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The activity of adenosine on some oral bacteria: An *in vitro* study.

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Abstract

Adenosine revealed to have a significant function in the avoidance of tissue destruction induced by stressful conditions including bacterial infection. throughout inflammation and infection, adenosine concentration increases quickly. Adenosine regulates a broad range of immunological, antibacterial and cellular defensive effects. This study involved evaluation the effects of adenosine on oral streptococci (isolated from saliva). Adenosine was melted in sterile deionized water 50mg/ml , two fold dilutions were made to obtain three concentrations of adenosine 50mg/ml,25mg/ml,12.5mg/ml. Susceptibility was tested by a broth microdilution method using spectrophotometer. This in vitro study showed that adenosine had significant antibacterial effect against oral streptococci suggesting its possible task in the treatment of the patient having oral streptococcal infection.

Key words : Adenosine, adenosine receptors, oral streptococci.

Abbreviations— Polymorphonuclear leukocytes (PMNs), deoxyribonucleic acid (DNA)

INTRODUCTION

Bacterial resistance is rapidly growing problem. Therefore, there is a more need for alternative antimicrobial agents with novel mechanisms of action[1]. Purines are heterocyclic aromatic particles which are important compounds in the biology[2]. One of them is adenosine which is an endogenous purine nucleoside produced by dephosphorylation of ATP. It have a role in different physiological functions through activating A1, A2A, A2B, and A3 adenosine receptors[3]. It is produced intra- and extracellularly with well controlled concentrations due to its multiple biological effects and it plays different roles in acute and chronic disease states[4]. Adenosine was assessed for its antibacterial activity [1], it can inhibit *Escherichia coli* and is involved in the interactions of immunocompetent cells with other cell types. So it could be useful for treatment of infection and sepsis[5]. Adenosine may reduce inflammatory processes associated with bacterial infections [6].and it can be involved in the modulation of inflammatory responses like in periodontitis which is an inflammatory disease caused by different types of bacteria characterized by genetic and acquired host susceptibility to these pathogens [7]. Adenosine and its receptors have been studied at site of inflammation and infection and used in medicine but little is known about adenosine in relation to dentistry and oral bacteria[5]. In vitro studies of adenosine have been done on different cells of inflammation and infection like neutrophils and macrophages, Adenosine (A1) receptor stimulation is the main inducer of proinflammatory effect, increased neutrophil chemotaxis , improved attachment to endothelial cells and "Fc+ receptor Y" initiated phagocytosis[8]. Development of some bacterial infection like Chlamydia trachomatis can be controlled by extended exposure of infected cells to extracellular adenosine[9]. While growing awareness rewarded to the extracellular adenosine effects on the inflammation, only few studies have been done to study the probable role of adenosine against the bacterial cells, infected cell and latent infections. The aim of this study was to focus on the activity of adenosine on oral streptococci by the in vitro assay method.

MATERIAL AND METHOD

This study was approved by scientific committee in the Dental Basic Sciences Department, Dental College, Mosul University.

Isolation and culturing of oral streptococci

Oral streptococci were isolated from saliva, 15 isolates were obtained using Mitis Salivarius Agar (Difco, USA), (MSA) with bacitracin (MSB) supplemented with 1% potassium tellurite and 2U/ml bacitracin (MSB) [10]. Further identification were carried out using gram stain technique showing gram positive streptococci, catalase negative, α or non-hemolytic on blood agar.

Preparation of Adenosine

A denosine pure powder were obtained from commercial suppliers. Adenosine was prepared on the day of testing, it is melted in sterile deionized water 50mg/ml , two fold dilutions were made to obtain three concentrations of Adenosine 50mg/ml,25mg/ml,12.5mg/ml.

Measuring susceptibility of oral streptococci to Adenosine.

Susceptibility was tested by a broth microdilution method according to (CLSI) rule with slight modifications using the spectrophotometer[11]. The Spectrophotometer measures the turbidity or optical density (OD) which is the gauge of the quantity of light absorbed by a bacterial suspension that may be affected by some medicaments. 18 hour bacterial inoculum is prepared equal to tube 0.5 MacFarland standerds ($1.5*10^8$) CFU[11].

Procedure

Five tubes each containing two ml Muller Hinton broth, one of the tube containing only 2 ml broth media (control -ve) ,the other tube containing 2ml broth only (control +ve) ,three tubes containing serial dilutions (double fold) of 2ml Adenosine (50mg/ml, 25mg/ml, 12.5 mg/ml) respectively, the tubes were inoculated with 100 µl bacteria (tube 0.5 MacFarland standerds) except control negative tube. The

tubes were incubated for 24 hour, then OD were measured by spectrophotometer at 600 nm and compared with the control positive, the experiment repeated three times for each isolate.

RESULTS

For all study groups, descriptive statistic of OD for each adenosine concentration was revealed in (Table 1). ANOVA test described significant differences between groups (Table 2). Duncan's Multiple Range Test showed significant differences between control, 50mg/ml, 25mg/ml and 12.5mg/ml study groups (0.10020 , 0.44927, 1.12760, 1.99700) respectively Table (3).

Table 1: Descriptive Statistics of all study groups.

Study groups (Adenosine concentrations)	N	Mean
Control	15	.100200
50 mg/ml	15	.449270
25 mg/ml	15	1.12760
12.5 mg/ml	15	1.99700

Table 2: ANOVA Test comparison of OD for all study groups.

Absorbance	'Sum of Squares'	df	'Mean Square'	F	Sig.
Between Groups	31.450	3	10.483	277.997	.000*
Within Groups	2.112	56	.038		
Total	33.562	59			

Table 3: Duncan's Multiple Range test comparison of OD for all study groups.

