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CORTICOSTEROID-INDUCED EXPRESSION OF MICROBIAL VIRULENCE CAN ENHANCE THE DEVELOPMENT OF HOST INFECTIOUS DISEASE

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Abstract: Corticosteroids regulate a number of physiological processes and are synthetic analogs of the natural steroid hormones produced by the adrenal cortex. As drugs, corticosteroids are non-inflammatory and are used for the treatment of plethora of conditions which include arthritis, kidney, skin, lungs or thyroid disorders, for the treatment and relief of symptoms of allergies and symptoms of some gastrointestinal disorders. In addition, glucocorticoids can regulate the effects of inflammatory disorders, including sepsis, autoimmune diseases, and allergies. These conditions are potentially fatal. Consequently, this drug class is among the most commonly prescribed globally. One representative of corticosteroid class of drugs is dexamethasone which is used to treat allergies, adrenal problems, arthritis, asthma, diseases of blood or bone marrow, inflammation, kidney diseases, different types of skin conditions, and episodes of multiple sclerosis. Virulence factors help bacteria colonize the host at the level of the cell. In their nature, these factors are secretory, associated with the membrane or present in the cytosol. Secretory factors allow bacterium to circumvent the host immune response, while membrane factors aid bacterium in adhesion to the host cell. Finally, cytosol factors help bacteria adapt metabolically, physiologically, and morphologically to their changing environment. One such factor is aspartyl proteinase, a protein that degrades other proteins and is a virulence factor in many pathogens playing a role in the host invasion process. Another important virulence factor is the ability to form biofilms, which can render bacteria resistant to antimicrobials. Despite the widespread use of corticosteroids, including dexamethasone, little is known about their possible influence on the expression of virulence factors such as aspartyl proteinase. If such a connection is to exist the use of corticosteroids could elicit pathogenesis in certain microbes. In the here-presented study we wanted to investigate the effects of dexamethasone on the growth, expression of aspartyl proteinase and biofilm formation in three E. coli strains that were previously isolated from patients suffering from urinary tract infection. To this aim, we amended the growth media with 0.5 mg/mL dexamethasone. Bacterial growth was measured over the period of 24 hours and the effect of dexamethasone was established at different time points. Administration of 0.5 mg/mL glucocorticoid drug dexamethasone did not significantly affect bacterial growth. However, it resulted in an increase in concentration of secreted E. coli virulence factor aspartyl proteinase, which increased up to 2.6-fold for some E. coli strains. In addition, we noted the increased biofilm formation in to three out of four studied strains. This study indicates dexamethasone as a possible trigger molecule for the expression of virulence factor aspartyl proteinase in E. coli.

Keywords: dexamethasone, E. coli, aspartyl proteinase, biofilm formation
Field: Medical science and Health

1. INTRODUCTION

Bacterial metabolism is a set of metabolic processes that characterizes these prokaryotic organisms, by which they survive in nature, grow, feed, adapt, and reproduce (Kerfeld et al., 2018). Microorganisms can cause disease under certain circumstances, with the activation of their pathogenicity mechanisms (Fierer et al., 2010). One of the most studied microorganisms in this regard is Escherichia coli (E. coli), a gram-negative bacterium that can contaminate food during its production and is one of the main causes of urinary tract and gastrointestinal tract infections (Kaper et al., 2004). Whether a pathogen will cause disease depends on the virulence factors. Virulence factors are most often different enzymes that are released as a metabolic product of microorganisms, including proteinases, hyaluronidases, collagenases, lipases, fibrinolytic enzymes, coagulases, and nucleases (Sharma et al., 2017).As a result of the activation of virulence factors, the infection spreads, the disruption of normal physiological functions of the cell occurs resulting in the appearance of various pathophysiological conditions, or the worsening of existing. (Pakbin et al., 2021)

During the development of pathogenesis, the invading bacteria must overcome a series of obstacles set by the host's immune system which responds to the presence of bacteria. To establish an efficient infection the pathogen must be sensitive to and respond to changes in the host environment,

