performed using a two-way ANOVA Bonferroni post test. The effects of hormones on biofilm formation were determined via one-way ANOVA Tukey's multiple-comparisons test.

Results and Discussion

Growth rate alterations

Effects of hormones on growth rates of MRSA, *P. aeruginosa* and UPEC C7 were found to be statistically not significant. Growth of *E. faecalis* was shown to be reduced in both 6 and 24-hour periods with the presence of all insulin concentrations ($p<0.05$). All estradiol and norepinephrine concentrations were shown to reduce the growth of *E. faecalis* in 6 hours ($p<0.05$). Two hundred µU insulin was shown to reduce the growth of *C. albicans* in 24 hours ($p<0.001$). 1700pg, 7500pg and 40.000pg norepinephrine were shown to enhance at 24^{th} hour ($p<0.001$).

Biofilm alterations

According to the results of biofilm formations, 20µU and 200µU insulin; 40.000pg norepinephrine were shown to enhance biofilm formation of *C. albicans* ($p<0.001$) (Fig. 1). Biofilm formation of *E. faecalis* was shown to be enhanced with the presence of 20µU and 200µU insulin; 20pg, 150pg and 400pg estradiol; 7500pg and 40.000pg norepinephrine ($p<0.001$) (Fig. 2). Two hundred µU insulin, 150 pg estradiol and 1700 pg norepinephrine were shown to enhance biofilm formation of MRSA ($p<0.001$) (Fig. 3).

Similarly to previous studies, our results have proven that all three hormones tested affected both growth rate and biofilm formation depending on the type of microorganisms. While mammalian cells can coordinate by synthesizing hormones to regulate and maintain their own homeostasis, microorganisms use chemical signaling to coordinate their community behavior. This chemical signaling is known as quorum sensing (QS), which influences the microorganism's defense, survival, virulence, and antibiotic susceptibility (Lyte *et al*., 2003; Sperandio *et al*., 2003; Williams, 2007; Hughes *et al*., 2008; Delcenserie *et al*., 2012; Sandrini *et al*., 2014). QS, therefore, provides communication both between bacteria-bacteria and bacteria-host, which is defined as "inter-kingdom signaling" (Sperandio *et al*., 2003; Williams, 2007; Hughes *et al*., 2008).

Some authors have reported that catecholamines' effect on bacterial growth could be mediated by accessing sequestered iron. In relation to that, the same catathecol moiety found in many siderophores also exists in catecholamines, NE, and E etc. (Coulanges, 1998; Lyte *et al*., 2003; Sandrini *et al*., 2014). Mammalian/bacterial insulin can play a role as a QS compound (homoserine lactones, autoinducer-2, autoinducer-3, and indole etc.) in a similar manner (LeRoith *et al*., 1981; Bassler, 1999; Sperandio *et al*., 2003). In addition to this mechanism, the adherence of *E. coli* K12 was shown to be enhanced by the effect of insulin in the presence of glucose (Plotkin *et al*., 2000). In the case of sexual hormones, steroid hormones could also serve as substitutes for vitamin K, and some bacteria (such as Prevotella intermedius) uptake progesterone and estradiol, which increase their growth rate in the absence of vitamin K (Kornman *et al*., 1982).

It is worthy to note that bacterial behavior in vivo can be different from that in vitro. Host's environmental conditions are different and more complex from in vitro conditions and change during infectious processes. At the present time, it is commonly known that hormones are one of the host's factors which affect the environmental conditions of a pathogen. Taken together, "microbial endocrinology" provides insights for understanding the power of hormones that determine microorganism-host interaction.

