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The Effects of Inhibitors and anti-GTF-I_b antibodyon Growth of Mutans Streptococci *Streptococcus sobrinus* (serotype G) N_{10} Strain

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Abstract: *In this study the effect of different concentrations of inhibitors (sodium fluoride, chlorohexidine, EDTA and ZAK mouthrins) was tested on the growth of Streptococcus sobrinusserotype G N10 strain by using broth dilution method (in liquid media) and diffusion method on solid media. It was found that different concentrations of anti- GTF-$I_b$antibody and EDTA were incapable to inhibit the growth of bacterial isolate whereas the inhibitors (sodium chloride and chlorohexidine) at concentrations (18 mM) and (20mM) respectively were capable to produce a complete bacterial inhibition, and ZAK mouthrins at concentration (12mM) was able also to inhibit the bacterial growth by using broth dilution method. Also the effect of the same concentrations of these compounds within which anti – GTF-I_b antibody were tested on the enzymatic activity of purified GTF-I_b enzyme which was isolated from the same bacterial isolate. It was found that different concentrations of these compounds and the anti- GTF-$I_b$antibody were able to inhibit the enzymatic activity of the purified GTF-I_b with an exception of EDTA.*

Keywords: *Streptococcus sobrinus, chemical inhibitors(sodium fluoride, cholohexidine, EDTA and ZAK mouthrins)against GTFs, anti GTF-$I_b$antibody*

I. INTRODUCTION

Dental caries is a bacterial disease of dental hard tissue which occur in certain localized sites of dentition.[1] This disease tend to remain untreated in many undeveloped country , leading to considerable suffering that is often alleviated only by the loss or extraction of infected teeth. [2]

Several molecular components has been used as an antigen to stimulate the immune system against cariogenic bacteria, GTF preparations were attractive possible vaccine that may constitute important target of the antibacterial mechanism[3]. Also a variety of compounds capable to controlling dental caries have been extensively used on the basis of the following criteria: Antimicrobial activity; inhibition of GTF by immunological neutralization; enzyme inhibitions and replacement of sucrose with other sweeteners [4].

So the aim of this study to determine the antibacterial and the antienzymatic activities of different concentrations of inhibitors and anti- GTF antibody on the growth of mutans streptococci *Streptococcus sobrinus*local Iraqi isolated bacterial strain from teeth and against the activity of purified GTF enzyme which that isolated and purified from the same strain.

II. MATERIALS AND METHODS

Bacterial isolate : *Streptococcus sobrinus* (serotype G) N_{10} was isolated from dental plaque and identified by Al-Mudallal*et al.*,[5] by growing on the surface of MS-agar, testing their tolerance to high concentration of sodium chloride, utilization of different carbohydrate sources , antibiotic sensitivity test and Latex test (PASTOREX STREP) for serotype identification.

Anti-GTF-I_b antibody: It was prepared also by Al-Jumaily*et al.*,[6]. The antigen was a concentrated purified GTF-I_b enzyme which was isolated and purified also by (Al-Mudallal*et al.,* 2011[7] from the previous bacterial isolate. The antigen preparation and the immunization schedule were done following the method describes by Wunder and Bowen, 2000 [8].

The effect of inhibitors and anti- GTF-$I_b$antibody on bacterial growth:

The effects of sodium fluoride, chlorohexidine, EDTA, ZAK mouthrins and anti-GTF-I_b antibody on the growth of bacteria were determine by the following methods:

The broth dilution method described by Carpenter, 1972 [9]: Briefly, brain heart infusion broth was supplemented with different concentrations of chlorohexidine (20, 15, 10, 5 and 3 mM) ; sodium fluoride (18, 16, 12, 8, 4, 2, 1, 0.5mM) ; EDTA (25,20,15,10,5 and 3 mM) and ZAK mouthrins (12,10,5,and 3mM) then sterile by autoclaving at 121°C. Also a sterile brain heart infusion broth supplemented with different concentrations of anti-GTF- I_b antibody ($1.5x10^{-3}$, $0.78x10^{-3}$, $0.51x10^{-3}$, $0.39x10^{-3}$, $0.31x10^{-3}$, $0.23x10^{-3}$, $0.21x10^{-3}$M) was also prepared. These broth cultures were inoculated with a stock culture of bacterial broth (O.D. =0.25) for each concentration of inhibitor and the GTF antibody. After incubation, the effects of inhibition of these compounds on the growth and metabolism of bacteria were determined using the spectrophotometer at (600nm).

