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To evaluate antimicrobial efficacy of ozone oil against streptococcus mutans and E faecalis microorganisms using disk diffusion method - An in vitro study

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Abstract

Oral streptococci & its strain of streptococcus mutans colonize on tooth surface & have the ability to synthesize polysaccharide from sucrose. E faecalis is a microbes commonly found in asymptomatic, persistent endodontic infections. Its prevalence in failed root canal treatment ranges from 25% to 75%. Now a days, application of biomaterials is gaining popularity & acceptance due to their high versatility & availability. Ozone is a Greek word derived from odorant, meaning to smell produced in upper atmosphere of earth, the stratosphere. Ozone is defined as triatomic, endothermic and thermodynamically highly unstable oxygen compound that dependent on system condition like temperature and pressure decomposes to pure oxygen with a short life time. Dr Edward Fisch was the 1st dentist to use ozone. The utilization of ozone in various forms has increased in the field of medicine & dentistry due to its high oxidative power stimulating the immune response & enhancing the blood circulation. It has been proved in vitro that ozone has efficacy in controlling microbial count in dental plaque. Pure plants extract like clove, aloe vera undergo

chemical reaction to form thick viscous oil or petroleum jelly. This happens when ozone gas in passed through this oils which solidifies them. This helps in sustained release for longer period of time.

Keywords: E faecalis, S mutans, ozone oil, disk diffusion, Endodontics, intra canal medicament

Introduction

Caries is a microbial disease of calcified tissue of teeth characterized by demineralization of the inorganic portion & destruction of the organic substance of the tooth. Bacteria ferment sugar to produce lactic acid. This acid dissolves the tooth surface. Enamel though the hardest substance in body is semi permeable & allows the acid to seep in affecting the pulp dentine complex. Among them streptococcus mutans has the ability to initiate & maintain microbial growth.

Oral streptococci & its strain of streptococcus mutans colonize on tooth surface & have the ability to synthesize polysaccharide from sucrose. It's based on glucosyltransferases (GTFB) produced by S mutans in combination with glucan binding proteins (GBP). GFTB plays important role in virulence of dental plaque & are responsible for glucan formation from sucrose. This glucan provides bacterial adhesion to enamel & each other. It has the ability to survive in acidic environment & interacts with other microbes to colonize into oral ecosystem. The ability of S mutans to form biofilm is significant from clinical point of view, mainly in context to caries etiology. Various anti-microbial agents are investigated to inhibit growth of this streptococcus but action of ozone oil has not been investigated.

E faecalis is a microbes commonly found in asymptomatic, persistent endodontic infections. Its prevalence in failed root canal treatment ranges from 25% to 75%. This can be attributed to its ability to compete

with other microbes, invade dentinal tubules deeper & resist nutritional deprivation for longer periods.

Now a days, application of biomaterials is gaining popularity & acceptance due to their high versatility & availability. The success of modern medicine is based on use of bio materials like hydroxyapatite, chitosan, ozone etc. Ozone is a Greek word derived from odorant, meaning to smell produced in upper atmosphere of earth, the stratosphere. It's heavier than air & thus starts falling earthwards combing with pollutants & cleaning the air. It's a naturally occurring gas & very strong, selective oxidant. It rapidly dissociates into water & releases a reactive form of oxygen that oxidizes the cell, thus having anti-microbial efficacy without inducing drug resistance. Following these principal the use of ozone therapy has been tested as alternative as well as complementary to NaOCl & Ca (OH) as a source of disinfection.

Ozone is defined as triatomic, endothermic and thermodynamically highly unstable oxygen compound that dependent on system condition like temperature and pressure decomposes to pure oxygen with a short life time. It has a half-life of 20 min & is 1.5 times more effective than chloride. The German chemist Christian Schonbein is considered father of ozone therapy. Dr Edward Fisch was the 1st dentist to use ozone. During the early 90s it was difficult to utilize the ozone due to ozone resistant materials of that time like nylon, Dacron. It's also called as super oxygen. Ozone can be naturally as well as synthetically obtained. Sometimes the oxygen splits into 2 separate atoms. Now these single atom is semi unstable & bonds with oxygen molecules to form ozone. This reactive third atom of oxygen is called has hungry atom. This atom break down from this semi unstable bond & react with any oxidizable compound (organic / inorganic).

