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Review Article

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Use of 5-Nitroimidazole drugs in the treatment of periodontitis

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Abstract Periodontal disease, a common cause of tooth loss, is an inflammatory response to the overgrowth of anaerobic and micro-aerophilic organisms in the subgingival plaque. Various 5-Nitroimidazole chemotherapeutic agents have been discussed in this review article with a special emphasis on periodontal gels because of their relatively fast rate of drug release, ease of preparation, easier administration, a high biocompatibility and mucoadhesivity, which in turn allow prolong adhesion to the dental mucosa and at the same time, rapid elimination. Scaling and root planning treatment along with use of 5-Nitroimidazole drugs like metronidazole, satranidazole, niridazole, ornidazole and tinidazole in different dosage form are very promising way of treatment of periodontitis.

Keywords Gel, Gingival crevicular fluid, 5-Nitroimidazole, Metronidazole, Periodontitis, Periodontal pocket, Satranidazole.

1. Introduction

Mouth provides a unique environment where oral tissues are exposed to a number of chemical and physical stimuli and yet, the most part of oral tissues other than dental tissues remain healthy. The periodontal diseases are inflammatory conditions affecting the physiological structural organs supporting the teeth. Gingival tissues detached from tooth used to form periodontal pockets which provides an ideal ecological niche for proliferation of anaerobes and thereby provokes a host response which may lead to local inflammation. The resulting tooth mobility is eventually reflected clinically in tooth loss.

Periodontal disease has plagued mankind since his first appearance on the earth. Bone lesions typical of periodontitis are observed in fossils from the Paleolithic culture of Neanderthal man. Periodontal diseases were described in ancient Chinese writings, and a form of suppurating periodontitis appears to have been one of the most common diseases of the Egyptians more than 4000 years ago [1]. No age group, ethnicity, race, gender or socio-economic group is immune to this disease, presenting a health problem worldwide [2-3].

According to the World Oral Health Report, oral diseases qualify as major public health problems owing to their high prevalence and incidence in all regions of the world. Dental diseases have always been considered a very important global oral health problem. Severe periodontitis, which may result in tooth loss, is found in 5–15% of most populations. It was found that in many developing countries, access to oral health services was limited and teeth are often left untreated or are extracted because of pain or discomfort. Throughout the world, losing teeth is still seen by many people as a natural consequence of ageing [4].

In treatment of periodontitis (Pyorrhea), use of systemic antibiotics can raise a lot of issues related with bacterial resistance and unpleasant or adverse side effects. Large doses must be taken in order to achieve sufficient

concentrations in the gingival crevicular fluid of the periodontal pockets; this brings with it the associated side effects of antibiotics and problems regarding antibiotic resistance [5].

2. Role and scope of periodontal diseases

Periodontal diseases are inflammatory conditions affecting the physiological structural organs supporting the teeth. The disease may have microbiological, immunological, nutritional, hormonal, cardiovascular or psychosomatic etiology. Any medical condition that affects host antibacterial defense mechanisms, such as human immunodeficiency virus (HIV) infection, diabetes and neutrophil disorders, will predispose the individual to periodontal disease [6]. Microbial overgrowth in the form of accumulated plaque that extends into the subgingival areas of periodontium is one of the primary reasons. This results into an inflammatory response in adjacent tissue, initially involving the gingival tissue and slowly progressing to the periodontium in developing periodontitis. If untreated, this results in loss of tooth support structures, imparting mobility to the tooth. If treatment is not initiated irreversible loss of tooth supporting structures take place resulting in tooth loss [7-9].