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including changes in pH, host secretions (for example, mucus), physical barriers, reduced oxygen, as well as responding to changes in the active immune response that seeks to prevent the pathogen to colonize the organism (Wilson et al., 2002). In addition, it is sometimes necessary for invading microbes to displace or utilize microbes found in the normal flora of the host organism. All this is necessary for pathogens to establish an efficient infection. Therefore, pathogenic bacteria are equipped with a set of strategies to evade host defenses and to be able to cause disease (Murray et al., 2015). Simple triggers such as temperature, osmolarity, pH, amount of nutrients, amount of oxygen or iron can turn on or off genes that control virulence (Johnson B.K & Abramovitch R.B., 2017). Inhibition of the regulatory system associated with virulence prevents the sensitivity and adaptability of bacteria to conditions within the host, making them a target for antibiotics and an efficient immune response (Diard & Hardt, 2017). Virulence is the degree of pathogenicity of microbe and is determined by invasiveness, infectivity, and pathogenic potential. Within the same species, there are different strains of bacteria that have different degrees of virulence. Virulence represents the capacity of a microorganism to cause damage in the host organism and is the result of a complex interaction between the parasite (microorganism) and the host (Sharma et al., 2017). Bacterial cell structures (capsule, peptidoglycan, teichoic acid, surface proteins, LPS) and extracellular products of bacteria (toxins and enzymes) can be classified as virulence factors. Virulence factors can be encoded by genes localized on the bacterial chromosome, plasmids, bacteriophages, or transposons (Alberts et al., 2008). Virulence factors play an important role in the pathogenesis of bacterial infection. Most bacteria possess several virulence factors and each of them has a certain role in different stages of the disease. Different experimental models are used in testing virulence factors, but it should be emphasized that not all clinical syndromes of human infections can be reproduced in animal models. Most of the studies of virulence factors were conducted in in vitro conditions, but even these conditions are significantly different from those present in the tissue of the infected host. In the clinical sense, virulence is a manifestation of the complex relationship between the parasite and the host, and depends on the characteristics of the microorganism on the one hand and the host's defenses on the other (Neher et al., 2008).Corticosteroids are synthetic analogs of the natural steroid hormones produced by the adrenal cortex and regulate a variety of physiologic processes (Peinado-Acevedo et al., 2023). They are among the most widely prescribed drug classes worldwide (Wallace et al., 2023). Indications for corticosteroid therapy include infectious and inflammatory disorders. Whether the corticosteroids influence bacterial metabolism has not been studied yet. In this study we used glucocorticoid dexamethasone. Despite their widespread use, little is known about the possible role of corticosteroid-based drugs on the expression of microbial virulence factors. In this study, we assessed the levels of metabolic activity of E. coli and the expression of its virulence factor aspartyl proteinase in the presence of glucocorticoid drug dexamethasone in defined concentration and at different incubation time-points.

2. MATERIALS AND METHODS

2.1. STRAINS AND GROWTH CONDITIONS

Three strains of E. coli (E. coli-C1, E. coli-C2 and E. coli-C3) with strong biofilm-forming ability were isolated from clinical samples of hospitalized patients suffering from urinary tract infections based on colony morphology on blood and MacConkey agar and standardized biochemical tests. One E. coli (E. coli -Ref) strain without the ability to form biofilms was included as a control in all performed experiments. All bacteria were grown in Trypticase soy broth (TSB) media, supplemented with 1% glucose, under aerobic conditions. To produce the growth curve, 10 µl from an overnight culture was inoculated into 3 ml of TSB growth media in sterile 15 ml plastic tubes. The cultures were incubated at 37°C for varying lengths of time: 0 h, 6 h, 12 h, 18 h, and 24 h. Prior to incubation, each test tube containing E. coli cells was enriched with dexamethasone at 0.05 mg/ml. The optical density of each test sample was measured at a wavelength of 600 nm.

2.2. PREPARATION OF SAMPLES AND QUANTIFICATION OF ASPARTYL PROTEINASE

Subsequent to incubation, 0.5 ml of bacterial culture was combined with 2 ml of BSA solution (1%) and kept at 37 °C for half an hour. To stop the reaction, 5 ml of trifluoroacetic acid (10%) was added. The samples were then centrifuged for 10 minutes at 1000 rpm and the concentration of aspartyl proteinase was measured. The trifluoroacetic acid (10%) and BSA solution (1%) were diluted with 0.1 M citrate buffer (pH 3.5).The quantity of aspartyl proteinase was assessed using a spectrophotometer by gauging the absorption of the test specimens at 260 nm and 280 nm. The conclusive estimations were obtained

through the formula: mg of protein/mL = 1.55 × A280 - 0.76 × A260.

2.3. ASSESSMENT OF THE ABILITY OF STRAINS TO FORM BIOFILMS

E. coli cultures, which were stopped in their growth at various time intervals (0 h, 6 h,12 h, 18 h, and 24 h), were removed from the tubes which were then rinsed with pH 7.5 phosphate-buffered saline (PBS). Subsequently, the tubes were inverted and left to air dry for 20 minutes, following which 1 ml of 0.1% crystal violet was added. After incubating for 5 minutes, the crystal violet stain was washed away with sterile distilled water, and the culture tubes were examined for the presence of biofilm. Only tubes exhibiting a visible violet film coating the walls were positive for biofilm formation. Based on the intensity of the biofilm formation, the biofilms were classified as weak, moderate, or strong.