The diffusion method on a solid media following method described by Silva *etal.*,1987[10]: Briefly, brain heart infusion broth stock culture bacteria was spreaded on the surface of brain heart infusion agar plates. Then each concentration of inhibitors and anti-GTF-I_b antibody was pipetted into prepared holes on the same agar plates and incubated for (24-48hrs.) at 37^0C. The diameters of clearance zone area were measured for each concentration and dilution

III. RESULTS AND DISCUSSION

The Effect of Inhibitors and Anti-GTF-I_b antibody on Growth of bacterial isolate

The susceptibility of N10 (*S . sobrinus*) (serotype G) to different concentrations of sodium fluoride, chlorohexidine dichloride, EDTA, ZAK (mouthrinse) and anti-GTF-I_b antibody with concentrations of ($1.5x10^{-3}$, $0.78x10^{-3}$, $0.51x10^{-3}$, $0.39x10^{-3}$, $0.31x10^{-3}$, $0.23x10^{-3}$, $0.21x10^{-3}$M) were determined by two methods . For the first method, susceptibility of the bacterial growth against these inhibitors and anti-GTF-I_b antibody was estimated by a broth-dilution method. Results showed, different concentrations of anti-GTF-I_b and EDTA were incapable to inhibit the growth of bacterial isolate. The absorbance at 600 nm remained the same as a control. For the sodium fluoride, chlorohexidine (CHX), ZAK (mouthrinse), results shown in tables (1), (2) and (3) indicate that, these inhibitors were capable to inhibit the growth of bacteria at concentrations (0.5mM), (3mM), and (3mM) respectively. The minimal inhibitory concentrations in which these concentrations were found to give the lowest growth rate or effect were noticed at (2mM), (15mM), (12mM) respectively. Complete bacterial growth inhibition was noticed only with sodium fluoride and chlorohexidine at concentrations of (4mM) and (20mM) respectively.

Table (1): The effect of sodium fluoride (NaF) on the growth of mutans streptococci N_{10} (*S. sobrinus*) (serotype G) using broth dilution method.

Absorbance at 600 nm of the control	Concentration of NaF (mM)	Absorbance at 600 nm with NaF
0.940	–	–
	0.5	**0.788**
	1	**0.459**
	2	**0.224**
	4	–
	8	–
	12	–
	16	–
	18	–

(–) no growth was detected.

Table (2): The effect of chlorohexidine dichloride (CHX) on the growth of mutans streptococci N_{10} (*S. sobrinus*) (serotype G) using broth dilution method.

Absorbance at 600 nm of the control	Concentrations of (CHX) (mM)	Absorbance at 600 nm with NaF
0.969	–	–
	3	**0.704**
	5	**0.584**
	10	**0.292**
	15	**0.075**
	20	–

(–) no growth was detected.

Table (3): The effect of ZAK (mouthrinse) on the growth of mutans streptococci N_{10} (*S. sobrinus*) (serotype G) using broth dilution method.

Absorbance at 600 nm of the control	Concentration of ZAK (mM)	Absorbance at 600 nm with ZAK
0.959	–	–
	3	**0.821**
	5	**0.633**
	10	**0.341**
	12	**0.174**

For the second method in which susceptibility of bacterial growth against the previous inhibitors and different concentrations of anti-GTF-$I_b$antibody was tested by diffusion method on solid media . Results shown in figures (1) and (2) indicated that, the highest zone of inhibition (50mm) was recognized with chlorohexidine at concentration of (20mM) followed by sodium fluoride with a zone of inhibition of (37mm) at concentration of (18 mM) (Figure 3). ZAK (mouthrinse) at concentration of (12 mM) was capable to give zone of inhibition of (27.5 mm)(Figure 4). Complete inhibition of the bacterial growth was unable to be recognized with any previous inhibitors at any concentration.

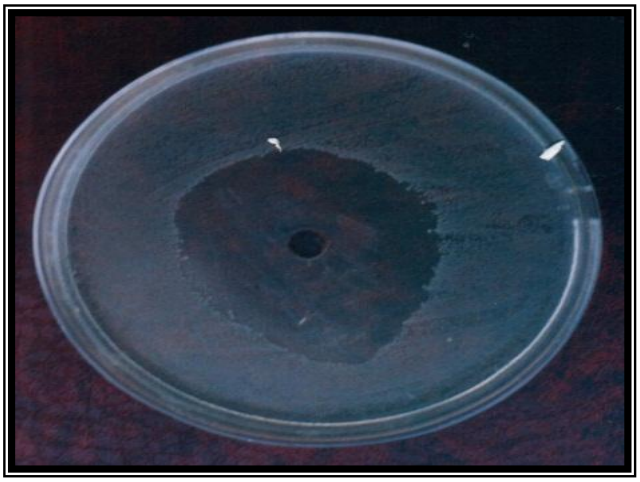

Figure (1):The highest zone of inhibition for the growth of mutans streptococci N_{10} (*S. sobrinus*) (serotype G) at (20 mM) of chlorohexidin (CHX).