3. Requirement of chemotherapeutic agents

Bacterial plaque is the main etiological factor in chronic inflammatory periodontal disease. Plaque removal is very important for control and prevention of this disease. Conventional treatment of periodontitis includes periodic mechanical debridement of plaque by scaling and curettage from tooth surfaces and repeated topical or systemic administration of antibacterial agents. The effectiveness of conventional treatments is limited by the lack of accessibility to bacteria in the periodontal pocket [10]. The popular local delivery systems are mouth rinses, ointments, dentifrices, chewing gums, local irrigation, mucoadhesive tablet, bioadhesive film, strip and gel. Mouth rinses are ineffective in controlling periodontal disease involving pocket formation presumably due to inadequate drug penetration and retention. Pitcher et al. showed that a plaque disclosing agent administered as a mouth rinse did not penetrate into periodontal pockets [11]. Controlled-release local delivery systems with or without a rate-controlling systems are classified as reservoirs [12]. Reservoirs that lack control include hollow fibers, gels, and dialysis tubing [13]. Examples of different drug delivery systems include: bioadhesive semisolid polymers for tetracycline [14], bioerodible polymeric inserts based on a blend of cellulose acetate phthalate and Pluronic for metronidazole [15], ethyl cellulose and acrylic strips for chlorhexidine or metronidazole [16, 17], ethylene vinyl acetate fibers for tetracycline [18], satranidazole 0.25% mucoadhesive gel [5], Elyzol a 25% suspension gel of metronidazole benzoate [19-21] and collagen matrix for metronidazole [22]. Elyzol is easy to apply and most research reports have applied the gel multiple times [23]. The reservoir systems can cause potential immunological reactions and tissue sensitization and mostly are retained poorly within the periodontal pocket [24].

Systemic administration can achieve therapeutic concentrations in the gingival crevicular fluid only with high doses [25]. Systemic administration have achieved therapeutic concentrations at the site of infection [26] but these concentrations are maintained for short periods of time after a single dose while repeated dosing has potential for side effects. Generally, systemic therapy is recommended in rapidly progressing or refractory periodontitis [27]. Another method of drug delivery is subgingival irrigation with antibacterial solutions directly into the periodontal pocket by specialized devices [28]. The duration of action is short and frequent application is required to maintain therapeutic concentration.

To overcome the above shortcomings, prolonged release intra pocket delivery systems have been developed. Goodson et al. pioneered the development suggesting a controlled release device within the pocket for administering antibacterial agents for periodontal therapy [29]. The most effective treatment may be achieved by a combination of delivery system, mechanical debridement and oral hygiene maintenance. After scaling and root planning, treatment with antibacterial agents (in biodegradable gel or film system) may then prevent pocket recolonization by suppression of marginal plaque without interfering with tissue regeneration. With the knowledge of *in-vitro* and *in-vivo* release characteristics, a device may be modified to provide desired drug concentration to eliminate subgingival infection and ultimately the periodontal pocket.

Chemotherapeutic agents can be administered systematically or delivered locally. By delivering chemotherapeutic agents directly to the periodontal tissues, greater concentrations are achieved and systemic side effects are reduced.

Vehicles for local delivery of chemotherapeutic agents include dentifrices, mouth-rinses, chewing gum, and slow-release devices [30-31]. Innovative methods of drug delivery via controlled-release devices represent an exceptional therapeutic potential. They allow higher concentrations of agents in dramatically reduced doses (when compared with a systemic load) for longer periods of time in sites that are difficult to reach. Clearly, treatment of well selected sites by local application of antibacterial agents is promising.

A variety of specialized local delivery systems (i.e., intrapocket devices) were designed to maintain the antibiotic in the gingival crevicular fluid at a concentration higher than that achieved by systemic administration. With respect to solid devices, semisolid (especially gel) formulations have some advantages, such as relatively faster release of the incorporated drug (particularly with respect to fibers or microparticles); easy preparation; easier administration and a higher biocompatibility and mucoadhesivity, allowing longer adhesion to the mucosa in the dental pocket and rapid elimination through normal catabolic pathways, decreasing the risk of irritative or allergic host reactions at the application site [5].

A pre-requisite for drug delivery systems for localized periodontal therapy is retention on the mucosal surface and controlled drug release at the site of action. A prolonged retention at the mucosal surface provides intimate contact between the dosage form and absorbing tissues that result in a prolonged period of drug exposure to the region. Therefore, an increased retention time is a desirable property of bioadhesive drug delivery systems. Retention time has been shown to be increased with an increase in the bioadhesivity measurement of the system [32-33]. Maximizing the bioadhesive forces of these systems therefore remains a significant goal in the development phase of mucoadhesive drug delivery systems. Mucoadhesive drug delivery systems have several advantages including ease of application and good retention within the periodontal pocket [34]. They extract water locally and form strong secondary chemical bonds with the dehydrated mucus [24, 35].