3. RESULTS

3.1. THE INFLUENCE OF DEXAMETHASONE ON THE PROLIFERATION OF E. COLI

The growth of E. coli strain C1 during 24 hours of incubation in culture medium at 37 °C is shown in Figure 1A. The results show that dexamethasone at a concentration of 0.05 mg/mL caused the bacterial culture to enter stationary phase of growth after 12 hours, earlier than in the control experiment. In E. coli strain C2, dexamethasone showed a stimulating effect on cell proliferation that reached maximum after 6 hours of incubation after which the culture again entered the stationary phase earlier than the control (Figure 1B). In the strain E. coli C3, dexamethasone did not influence the growth rate as it closely followed that of the control experiment (Figure 1C). Likewise, the growth of E. coli reference strain did not change significantly upon dexamethasone addition (Figure 1D). We conclude that the addition of dexamethasone does not significantly impact the growth of E. coli in the culture.

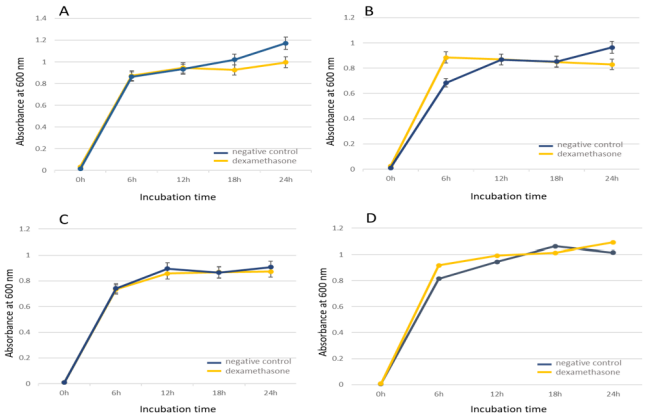
3.2. CHANGES IN ASPARTYL PROTEINASE SECRETION UNDER THE INFLUENCE OF DEXAMETHASONE

Administration of dexamethasone at a concentration of 0.05 mg/mL showed stimulatory effect on the metabolic activity of E. coli-C1 and resulted in significantly increased secretion of aspartyl proteinase by 2.6-fold compared to the negative control, after 18 hours of incubation at 37 °C (Figure 2A). Administration of dexamethasone at a concentration of 0.05 mg/mL showed a significant stimulating effect on the metabolic activity of E. coli-C2 and increased secretion of virulent protein aspartyl proteinase after 12 hours of incubation at 37 °C with 1.47 increase in this protein production (Figure 2B). Dexamethasone at a concentration of 0.05 mg/mL did not show a significant stimulating effect on the metabolic activity of E. coli strain C3, on the contrary, the concentration of the enzyme was lower than in the negative control (Figure 2C). Administration of dexamethasone at a concentration of 0.05 mg/mL did not show a stimulating effect on the metabolic activity of the E. coli reference strain compared to the negative control after incubation at 37 ° C (Figure 2D).

3.3. ABILITY OF STRAINS TO FORM BIOFILM

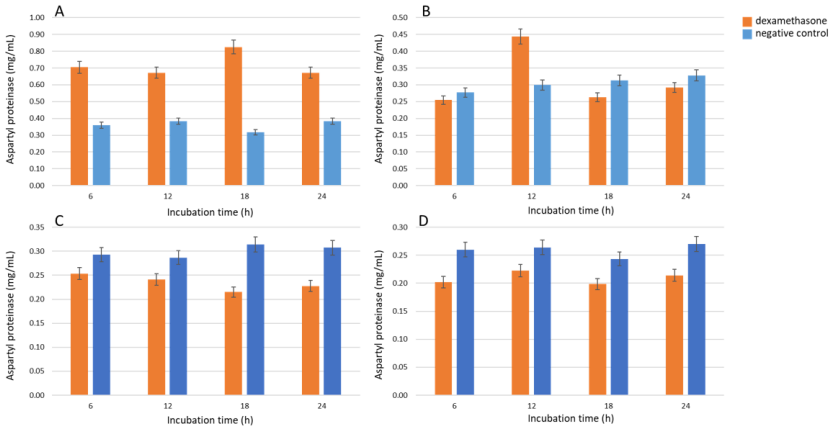
Finally, we assesed the ability of strains to form biofilm, another important viruence factor. The results of our analysis are presented in Table 1. Strain E. coli C1 showed moderate ability to form biofilm after 18 and 24 hours of incubation. Strain E. coli C2, showed moderate ability to form biofilm after 24 hours of incubation, strain E. coli C3 showed strong ability to form biofilms after 24 hours of incubation. Control strain showed no ability to form biofilm.