Study groups (Adenosine concentrations)	N	A	B	C	D
Control	15	0.10020			
50 mg/ml	15		0.44927		
25mg/ml	15			1.12760	
12.5mg/ml	15				1.99700

Means with the different letters were statistically significant.

The OD of different adenosine concentrations showed significant decrease in OD by increasing the concentration of adenosine which indicate the antibacterial effects of adenosine in vitro Figure (1).

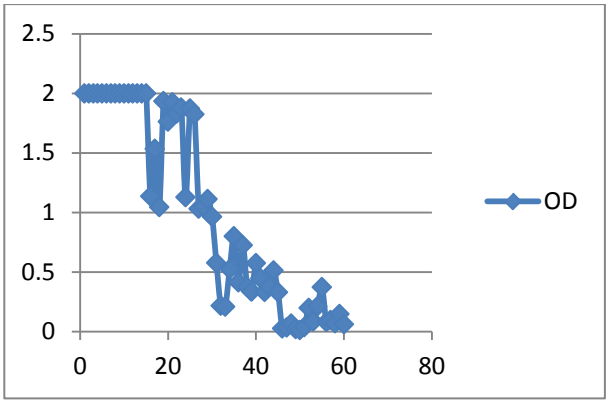

Figure (1):The OD of different adenosine concentrations.

DISCUSSION

More than hundreds of different types of bacteria have been recognized to habit within the oral environment. Among them, many species belonging to the genus *Streptococcus*[12]. Adenine nucleoside can interfere with deoxyribonucleic acid "DNA" synthesis in bacteria, bacteriophages and mammalian viruses. In the evaluation of a numbers of adenosine nucleosides and nucleotides for antimicrobial activity, it was revealed that they have activity in vitro against a diversity of bacteria and also was effective in vivo in infected mouse. In this study, adenosine showed significant in vitro activity against oral streptococci which may be explained by the ability of adenosine nucleosides to inhibit 'DNA' synthesis of this bacteria[15]. 'DNA' ligase is a new target for the finding of antibacterial agents with a new mechanisms. The bacterial DNA ligases are indispensable for viability of all Gram positive and Gram negative bacteria. A study was showed that the 'DNA' ligase from *Streptococcus pneumoniae* was inhibited by compounds with an adenosine substructure that have antibacterial activity[14].The adenosine bactericidal activity and inhibition of *S. pneumoniae* NAD_-dependent 'DNA' ligase resulted in concentration independent antibacterial effect. Further, the activity of adenosine against bacteria was selective, it has no cytotoxic activity against other type of cell [15]. Adenosine have significant roles in the host response to pathological microorganisms. The (A2a) adenosine receptor has been well considered for its action in immunity and inflammation where adenosine serves as a ''danger signal'' . The (A2b) adenosine receptor has a less affinity for adenosine compared to (A2a) which mean that (A2b) should act in field of high adenosine concentrations [9]. Epithelial cells have low ability for adenosine synthesis. During normal adenosine homeostasis, adenosine concentrations extracellularlly can be less than "1 µM" [16]. In response to cellular destruction and inflammation, adenosine concentrations are rapidly elevated due to "ATP" and adenosine secretion in inflammatory and other various cell types. Inflammation and injury manage is thought to be secondary to (A2a) adenosine receptor stimulation in area of injury [17]. In a study carried out by Yuesheng Li *et al* , (A2a) adenosine receptor knockout mice had worse colitis and higher death incidences from *C. difficile* infection which indicated that endogenous adenosine gives protection from infection by the (A2a) adenosine receptor [18]. Bacteria are contained in the mucosa of the gingival epithelial cells during infection which convey adenosine receptors " A1, A2a, A2b, and A3 " inhibition[19]. Researchs explain that (A2a) adenosine receptor may stimulate negative effect against *Porphyromonas gingivalis* " a major opportunistic periodontal bacterium" for its extended persistence in the oral mucosa. Additional work is wanted to reveal the relations between different adenosine receptor subtypes during oral infections[19]. A study explain the synthesis and assessment of adenosine analogs for in vitro activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* found that these analogs had significant antibacterial effects

than ciprofloxacin by their action on tyrosyl-tRNA synthetase of bacteria [20]. which may explain the significant antibacterial effect of adenosine in the present study. Another parameter is the regulation of oxidative metabolism by adenosine. Some nucleosides decrease and other increase superoxide and hydrogen peroxide synthesis by PMN. In vivo superoxide generation by activated human PMN can be regulated by adenosine through its binding to external cell membrane nucleoside receptors (either stimulatory (A2) or inhibitory (A1)) in manner better than of microbial particle-induced effect[21]. On other hand, Thammavongsa W *et al* stated that synthesis of adenosine by staphylococcal can help this bacteria to escape from phagocytic clearance [22], and could be rescued by an exogenous supply of adenosine which can exert deleterious effects during active infections by inhibiting the innate immune response and may contribute to the establishment of bacterial and parasitic infections[23 and 24].

Further studies of adenosine antibacterial effect could lead to breakthroughs in treatment of infections and decreased the bacterial burden in the infected tissue, thus validating adenosine as antibacterial therapy.

CONCLUSION

In this work , adenosine have been shown to have significant antibacterial effect on oral streptococci suggesting that pharmacological modulation of adenosine by further " in vitro and in vivo " studies is necessary for dental applications in the context of oral infections . The exact effects of adenosine in vivo on infection activity in humans are unknown. To decide whether adenosine have potentially therapeutic implications as antibacterial agent in humans, in vivo studies are necessary.

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