4. Role of microorganisms

Approximately 60-70% of the tooth loss in the United States after age 40 is caused by periodontal disease [36], whereas in the India the disease is responsible for about 80% of the teeth extracted after age 30. Studies have indicated a strong association of microorganisms of *Actinobacillus, Porphyromonas, Bacteroides gingivalis, B. melaninogenicus, Fusobacterium, F. nucleatum, Prevotella, Wolinella recta* and *Capnocytophaga* species in periodontitis of all stages [36]. The damage to the periodontium results from the direct toxic effects of sub gingival bacteria and due to the destructive effects of the host inflammatory response. Collagenase and other enzymes originating from bacteria can destroy the connective tissue and ligaments of the periodontium. Toxins of bacteria contribute to the progress of periodontal diseases.

The Minimum Inhibitory Concentration to kill 90% of the bacteria (MIC90) of satranidazole against 50 clinical isolates of anaerobes was found to be 0.25 mg/liter which was four-fold lower than the MIC90 of metronidazole, tinidazole and ornidazole (MIC90 = 1.0 mg/liter). In a fatal murine infection with Fusobacterium necrophorum, ATCC 27852, the Effective Dose (ED50) of satranidazole was 2.1 ± 0.62 mg/kg while for metronidazole, ornidazole, tinidazole and clindamycin, the values were 11.31 ± 1.99, 8.70 ± 2.21, 13.19 ± 2.39 and 7.10 ± 1.73 respectively. In a subcutaneous *Bacteroides fragilis* abscess in mice, satranidazole alone produced a three log reduction in colony forming units of the infecting organism at 10 mg/kg, the lowest dose tested [37].

Bansal et al. evaluated periodontal delivery of satranidazole which is a 5-nitroimidazole substituted at the 2- position and more active against aerobic, microaerophilic, and anaerobic bacteria than metronidazole [5]. In the healthy mouth, more than 350 species of microorganisms have been found. Periodontal infections are linked to fewer than 5% of these species. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* bacteria appear to cause aggressive periodontal disease. The MIC90 of satranidazole for *Salmonella typhimurium* (2 to 128µg/ml), *Campylobacter spp*. (0.06 to 16µg/ml) and *Bacteroides spp*. (0.03 to 0.25µg/ml) are definitely lower than those of metronidazole. The MIC90 of satranidazole for strain ATCC 25285 of *Bacteroides fragilis* is 0.03µg/ml and it is much lower than that of metronidazole (0.25µg/ml). According to the Structure Activity Relationship (SAR) technique of synthetic chemistry, the modification of 5-nitroimidazole drugs at 2nd position increases not only its antitrichomonal activity [38-39] but also its antibacterial activity.

5. Role of periodontal pocket in disease aggravation

About 50% of the adult population has gingivitis (gingival inflammation without any bone loss about teeth and no pockets deeper than 3 mm) around three or four teeth at any given time, and 30% have periodontitis as defined by the presence of three or more teeth with pockets of ≥4 mm [40-41]. This pocket can extend from 4 to 12 mm and can harbor, depending on its depth and extent, from 10^7 to almost 10^9 bacterial cells [42]**.** Between 5 and 15% of those with periodontitis have advanced forms with pockets of ≥6 mm [43]. Another 3 to 4% of individuals will develop an aggressive form of periodontal disease, known as early onset periodontitis, between the ages of 14 and 35 years [6].

Changes in periodontal pocket depth are used to assess disease progression. The primary aqueous environment is represented by gingival crevicular fluid. Healthy sites are associated with small volumes (0.04 µl) and low gingival crevicular fluid (GCF) flow rates (0.03 µl per minute). At diseased sites there is increased GCF production and serum like protein composition, indicating exudates formation. Volumes of about 0.5 ml and flow rates of 0.33-0.5 µl/min have been reported [44]. Turnover rate of GCF has been calculated to be 40 times per hour and this accounts for rapid clearance and short duration of action observed with irrigations. The gingival crevice fluid flow occurs at extremely low levels in healthy gingival sulci, but being an inflammatory exudates increase enormously to 3.5 ml per day or more [45].