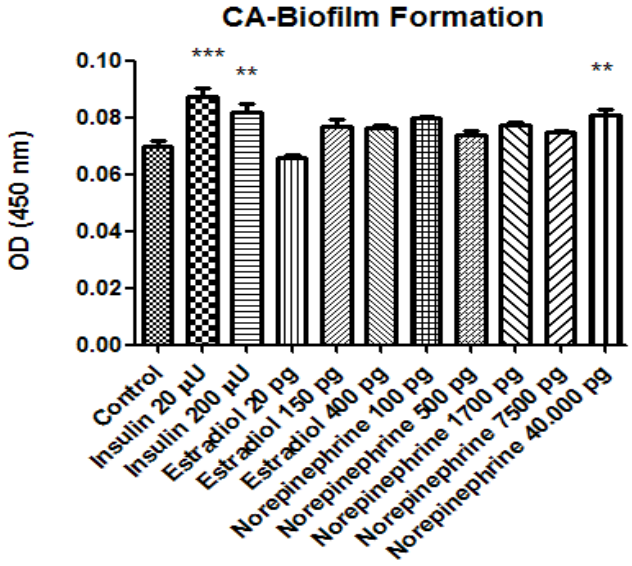

Fig. 1. Effects of different concentrations of hormones on *C. albicans* biofilm

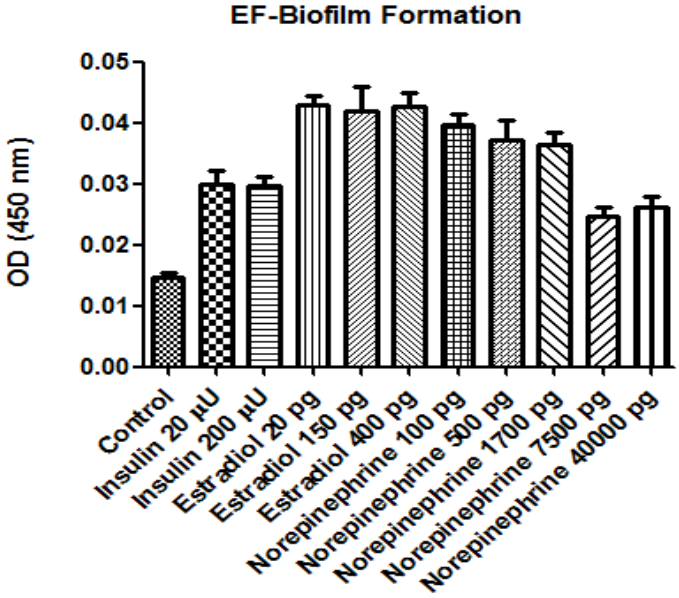

Fig. 2. Effects of different concentrations of hormones on *E. faecalis* biofilm formation

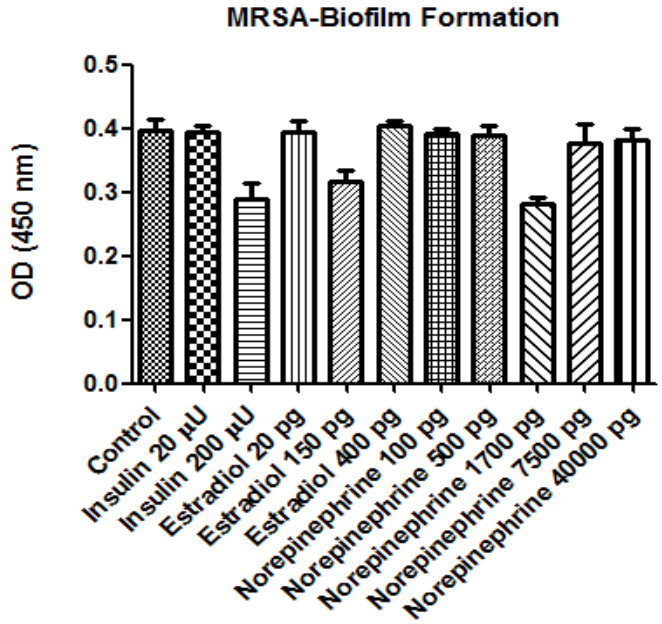

Fig. 3. Effects of different concentrations of hormones on MRSA biofilm formation

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