The utilization of ozone in various forms has increased in the field of medicine & dentistry due to its high oxidative power stimulating the immune response & enhancing the blood circulation. It has been proved in vitro that ozone has efficacy in controlling microbial count in dental plaque. It's very volatile & thus cannot be stored. It breaks up into oxygen in 20 minutes. It's very susceptible to heat & should be kept away from inflammable substances. So to sum it up, ozone when exposed to oral environment eliminates pathogens, restores oxygen metabolism, induces friendly ecologic environment, increases blood circulation, activates immune system & stimulates humoral anti-oxidation system.

Though there are many irrigants & intra canal medicaments available today for endodontic use there is need of more effective & natural alternative. The aim of this study is to evaluate the antimicrobial property of ozone oil against caries causing streptococcus mutans & against the enterococcus faecalis which causes root canal failure.

Material & Methods

This in vitro study was conducted in Dayananda Sagar College of Dental Sciences, Bengaluru.

The Ozone oil to be tested was obtained from Dr Sudhir Dole Ozone center, Mumbai.

2 test groups were formed as follows:

Group 1 – Streptococcus mutans

Group 2 - Enterococcus faecalis

Materials Required

Petri dish (C Bangalore refinery)

Micro pipette (Singh Science Systems)

UV chamber

Ozone oil (C Bharat Enterprises)

Muler agar (PPMS Sterile)

S mutans & E faecalis (Biomed Central)

Ozone Oil Preparation

Pure plants extract like clove, aloe vera undergo chemical reaction to form thick viscous oil or petroleum jelly. This happens when ozone gas in passed through this oils which solidifies them. This helps in sustained release for longer period of time.

Methodology

Pure strain of E. faecalis & S. mutans was obtained from Azyme Bioscience Institute, Bengaluru, India. Mueller Hilton agar was prepared on sterile petri dishes & kept for sterility check at 37 degree for 2 Hours. After sterility check, the samples of E faecalis & S mutans were used to make culture on Mueller Hilton agar plates.

Wells of 4 mm diameter & 4 mm depth was prepared in the sterile agar plates. 200 ml of experimental solution (ozone oil) was placed into punched out wells in agar plates using micropipette. The agar plates were then placed inside UV chamber for 1 hr. for solution to diffuse through the culture medium. Later the organism were taken from culture medium & were streaked on agar plates which was previously diffused with ozone oil. The medium was left undisturbed for 48 hours, after which the zone of inhibition was measured & values were noted.

Results

Ozone oil shows total inhibition against S mutans & E faecalis in vitro by disc diffusion method.

Statistical Analysis

The level of significance was set at P < 0.05 (P Value) Independent student t test was done.

Table 1

Group 's	E faecalis	S mutans	P value
Ozone oil	No growth	No growth	0.000

P value highly significant

Discussion

This study was done to investigate a better, alternative & natural irrigant as intra canal medicament against most common endodontic pathogens. The results obtained

shows that ozone oil has total inhibition of S mutans & E faecalis even after 24 hours of incubation It works in combination by inducing modification of intracellular contents & damaging the cytoplasmic membrane of bacterial cells. As this process goes on ozone acts on the glycoprotein, glycolipids that are present in cytoplasmic membrane of microbes and other amino acids. It inhibits and blocks the enzymatic control system of the cell resulting in an increased membrane permeability which is the key element of cell viability thereby causing immediate functional cessation. Thus, ozone molecules in oil form have sustained release & they do easily enter cell & causes apoptosis.

The oxidation process of unsaturated lipids & proteins generates a quantitative conversion of present olefinic bonds to reactive species of lipid oxidation products. These reactive species, named ozonide signal & trigger metabolic changes that yield distant microbicidal effects. The ozone will punch a hole in membrane of organism. Now the contents of pathogens are exposed to internal environment that allows the immunologic system to start its physiologic cascade. Now antibodies in addition to identification of pathogens also kill them with ozone.