The size of the periodontal pocket limits the size of device and consequently the amount of drug that can be delivered. The periodontal pocket is naturally irrigated by GCF. In subjects with periodontal disease, a mean rate of GCF flow at individual sites is approximately 150 µl per hour [46]. A high flow of GCF will result in a faster rate of drug diffusion from the device. Consequently, this will lead to a faster evacuation of the already released drug form the pocket to the mouth, thereby depleting the drug concentration in the pocket. Therefore, the rate of release should be higher in the initial stage to achieve an immediate therapeutic level in the pocket. The next stage of release should maintain the therapeutic levels in the pocket and thus a moderate release profile is required. Another parameter to be considered is the absorbency of drug. A poorly absorbent drug that has low penetration through the mucosal tissues enables the level of drug to reach higher concentration and prolong higher release in the pocket.

6. Chemotherapeutic therapy for Periodontitis

In antibiotic therapy of periodontal disease, maintaining an effective drug concentration in the periodontal pocket fluid for a sufficient period is considered to be more important than increasing drug concentration in the periodontal tissues. Evidence from longitudinal repeat periodontal probing studies indicates that periodontitis occurs as acute episodes which undergo spontaneous remission, often with a net loss of connective tissue attachment [47]. Furthermore it appears that attack of periodontal disease used to occur in bursts, more or less randomly distributed at different teeth sites throughout the mouth [6].

6.1 5-Nitroimidazole agents and gels for periodontal use

A mucoadhesive periodontal gel of metronidazole was prepared with hydroxyethyl cellulose (3%, 5% w/w), carbopol (3 and 5% w/w), polycarbophil (1 and 3%, w/w) and metronidazole (5%, w/w) at pH 6.8. Carbopol and polycarbophil have been reported to possess good bioadhesive properties, whereas the adhesive properties of hydroxyethyl cellulose are lower [34].

Sato et al. evaluated 15% metronidazole gel in the gingival crevicular fluid (GCF) of periodontal pockets of dogs. Periodontal pockets of 4 mm or deeper was treated. A single administration of the 15% metronidazole gel was found to release the drug in the GCF in levels several-fold higher than the minimum inhibitory concentration and metronidazole could be detected in the GCF even after 48 hours of gel application [48].

Smart gel periodontal drug delivery systems containing gellan gum (0.1-0.8% w/v), lutrol F127 (14, 16, and 18% w/v) and ornidazole (1% w/v) were designed for the treatment of periodontal diseases. Drug release data from all formulations was fitted to different kinetic models and the Korsemeyer-Peppas model was the best fit model. Drug release was significantly decreased as the concentration of each polymer component was increased. Increasing the concentration of each polymeric component significantly increased viscosity, syringeability, and time for 50%, 70%,

and 90% drug release. The formulation containing 0.8% w/v of gellan gum and 16% w/v of lutrol F127 exhibited superior physical characteristics [49].

A 5% metronidazole bioadhesive periodontal gel was developed using carboxymethylcellulose, methylcellulose, hydroxyethyl cellulose, polyvinylpyrrolidone, and carbopol. Increased concentrations of the polymers decreased the drug release rate and enhanced syringeability, yield value, and adhesiveness but decreased the spreadability. The gel containing 20% HEC, 20% polyvinylpyrrolidone and 1% carbopol exhibited zero order drug release kinetics and suitable physical properties for drug delivery to the periodontal pocket [24].

Amoxicillin and metronidazole were found effective as adjuvant therapy to scaling root planing (SRP) for the treatment of chronic periodontitis [50] by Sgolastra et al and important clinical parameters like clinical attachment level (CAL), reduction in probing depth (PD), secondary outcomes, and adverse events were analyzed.

The efficacy of metronidazole on cyclosporine-induced gingival overgrowth was evaluated and a prospective intra-subject double-blind longitudinal study was performed on six heart transplant patients with gingival overgrowth. Plaque index (PI), bleeding on probing (BP) and probing depth (PD) were recorded for all teeth of the four anterior hemi-sextants before and at 1, 2, 3, and 4 months after gel application. The PD significantly decreased after 1 month following both treatments. Analogous results were obtained with respect to parameters PI and BP. Long-term results showed greater efficacy of metronidazole with respect to placebo in controlling cyclosporine-induced gingival overgrowth [51].