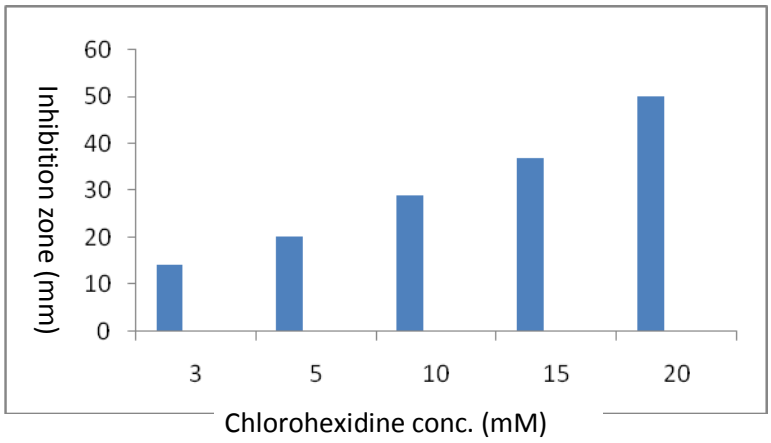

Figure (2): The effect of chlorohexidine (CHX) on the growth of mutans streptococci N_{10} (*S. sobrinus*) (serotype G) using diffusion method on solid medium

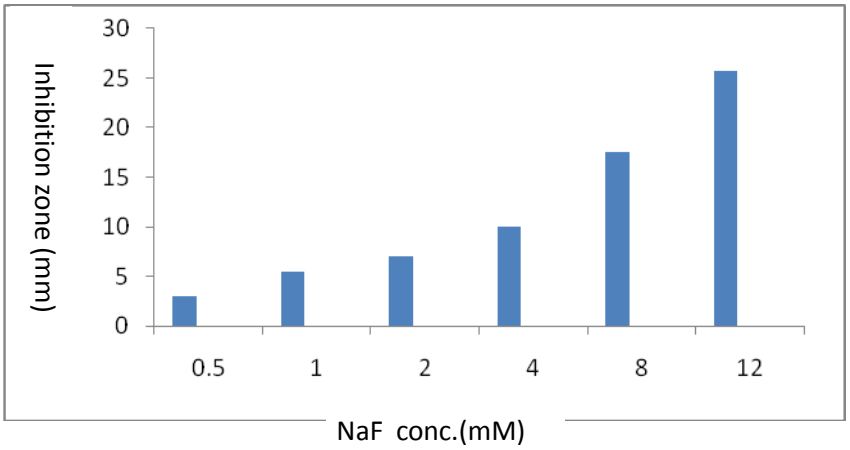

Figure (3): The effect of sodium fluoride (NaF) on the growth of mutans streptococci N_{10} (*S. sobrinus*) (serotype G) using diffusion method on solid medium.

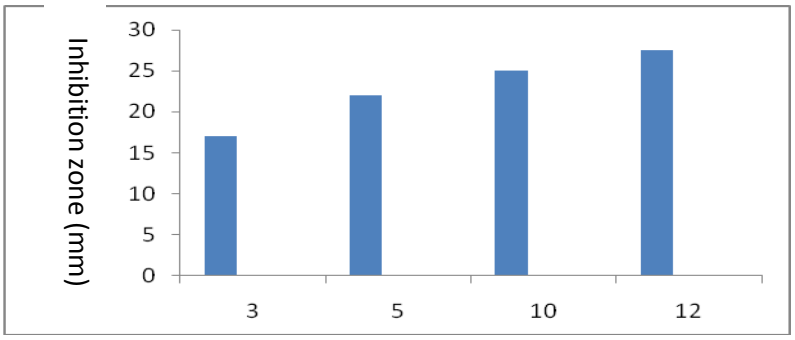

Figure (4): The effect of ZAK mouthrinse on the growth of mutans streptococci N_{10} (*S. sobrinus*) (serotype G) using diffusion method on solid medium.