Another interesting fact is that anaerobic type microbes produce positive charged infective environment. Since oxygen is the only gas that can carry an electrical charge, this opposite charge phenomena attracts ozone to that area. Thus this helps ozone to attack biomolecules like cysteine, methionine & histidine residues of proteins. By oxidizing the biomolecules featured in dental diseases, ozone has severe disruptive effect on cariogenic bacteria resulting in elimination of acidogenic bacteria.

Abu Naba et al conducted in vivo split mouth randomized control clinical trial of 90 patients with atleast 2 primary pit & fissure lesion in permanent posterior tooth. The results showed significant decrease in caries rate with ozone use. Ozone has showed more efficiency hen there is less organic debris left inside root canal. So it's advised to use conventional irrigant initially & finally irrigate with ozone oil as intracanal medicament. It is effective in killing root canal star survivor E faecalis but the resistance of bacterial cells in biofilms must be attributed to depletion of ozone as it diffuses into biofilm by virtue of its organic composition. Thus a fresh generation of solution is needed as it rapidly dissipates.

In a study done by R S Hems et al it was found that ozone water is less effective in killing E faecalis when embedded in biofilm matrix. Ozone gas has its own limitations. Thus a better vehicle is needed to carry ozone with no / minimal side effects. Virgin olive oil acts as a good vehicle along with having additive anti-bacterial properties overcoming the limitations of gaseous form.

Nagayoshi et al 2004 found nearly same antimicrobial activity against E faecalis & S mutans & lower level of cytoxicity of ozone water compared to 2.5% NaOCl. But there are controversies regarding concentration of NaOCl used & its comparison with various forms of ozone used. 5% NaOCl was found to be superior then gaseous ozone in a study done by Muller et al 2007 in eliminating endodontic pathogens. Similar results were found by Estra et al where 2.5% NaOCl, 2% Chlorhexidine & gaseous ozone for 20 min was compared with ozone as fairing last.

Viscous vehicle have higher clinical value as they not only offer greater healing (due to sustained release) but also reduces the number of dental visits in case of chronic infection & large lesion. When olive oil is ozonized suitably & sufficiently, density of oil increases in direct proportion to ozonation time. So it will be more viscous than extra virgin olive oil. This will have slower ionic liberation which support our results.

Olive oil itself is rich in aloins & aloe emodin. Presence anthrax quinine makes it effective of against Streptococcus & E faecalis. Its effective antibacterial due to protein synthesis in bacterial cells. It is rich in vitamins, minerals, enzymes, lignin, saponins, salicylic acid & amino acid. It acts as good carrier along with being antibacterial itself. Because of viscous nature it has high density. So when ozone is passed through this viscous olive oil, it stores ozone for later sustain release. Along with having good biological properties olive virgin oil is a good lubricant thus helpful in being used as root canal medicament. It is easy to remove from the canal & comes in contact with all the surfaces of root canal. In a study done by Anuj Bhardwaj et al 2012, he compared antimicrobial efficacy of morinda citrofilia, papain, olive oil & 2% Chlorhexidine gel against E faecalis in an in vitro study. Virgin olive oil showed 79% inhibition.

Advantages

Eliminates the use of drill. Eliminates 99% bacteria. Excellent for anxious patients. Prevents caries progression. The oxidation process is self-contained & gets over from 40 sec to within 20 minutes. Also ozone doesn't have any influence on physical properties of enamel & thus neither hinders nor influences bonding.

Limitations

Ozone gas inhalation for prolonged period of time can cause lung damage & has a risk of pulmonary embolism thus ozone oil was used in this study. Known side effects are upper respiratory tract irritation, cough, head ache.

It's contraindicated in pregnancy as well as patients with hyperthyroidism, severe anemia, myasthenia, active hemorrhage, asthma. In event of ozone intoxication the patient must be placed in supine position, inhale humid oxygen & take ascorbic acid, vitamin E & nacetylcsteine.

Conclusion

Within the limitations of this study, it can be concluded that ozone oil has total inhibition of S mutans & E faecalis. It can be used as an intra-canal medicament & irrigant because of its superior anti-bacterial properties & sustained release without any side effects due to its natural availability. But further in vivo research has to be done to check for the biocompatibility & efficacy.

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