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FORMULATION OF 0.5%AZITHROMYCIN GEL AS LOCAL DRUG DELIVERY AGENT FOR PERIODONTAL THERAPY- INVITRO DRUG RELEASE STUDY

Periodontics

Dr. Kranthi Kosuru*	M.D.S Reader, Department of Periodontics, Meghna institute of dental science, Nizamabad, Telangana. *Corresponding Author
Dr. Abhishek Reddy	M.D.S Reader, Department of Oral and Maxillofacial surgery, Meghna institute of dental science, Nizamabad, Telangana.
Dr.Vinuthna	M.D.S Assistant Professor, Department of Dentistry, Government Dental College, Nizamabad, Telangana.
Dr. V.Shakuntala Soujanya	M.D.S Reader, Department of Oral Medicine and Radiology, Meghna institute of dental science, Nizamabad, Telangana.
Dr. Durgakeerthi.P	M.D.S Senior lecturer, Depatment Of Orthodontics, Meghna institute of dental science, Nizamabad, Telangana.

ABSTRACT

Context: Non surgical periodontal therapy is the gold standard treatment for periodontitis. But the invasive nature of subgingival microorganisms makes the use of antimicrobials inevitable. These antimicrobials can be used systemically and locally. Due the side effects posed by systemic administration of antibiotics local drug delivery is more favourable. Various local drug delivery agents are commercially available for periodontal therapy. Studies have shown that azithromycin is effective against periodontal pathogens so, it can be used in periodontitis treatment. But the use of azithromycin as local drug delivery agent is rare.

Aims: The present study aims at formulation of 0.5% azithromycin gel as local drug delivery agent for periodontal therapy with PLGA as vehicle and invitro drug release evaluation in artificial saliva.

Settings and Design: Formulation of 0.5%azithromycin was done and artificial saliva prepared. Azithromycin gel was placed in a dialysis tube. The dialysis tube was then placed in a beaker containing 100ml of artificial Saliva. A total of eight samples were collected for a period of seven days. The amount of drug release was estimated using HPLC.

Results: The results showed that the concentration of azithromycin in samples collected was greater than the minimum inhibitory concentration of most the periodontal pathogens.

Conclusions: The formulation of 0.5% azithromycin can be used as local drug delivery agent adjunct to scaling and root planning.

KEYWORDS

Azithromycin, local drug delivery, PLGA

INTRODUCTION:

Periodontal disease is one of the most common inflammatory diseases of microbial origin which involves the destruction of the supporting structures of the teeth including the periodontal ligament, bone and soft tissues.[1, 2, 3] Even though dental plaque seems to be primary etiological agent, the severity and progression of periodontal disease is influenced by several local and systemic factors. [4, 5]

More than 500 microbial species have been identified in the sub gingival plaque. It consists of microorganisms involved in periodontal health and disease. The main putative periodontal pathogens include *Porphyromonas gingivalis(P.gingivalis), Tannerella forsythia (T.forsythia), P. intermedia, Campylobacter rectus(C.rectus), Eikenella corrodens, F. nucleatum, Aggregatibacter actinomycetemcomitans (Actinobacillus actinomycetemcomitans* previously) (A. *actinomycetemcomitans*)*, P. micros, and Treponema spp*. But *P. gingivalis, T. forsythia, P. intermedia, C. rectus* and *F. nucleatum* have been reported at higher levels in sites with active disease or with progressing disease. The majority of organisms in chronic Periodontitis are gram negative anaerobes.[6] Highly organized bacterial populations form the apically advancing front of periodontal pockets in close proximity to connective tissue and cause alveolar bone destruction.

Elimination or adequate suppression of putative periodontopathic microorganisms in the sub gingival microbiota is essential for periodontal healing. The periodontal healing can be achieved by non surgical and surgical therapies.

The nonsurgical periodontal therapy is the gold standard for periodontal therapy. The nonsurgical periodontal therapy includes scaling and root planning which involves removal of subgingival plaque and calculus that reduces bacterial load, shrink swollen and inflamed gingivaand recondition the sub gingival ecology, making it biologically compatible with optimal healing and allow reattachment of epithelium to root surface.[7,8]

In spite of meticulous scaling and root planing procedures, the reduction in probing depth and gain in clinical attachment level is not happening in moderate to deep periodontal pockets (pocket depth ≥5 mm) because of the invasive potential of the putative periodontal pathogens into gingival epithelial cells and sub epithelial connective tissues, and their high affinity for crevicular epithelium and dentinal tubules.[1, 4, 9]

A microbiological approach to periodontal therapy aiming primarily at suppressing specific pathogenic bacteria and permitting a subsequent recolonization of a microbiota compatible with health is effective. The antimicrobials can be given systemically and locally.