Accordingly, the best inhibitors of growth of bacteria by using the broth-dilution method were sodium fluoride at concentration of (4 mM) followed by chlorohexidine at concentration of (20mM) in which complete inhibition of bacterial growth was noticed with a minimal inhibitory concentrations that gave the lowest growth rate at (2mM) and (15mM) respectively. ZAK (mouthrinse) has a good degree of inhibition on the growth of bacteria with minimal inhibitory concentration or effect on bacterial growth at concentration (12mM) but no complete growth inhibition was recognized with this inhibitor. For the diffusion-method, the best inhibitor for the bacterial growth was chlorohexidine at (20mM) which capable to give the highest zones of inhibition on the agar followed by sodium fluoride with concentration of (18mM). ZAK (mouthrinse) was also capable to produce a zone of inhibition about (27.5mM) at concentration of (12mM).

In these methods, no effect on the bacterial growth was detected by the use of anti-GTF-I_b antibody and EDTA inhibitor.The susceptibility of the bacteria to the inhibitors was determined clearly by broth dilution method more than the diffusion-method in agar media because the first method gives a quantitative assessment for the effective concentration of inhibitors which was given a complete inhibition (bactericidal or bacteriostatic action). Also the minimal inhibitory concentrations of the previous inhibitors can also be determined by this method.

The inability of the anti-GTF-$I_b$antibody to inhibit the growth of bacterial isolate was related to this antibody which can decrease or inhibit the adsorption of these bacteria on the tooth surface *in vivo* or *in vitro* on the surface of saliva-coated hydroxyapatite. So this antibody was capable to block the synthesis of the insoluble-glucan which was very important for the adsorption and attachment only, so the bacteria was remained capable to grow and multiply (i.e. the number of the bacteria was remained the same). This information was recorded after an experiment in animal model *in vivo* and from the recognition of the behavior of bacteria on the surface of saliva-coated hydroxyapatite *in vitro* after the usage of an antibody specific for GTF enzyme.[11,12]

Chlorohexidin had a bactericidal activity against both gram-positive and gram-negative bacteria. It was capable to reduce the number of mutans streptococci in the mouth greater than *S. sanguis*and lactobacilli. [13] Because chlorohexidin was positively charged, it was bind to various surfaces including enamel pellicle, hydroxyapatite and mucous membranes. It was also bind to the negatively charged bacterial surface and disrupt

bacterial cytoplasmic membranes, including leakage of low molecular weight components and the precipitation of cell contents, chlorohexidine also inhibite the key metabolic enzymes such as glucosyltransferase and phosphoenolpypruvate [14]. The side effect of chlorohexidin was the discoloration of the teeth and taste disturbance. In spite of the poor penetration ability into plaque associated with pits, fissures and proximal surfaces, nowadays chlorohexidin is used in the form of chlorohexidin chewing gum or as a complement to tooth-brushing in order to increase the normal oral hygiene [15].

Fluoride was widely used as a highly effective anticaries agent. This action was related mainly to effect on the mineral phases of teeth and on the process of remineralization. Fluoride had important effects on the bacteria of dental plaque, which were responsible for the acidification of plaque that result demineralization. Fluoride can affect bacterial metabolism through a set of actions with fundamentally different mechanisms. It can act directly as an enzyme inhibitor ex: capable to inhibit the mutans streptococci enzyme enolase, leading to reduce the uptake of glucose through the phosphotransferase system, fluoride was capable to reduce the cariogenecity of dental plaque bacteria by enhancing the membrane permeability to protons and comprimisity the function of F-ATpases in exporting protons, thereby inducing cytoplasmic acidification and acid inhibition of glycolytic enzymes. Fluoride acted by reducing the aid tolerance of the bacteria. It was most effective at acid pH values, in the acidic conditions of cariogenic plaque, fluoride at levels as low as 0.1mM can cause complete arrest of glycolysis by intact cell of *S. mutans*. [16.17]. Fluoride in toothpaste and other oral products was believed to be the major reason for the substantial defect in caries incidence in many countries. Fluoride can be administrated systemically (Tabletes), applied topically (toothpastes or mouthwashes) or applied by dentists in the form of solutions, gels and varnishes. In some parts of the world, fluoride was added to drinking water [17]. It was found that the combination of chlorohexidin with fluoride or with metal ions such as Zn^{2+} capable to increase the anti-cariogenic activity. This action was bactericidal [17]. In this study the bactericidal effect by ZAK mouthrinse on the growth of bacteria was unrecognized perhaps due to its low concentration as compared with chlorohexidin and sodium fluoride alone, or due to the interference between these material and other component of the mouthrinse capable to reduce its effect.

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