Figure 1. Growth curves of E. coli strain C1 (A), strain C2 (B), strain C3 (C) and reference strain (D) with and without dexamethasone supplementation



Source: Authors research

Figure 2. Excretion of the enzyme aspartyl proteinase under the influence of dexamethasone and in different incubation periods in E. coli strains C1 (A), C2 (B), C3 (C) and reference strain (D).



Source: Authors research

Table 1. Biofilm formation in tested E. coli strains
Legend: (-) no biofilm formation; (+) weak biofilm formation; (++) moderate biofilm formation; (+++) strong biofilm formation

Biofilm formation					
E. coli C1	0h	6h	12h	18h	24h
Dexamethasone	-	+	+	++	++
Negative control	-	+	++	+++	+++
E. coli C2					
Dexamethasone	-	+	+	+	++
Negative control	-	+	++	++	+++
E. coli C3					
Dexamethasone	-	-	+	+	+++
Negative control	-	+	+	++	+++
E. coli reference strain					
Dexamethasone	-	-	-	-	-
Negative control	-	-	-	-	-

Source: Authors research

4. DISCUSSION

Corticosteroids are often used to combat ear, nose, and throat infections in addition to antibiotics in order to reduce inflammation and swelling. These infections often occur in airtight cavities and ducts where growth conditions favor microbial growth. Corticosteroids reduce inflammation through specific receptor-mediated binding to intracellular receptors.

In this study, the effects of addition of low concentration of dexamethasone, a well-known synthetic and inexpensive anti-inflammatory drug, on bacterial growth and secretion of the virulent factor aspartyl proteinase in three clinical strains of E. coli and the reference strain of E. coli was investigated.

With respect to the influence of dexamethasone on growth of selected E. coli strains, the results showed that dexamethasone has a strain-dependent and only a minor effect on the growth of the strains. Little is known about the effects of corticosteroids on bacterial growth. Dexamethasone showed antimicrobial activity against Streptococcus milleri and Aspergillus flavus (Neher et al., 2008). However, in line with our observations, dexamethasone did not inhibit the growth of bacteria and fungi isolated from keratitis (Mejia-Lopez et al., 2013).

When growth media was supplemented with 0.5 mg/mL dexamethasone, two out of three tested E. coli strains showed increased excretion of the virulent protein aspartyl proteinase after the first 6 hours of incubation in the culture medium at 37 °C. This implicates that the bacterial virulence can increase as a consequence of dexamethasone addition which can have serious consequences for the patient. In addition, an increased secretion of aspartyl proteinase coincided with the logarhytmic phase of growth (6-12 hours) in which the production of biofilm, another important virulence factor, was also seen. This finding is in line with the observations by Santos et al., who showed that an increased metabolic activity of bacteria leads to an increase in overall protein production, some of which act as virulence factors and serve as modulators of bacterial pathogenicity (Santos et al., 2021).

Formation of biofilms of uropathogenic E. coli is linked to chronic inflammation that can cause severe or recurrent urinary tract infections. In the environment of a biofilm, antibiotic resistance is common and the virulence genes are transmitted horizontally which promotes the growth of multidrug-resistant microorgansims (Katongole et al., 2020). In this study, the three uropathogenic E. coli were found to form biofilms. Under the influence of dexamethasone, strains E. coli C1 and C2 showed slower biofilm development with moderate formation after 24 h of incubation as opposed to strong biofilm formation under the condition of no added dexamethasone. The third strain, E. coli C3 showed strong biofilm formation under both conditions. This shows that biofilm formation was again strain-specific and that can be partially inhibited by the addition of this corticosteroid drug. While we found no data on dexamethasone interactions with biofilm development in E. coli, a study on Staphylococcus aureus showed that subinhibitory concentrations (1/8 MIC) of dexamethasone reduced the production of virulence factors, including biofilm formation (Saleh et al., 2022).

5. CONCLUSION

The here presented study indicates that the expression of aspartyl proteinase and biofilm production could be enhanced by supplementing the growth media with 0.5 mg/mL dexamethasone. This shows that this anti-inflammatory drug can not only reduce human immune response, but also increase virulence potential of opportunistic/pathogenic strains which in turn could cause some serious pathogenic conditions. The results of our study indicate the need to continue research into the metabolic activity of microorganisms and their proteins as potential virulence factors and modulators of microbial pathogenicity.

To conclude, microorganisms employ a wide array of virulence factors to successfully thrive within their hosts leading to the development of infections that can often be fatal. The findings of this study show that dexamethasone has a minor effect on the growth of microorganisms, can induce the production of virulence factor aspartyl proteinase and can reduce the amount of formed biofilm. This suggests that the usage of dexamethasone as adjuvant to treat E. coli infections should be evaluated further.

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