Original Study

CREATING EXCELLENT ANTIBACTERIAL PROPERTIES IN FLOWABLE DENTAL COMPOSITES WITH A NEW ANTIBACTERIAL COMPOUND

Eskandarizadeh A,[1] Khabazzadeh H,[2] Mohammadzadeh I,[3] Khaleghi M,[4] Askari A,[5] Saidi A[6]

1. Associate Professor, Department of Oral Medicine, Oral & Dental Disease Research Center, Kerman University of Medical Sciences, Kerman, Iran
2. Associate Professor, Department of Chemistry, Shahid Bahonar University of Kerman, Kerman, Iran
3. PhD Student, Department of Oral Medicine, Oral & Dental Disease Research Center, Kerman University of Medical Sciences, Kerman, Iran
4. Assistant Professor Department of Biology, Shahid Bahonar University of Kerman, Kerman, Iran
5. Reader, Department of Pathobiology, Shahid Bahonar University of Kerman, Kerman, Iran
6. Professor, Department of Mechanical Engineering, Shahid Bahonar University of Kerman, Kerman, Iran

ABSTRACT

Background and Objective: One of most important problems of dental composites refers to weakness of its anti-bacterial activity and bacteria can grow on the surface of this composite which leads to secondary caries. The aim of this study is to synthesize a derivative of pyrazole that called 3,5-dimethyl-1-thiocarboxamide pyrazole and its anti-bacterial properties with its usage it to create anti-bacterial flowable dental composites.

Methods: Synthesis of 3,5-dimethyl-1-thiocarboxamide pyrazole by reaction between Thiosemicarbazide (tsc) and 2,4-pentandione (Hacac) and antibacterial activity of this compound was evaluated by Agar diffusion method. Then it added to flowable dental composite with 0-5 wt%. Also anti-bacterial properties of cured resin were studied by direct contact and agar diffusion methods.

Results: According to our findings, 3,5-dimethyl-1-thiocarboxamide pyrazole has significant antibacterial effect against Streptococcus mutans so that it has ability to make this property in flowable composites ($p < 0.05$).

Conclusion: Incorporation of 3,5-dimethyl-1-thiocarboxamide pyrazole into flowable resin composites can reduce activity of Streptococcus mutans.

Key word: 3,5-dimethyl-1-thiocarboxamide pyrazole, Antibacterials Activity, Dental Caries, Flowable Composites, Streptococcus mutans.

Introduction

In recent years the use of dental composites has increased significantly.[1] Unfortunately, one of most important problems of this composite refers to weakness of its anti-bacterial activity and bacteria can grow on the surface of this composite which leads to secondary caries[2,3] and ultimately it reduces the life of dental restoration and need of replacing. [4,5] This problem has led researchers to create a material with anti-bacterial properties to decrease the secondary caries. One of these methods is to use anti-bacterial materials such as Chlorhexidine and Fluoride.[6-8] Although these compounds have well antibacterial effects but they do not last for long time.[9] Another way to create anti-bacterial properties in the composites is to use metal oxides. Since these compounds cause tooth discoloration so their usage is limited.[10] One of the main bacteria that causes tooth decay is Streptococcus mutans.[11]

Pyrazoles are in class of heterocyclic compounds that are used in the pharmaceutical industry.[12-15] Some of these applications refer to their anti-bacterial properties.[16]

In this study, a derivative of pyrazole which is called 3,5-dimethyl-1-thiocarboxamide pyrazole with same color as tooth color is synthesized then review of its anti-bacterial properties in flowable composites is performed.

Materials and Method

All the chemicals were purchased from Sigma-Aldrich and used without purification.

Synthesis of 3,5-dimethyl-1-thiocarboxamide pyrazole.

Synthesis of 3,5-dimethyl-1-thiocarboxamide pyrazole by reaction between Thiosemicarbazide (tsc) and 2,4-pentandione (Hacac) with molar ratio of 1: 1 was prepared then five grams of tsc in 250 ml of cold water with 1ml of concentrated HCL was dissolved and resulted solution was passed through filter paper then the 5 ml of Hacac was added to it. It was stirred for 30 minutes and then was left at rest for hours. White precipitate of 3,5-dimethyl-1-thiocarboxamide pyrazole was formed and it was purified by ethanol.[17] [Figure 1]