Systemic antimicrobial agents enter periodontal pockets following their intestinal absorption and passage from the bloodstream into oral tissues, gingival crevicular fluid and saliva. This route provides a ready exposure of all periodontal sites to the antimicrobial agent but poses a risk of adverse effects such as drug toxicity, acquired bacterial resistance, drug interaction and patient's compliance, limits the use of systemic antimicrobials.[9, 10]

To overcome these shortcomings, local delivery of antimicrobial agents is extensively studied. It was Dr. Max Goodson[8] in 1979 that championed and developed local delivery of therapeutic agents into a viable concept. Local antimicrobial therapy in Periodontitis involves direct placement of an antimicrobial agent into sub gingival sites, minimizing the impact of the agent on non-oral body sites, limiting the drug to its target sites and hence achieving a much higher concentration. For local delivery in subgingival areas various antimicrobials have been used such as Tetracycline, Chlorhexidine, Metronidazole etc. and clinical studies [11, 12, 13] have shown that these agents are effective when used as an adjunctive to mechanical debridement.[6]The present study is devoted to formulation of 0.5% Azithromycin gel as local drug delivery agent to be used as adjunct to scaling and root planing in chronic periodontitis patients and invitro analysis of drug release in artificial saliva.

Aim:

1) To formulate 0.5% azithromycin gel to be used as local drug delivery agent.
2) Invitro drug release study of sustained release azithromycin *in situ* gel formulation.

MATERIALS AND METHODS:

Formulation of Azithromycin gel:

The azithromycin *gel* was prepared as described by the shah. et.al[14]

Ingredients:

- Azithromycin –drug
- Polylactic-co-glycolic acid (PLGA 75:25)-polymer
- N-methyl 2-pyrrolidone (NMP)-solvent

Procedure of the preparation:

- N-methyl 2-pyrrolidone (NMP) in a quantity of 5ml was taken in a 100ml beaker and heated on a magnetic stirrer till a temperature of 60°c was attained. Care was taken to ensure that the solvent loss due to evaporation was minimized by covering the beaker with aluminum foil. Poly lactic-co-glycolic acid (PLGA 75:25) (molecular weight 66,000- 1,07,000) in a quantity of 1.8gm was accurately weighed and added to the hot solvent and stirred on the magnetic stirrer till a clear solution is observed indicating the complete solubilization of PLGA. Azithromycin in a quantity of 0.09gm was added to the polymer solution, which rapidly dissolved to give a homogeneous phase of the drug, polymer andthe solvent. The resultant solution was transferred to a glass vial and stored under cold condition which was further subjected to sterilization (The formulation was subjected to a minimum dose of 25kGy gamma irradiation so that the formulation can be used for clinical trials).

In-vitro analysis:

In-Vitro Drug release studies of sustained release Azithromycin gel formulation were done.

MATERIALS FOR ANALYSIS:

Dialysis Membrane (figure 1), Methyl-p-hydroxybenzoate, Sodium carboxymethyl cellulose (SCMC), Potassium Chloride (KCL), MgCl2.6H2O, CaCl2.2H2o, K2HPO4, KH2PO4.

Preparation of Artificial saliva

The artificial saliva used in this present study was prepared according to Macknight-Hane and whitford (1992) formula.