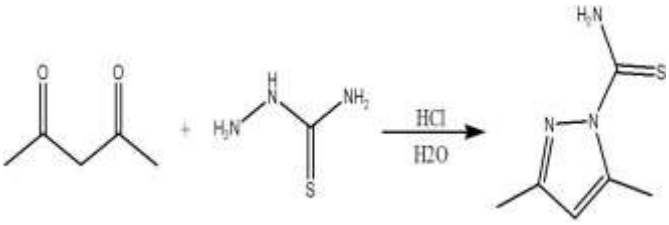

Figure 1: Synthesis of 3,5-dimethyl 1-thiocarboxamide pyrazole

The growth condition in bacterial microorganism that we used in this study was a reference strain of streptoccusmutans. It was grown overnight in Brain Heart Infusion medium (BHI)

Microorganism strains and culture

In this study, we employed four standard bacteria (Pseudomonas aeruginosa ATCC 27853, streptococcus mutans PTCC 1683, Staphylococcus aurous PTCC 1112, Bacillus cereus PTCC 1015 and Escherichia coli PTCC 1330) with two bacteria that isolated from clinical samples (Listeria monocytogenes and Enterococcus faecalis). Brain Heart Infusion medium (BHI-Merck) was used for L. monocytogenes and E. faecalis, whereas the other bacteria were cultured on Mueller Hinton medium (Merck).

Agar diffusion test

Agar diffusion test was used to study the effect of antibacterial activity in the 3,5-dimethyl-1-thiocarboxamide pyrazole and composites containing 3,5-dimethyl-1-thiocarboxamide pyrazole on bacterial growth.[18]

Determination of antimicrobial activity of 3,5-dimethyl-1-thiocarboxamide pyrazole by agar diffusion test

The agar media were inoculated with 100 µl of the inoculums which were prepared using an overnight culture for each microorganism (18–24 h) adjusted to a turbidity equivalent to a 0.5-McFarland standard. Then, Wells were cut and 50 ml of compounds (10 mg/mL; DMSO) that was used as solvent were added to them. Each compound was tested in triplicate along with standard ciprofloxacin. The plates were incubated at 37 C for 24 h. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well. The diameter of the zone of inhibitions was measured by measuring scale in millimeter (mm). The DMSO was used as a negative control factor.

*Determination of antimicrobial activity of composites containing 3,5-dimethyl-1-thiocarboxamide pyrazole***.**

Preparation of test specimens

Six groups of specimen were prepared by mixing of 3,5-dimethyl-1-thiocarboxamide pyrazole with resin composite (Tetric flow, Ivoclar vivadent, USA) in 1,2,3,4 and 5 wt% and 0 wt% as the control specimen group. Mixing was performed in a dark room for 15 min and in room condition using a spatula.

Agar diffusion test

This test was to investigate the effect of antibacterial activity of composites containing 3,5-dimethyl-1-thiocarboxamide pyrazole on bacterial growth. Discs with 2 mm thickness and 8 mm diameter were prepared using each of composites specimen groups. These discs were polymerized using a light cure device from two sides (bottom and top) for 40 s. In six groups, 200 µL of bacterial suspension was spread on blood agar and Then discs were placed on the surface of each of plates. After 24 hours of maintaining of plates in 37^0 C, inhibition zone diameter around each disc was measured. These tests were repeated 3 times each to ensure their accuracy.[19]

Direct contact test (DCT test)

This test performed to investigate the antibacterial properties of free surface of resins containing 3,5-dimethyl-1-thiocarboxamide pyrazole for this purpose walls of 500 µl microplates were covered by 200 µl of un-polymerized resin. Then resin layers were polymerized using a light cure device for 40s. 10 µl of 0.5 McFarland standard solutions of Streptococcus mutans (about 10^6 bacteria) were added to each microplate afterward and samples were kept in 37^0 C for one hour. During this one hour, bacterias were in direct contact with resin surface and the solvent was evaporated. Then 300 µl of BHI (Brain-heart infusion medium) were added to each microplate. Caps were completely closed and samples stored in 37^0 C. in time periods of 24, 48, 72, 96 & 120 hours, 50 µl of the mixture (bacteria+BHI broth) was placed on culture medium and after 24 hours' number of appeared bacteria colonies was counted using colony counter apparatus. This test were repeated 3 times for each of test group to ensure its accuracy.[20,21] Results were expressed as $\log_{10}$ (CFU).