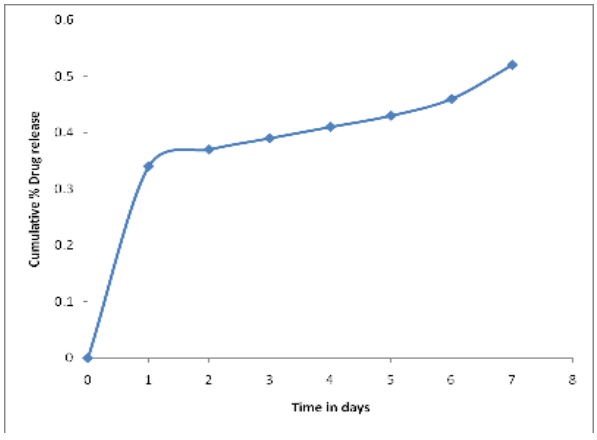
1. Methyl-p-hydroxybenzoate in a quantity of 2 gms is dissolved in 800 ml of distilled water. 20 ml of this solution was kept aside and used as the solvent for other agent used in the preparation of artificial saliva.
2. Then 200 ml of distilled water is boiled with slow adding of SCMC in to boiling water with constant stirring until all the SCMC was dissolved.
3. The cold Methyl-p-hydroxybenzoate solution (From step-1) is poured into SCMC (Step-2) and mixed thoroughly until they are uniformly mixed together.
4. KCL in the quantity of 0.625 gms was dissolved in Methyl-p-hydroxybenzoate solution (From step-1) and then mixed with the solution of step-3.
5. Then 0.0059 gms of MgCl2.6H2O was dissolved in Methyl-p-hydroxybenzoate solution (From step-1) then mixed thoroughly with the solution of step-4.
6. CaCl2.2H2O in the quantity of 0.166 gms was dissolved in Methyl-p-hydroxybenzoate solution (From step-1) then this solution was mixed thoroughly with the solution of step-5.
7. Then 0.804 gms of K2.HPO4 is dissolved in Methyl-p-hydroxybenzoate solution (From step-1) then this solution was mixed thoroughly with the solution of step-6.
8. KH2PO4 in the quantity of 0.326 gms was dissolved in Methyl-p-hydroxybenzoate solution (From step-1) then mixed thoroughly with the solution of step-7.
9. The pH of the solution obtained from the step-8 is adjusted to 6.75 with KOH, and then the artificial saliva (figure 2) obtained in above procedure was used for drug release study.

In-Vitro Drug release studies dialysis process

Dialysis tubing cellulose membrane was used in this process (Figure 1: Dialysis tubing cellulose membrane). The dialysis membrane bag with a molecular weight 10 kDa cut-off was soaked for overnight in saline solution. Typically, 1g of azithromycin gel was placed in a dialysis tube (figure 3). The dialysis tube was then placed in a beaker containing 100ml of artificial Saliva (figure 4). The entire system was agitated by a magnetic stirrer at 100 rpm and temperature was maintained at 37±0.5°C throughout the study. Aliquots of 5 ml were withdrawn periodically at intervals of 1 day (24 hrs) for a period of 7 days and each time equal volume was replaced with fresh artificial saliva previously maintained at 37±0.5°C to mimic the sink conditions in oral cavity. The amount of drug release was estimated using HPLC (high performance liquid chromatography). The results of the analysis were as follows

Table: 1 Concentration (%) of drug release at each interval during in-vitro analysis

S.No	Time in days	Cumulative % Drug release
0	0	0
1	1	0.34
2	2	0.37
3	3	0.39
4	4	0.41
5	5	0.43
6	6	0.46
7	7	0.52



Graph: 1 Drug release pattern during in-vitro analysis

PHOTOGRAPHS

figure1: Dialysis tubing cellulose membrane.

figure 2: Artificial Saliva in a beaker

figure 3: Dialysis tubing cellulose membrane with AZM gel

figure 4: Dialysis tubing cellulose membrane suspended in

RESULTS:

In the present in vitro drug release evaluation of azithromycin gel formulation, a total of 8 samples were collected at an interval of 24 hours for seven days. Then HPLC analysis was done. The first artificial saliva sample which was collected at the start of evaluation doesn't show any trace of drug present. The second sample collected after 24 hours after immersion of the dialysis tubing containing formulation showed 0.34% of drug released. The third sample showed 0.37%of drug released. The fourth sample showed 0.39% release of drug. The fifth sample showed 0.41% release of drug. In the sixth and seventh samples 0.43%, 0.46% was seen respectively. The final sample collected on the seventh day showed 0.52% of drug release. The consecutive samples showed a sustained release pattern of drug (table:1 and graph:1) .

DISCUSSION:

Azithromycin is a semi synthetic acid stable antibiotic and represent the protype of a novel class of macrolides called azalides. Azithromycin has a wide antimicrobial spectrum of action towards anaerobic bacteria as well as gram negative bacilli. It is effective against periodontal pathogens like *A. actinomycetemcomitans* and *P. gingivalis* and this antimicrobial activity supports its use in periodontal infections. Azithromycin has significantly less bacterial resistance to the sub gingival microflora of chronic Periodontitis compared to other commonly prescribed antibiotics. Azithromycin also has a long half –life and good tissue penetration. It gets concentrated in fibroblast and phagocytes and is transported to the areas of inflammation as a result of chemotactic effects exerted on the phagocytes, thus delivering the drug at those target sites.[15, 16] Various studies[17, 18] have found that scaling and root planing with adjunctive use ofsystemic Azithromycin demonstrated improvement in clinical parameters. But the use of Azithromycin as local drug delivery agent is rare.

The dilution effect on systemically administered antibacterial agents poses a severe restriction on their utility. An antibacterial agent administered systemically is dissolved in total body water (42 liters) and suffers 50% or more loss through other compartments (bone, kidney, liver, etc.). A typical systemic antibiotic would achieve a peak concentration of 3 µg/ml in blood and gingival fluid (0.0003%). To inhibit the growth of periodontal pathogens, a concentration between 1 and 16 µg/ml must be achieved.[19] systemic administration also shows effect on non target body tissues along with certain side effects like mild gastric upset, abdominal pain, headache and dizziness. To avoid effect on non target tissues, the concept of local drug delivery was developed.

In the present study the formulation of azithromycin gel was done using 75:25 PLGA as the delivery system. PLGA is an acronym for poly D, L-lactic acid-co-glycolic acid which is a copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA). Notation 75:25 PLGA means 75%of the copolymer is lactic acid and 25% is glycolic acid. PLGA is synthetic biodegradable FDA approved polymer which is highly biocompatible and extensively studied as drug delivery vehicles for drugs, proteins and various other macromolecules such as DNA, RNA and peptides. PLGA was also successfully used in the field of periodontics with good results. Kurtis et al. conducted a study using PLGA loaded with and without metronidazole for guided tissue regeneration in dogs and observed successful regeneration without toxicity and adverse reaction.[20]

PLGA copolymer undergoes degradation by hydrolysis or biodegradation through cleavage of its backbone ester linkages into oligomers and finally monomers. The mechanism ofbiodegradation of PLGA is unclear whether it is by complete hydrolysis or some enzymatic activity is also involved. Some investigators has suggested that PLGA polymers biodegrades into lactic and glycolic acid. The lactic acid enters into tricarboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbondioxide and water. Glycolic acid is either excreted unchanged in the kidney or it enters tricarboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbondioxide and water.[20]

The release of drug from the PLGA matrix has biphasic curve. Initially burst of drug release is seen which is related to drug type, drug concentration and polymer hydrophobicity. Drug on the surface in contact with the medium, is released as a function of solubility as well as penetration of water into polymer matrix. In the second phase, drug is released progressively through the thicker drug depleted layer. The water inside the matrix hydrolyzes the polymer into soluble oligomeric and monomeric products. This creates a passage for drug to be released by diffusion and erosion until complete polymer solubilization. Drug type also plays an important role in attracting the aqueous phase into the matrix.[20]

When the formulation of azithromycin was subjected to in-vitro release studies by HPLC, it was found that a similar type of drug release was present. A quantity of 0.09 gm of azithromycin was added in the formulation to attain a concentration of 0.5%. In the in-vitro drug release analysis it was observed that in the first sample, which was collected after 24 hours after immersion of dialysis tubing containing formulation into artificial saliva, 0.34% of drug was released. Later a sustained release of the drug was observed for 7 days where in the last sample 0.52% of drug release was observed.The advantages of using PLGA as vehicle is easy placement, bioabsorable, do not require a periodontal dressing for retention.The MIC(minimum inhibitory concentration) of azithromycin against standard and clinically isolated strains of bacteria associated with periodontal diseases such as *P. gingivalis, P. intermedia, A. actinomycetemcomitans, Eikenella corrodens*, and *F. nucleatum*, is between 0.025 and 2.0 µg/ml. [16] In the in-vitro analysis the concentration of azithromycin that was obtained was 0.34% in the first sample that was collected, which is equivalent to 0.003mg (approximately 3µg) which is greater than the MIC of the most common microorganisms found in the chronic periodontitis. It was also observed that the concentration of azithromycin was maintained above the MIC of periodontal pathogens for about 7 days in the in-vitro study. The results of the analysis showed that the concentration of the azithromycin released from the formulation was above the MIC of common periodontal pathogens which was maintained for about 7days showing that sufficient subgingival drug-microbial contact time could be expected.

CONCLUSION:

The present study on invitro evaluation of the 0.5% azithromycin gel has showed that the concentration of the drug released from the formulation is greater than the MIC of common periodontal pathogens for adequate amount of time indicating that azithromycin gel can be used as local drug delivery agent in periodontitis patient. However the clinical trials that were done later showed statistically significant improvement in clinical parameters when azithromycin gel was used as an adjunct to scaling and root planning compared to scaling and root planning alone.

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