Data analysis

The data were analyzed by one-way ANOVA, and the tukey post hoc HSD multiple comparison test. The level of significance was determined as p=0.05

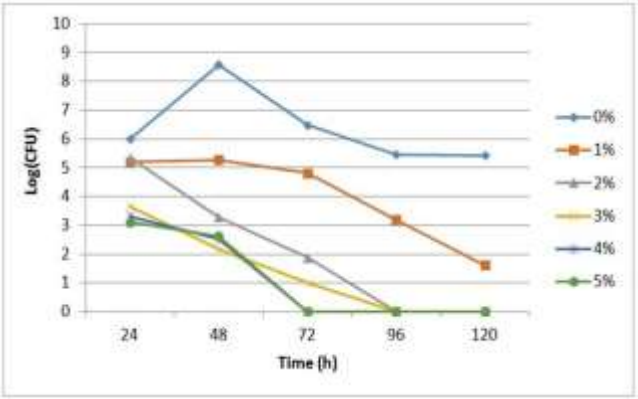

Figure 2: Colony forming unit following direct contact between S.mutans and dental resin composites containing 0-5 wt.% 3,5-dimethyl-1-thiocarboxamide pryrazole. Decrease in Log CFU indicates destruction of bacteria.

Results

Determination of antimicrobial activity of 3,5-dimethyl-1-thiocarboxamide pyrazole by agar diffusion test

According to the results that are given in Table 1 the 3,5-dimethyl-1-thiocarboxamide pyrazole compound has well anti-bacterial properties against many bacteria, such as streptococcus mutans.

Microorganism	Inhibition Zone Diameter (mm)	
	3,5-dimethyl-1-thiocarboxamide pyrazole	Ciprofloxacin
S. aureus PTCC1112	18	25
E. coli PTCC 1330	11	27
B. cereus PTCC 1015	12	24
S. mutans PTCC 1683	18	22
Listeria monocytogenes*	22	0
E. faecalis*	9	0
Pseudomonas aeruginosa *	9	0
*Isolated clinical samples		

Table 1: In vitro antimicrobial activity of the 3,5-dimethyl-1-thiocarboxamide pyrazole

Agar diffusion test for composites containing 3,5-dimethyl-1-thiocarboxamide pyrazole.

There was no inhibition zone around the samples in agar medium containing s.mutans strain.

Direct contact test (DCT) for composites containing 3,5-dimethyl-1-thiocarboxamide pyrazole.

The resuls of bacterial colony count (colony for ming unit,cfu) is shown in figure 2. It is clear that with increase in w% of 3,5-dimethyl-1-thiocarboxamide pyrazole, antibacterial activity of composite increases significantly ($p<0.05$) and also time has a meaningful effect on antibacterial properties of resin.

Discussion

The agar diffusion test and the minimum inhibitory concentration are important traditional test methods for evaluating and investigating of antibacterial properties and behavior of many pharmaceutical materials. Agar diffusion test works based on solvability of materials as tested material diffuses from the bulk surface and eliminates microorganisms. So this method can't be used for the materials with low solvability in water. Giving that one of the most important and necessary properties of proper dental material is low water solvability, the Agar diffusion test cannot be used and it is not suitable test method for dental material. Instead of that DCT in other hand, have a very low sensitivity toward test subject solvability properties. Knowing that it is an efficient method for evaluating antibacterial properties, we can use it for investigating of antibacterial properties of materials with very low water solvability. As it is shown in Figure 2, DCT result indicated that 3,5-dimethyl-1-thiocarboxamide pyrazole compound gives excellent antibacterial properties to the resin that increases with time and in higher concentration. The antibacterial property of 3,5-dimethyl-1-thiocarboxamide pyrazole reaches its maximum at 5% w and after 24 hours. Its non-toxic properties and also very its low aqueous solvability make 3,5-dimethyl-1-thiocarboxamide pyrazole a very suitable material to be used in oral environment. In addition to mentioned advantages, 3,5-dimethyl-1-thiocarboxamide pyrazole is white and it has the same color as tooth enamel. So it can improve the antibacterial properties of the dental composites and the same time preserving beauty characteristics of them.

According to previous researches, 3,5-dimethyl-1-thiocarboxamide pyrazole probably uses the following mechanism to prevents bacterial growth. This compound is a derivative of pyrazoles, one of most important nitrogenated heterocycle compound group. pyrazols prevent bacterial activity by possessing electron rich property and having non-covalent interaction with microorganism.[22]

Next step in this research is investigating of effect of this compound on mechanical properties of dental composite. We decided to conduct this research in near future.

Conclusion

Within the limitation of this study can be conclude that Incorporation of 3,5-dimethyl-1-thiocarboxamide pyrazole into flowable resin composites can reduce activity of Streptococcus mutans.

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Corresponding Author

Dr. Iman Mohammadzadeh
PhD Student of Dental Materials,
Department of Oral Medicine,
Oral & Dental Disease Research Center,
Kerman University of Medical Sciences,
Kerman, IRAN
Email Id: - iman.mz@gmail.com