Mody et al. filed a patent in 1997 for pharmaceutical dental formulation of metronidazole benzoate and chlorhexidine gluconate (about 0.01% to 0.5%). The formulation is for topical application in the form of an aqueous gel in the treatment of periodontal diseases including gingivitis, stomatitis, apthous ulcers and post-extraction infection. The results are better with polymer 1.5% w/w. Disodium edetate 0.025% used as chelating agent. The penetration enhancer used for the composition is propylene glycol (2% to 10%). Polymers used were carbomer 940, carbomer 934, hydroxypropyl methylcellulose, sodium carboxymethyl cellulose [52].

Miani et al evaluated a metronidazole-based gel where 15% metronidazole gel was applied in addition to SRP. Subgingival microbiological profile showed lower microbiological count when compared to active control treatment of SRP alone [53].

Mucoadhesive tablets were developed using different mixture of cellulose and polyacrylic derivatives containing metronidazole for periodontal disease treatment. All tablets were characterized by swelling studies, *ex vivo* and *in vivo* mucoadhesive time, mucoadhesion force and *in vitro* drug release. The best mucoadhesive performance and the best *in vitro* drug release profile were achieved by using hydroxyethyl cellulose (HEC) and carbomer 940 in 2:2 ratios. The tablet containing 20 mg of metronidazole showed 12 hour drug sustained release with buccal concentrations always higher than its MIC90 [54].

Liew et al. evaluated tinidazole orally in ten adult patients with moderate to advanced periodontitis. Samples were assayed by high performance liquid chromatography. The mean concentration of tinidazole in serum at 24 hour (13 ± 3.0 µg/ml) is greater than the MIC90 for anaerobic bacteria. The present data suggest that a single 2 gm oral dose of tinidazole may lead to the presence of potentially bactericidal levels of tinidazole for up to 24 hour in the periodontal pockets of patients [55].

Periodontal pocket inserts of niridazole Resomer R were developed and drug release from the prepared inserts was evaluated using a static dissolution setup. Pilot-scale clinical trials in 12 patients indicated improvements in clinical indices from the baseline values. The average pocket depth was reduced significantly from 6.34 ± 1.86 mm at baseline to 5.94 ± 0.28 mm after 28 days of treatment [56]**.**

The antibacterial effects of a short-term topical application of ornidazole on anaerobic microorganisms were investigated. The antibacterial activity of ornidazole on primary molars with infected pulps caused significant changes in growth rate of microorganisms (94.53% reduction) [57]. Subgingival irrigation in deep pockets showed significant improvement in all four clinical parameters (PI, PD, BP and gingival index). Therefore, subgingival irrigation with ornidazole in deep pockets is more effective than metronidazole or chlorhexidine in nonsurgical

periodontal therapy [58]. Super granules of ofloxacin 200 mg and ornidazole 500 mg (Zanocin OZ®, Manufactured by Ranbaxy, India) are also available in market for treatment of mixed dental infections.

7. Conclusion

The inflammatory components of chronic periodontitis can be managed effectively for the majority of patients with a plaque control program along with surgical root debridement coupled with continued periodontal maintenance procedures. Uses of chemotherapeutic agents is effective at both clinical and microbiological levels. Periodic monitoring of periodontal status and appropriate maintenance procedures are essential components of treatment of periodontal diseases. The use of 5- nitroimidazole drugs like metronidazole, satranidazole, niridazole, ornidazole and tinidazole in the treatment of periodontal diseases seems very promising. The current practice for the treatment of gingivitis and periodontitis involve the removal of plaque by scaling and root planning, along with application of 1% w/w metronidazole gel directly on the gums several times. The only dosage form of satranidazole available in the Indian market is immediate release tablet having 300 mg strength for treatment of amoebiasis and no modified release dosage form is available to further explore its therapeutic efficacy and the ease of use. Although satranidazole in gel dosage form has been investigated for periodontitis and was found effective in 0.25% w/w concentration, but this formulation is not available in the market till date [5, 59]. The microbiological [37] and clinical data [5] suggests superiority of satranidazole over metronidazole. The mucoadhesive gel of satranidazole adheres with gums for a prolonged period of time, reduces the dosing frequency, and lowers the bitterness of the periodontal gel. However, it is necessary to conduct more studies, like comparative studies with other modes of treatment, and formulations to assess comparative therapeutic efficacy. There is a scope for the pharmaceutical researchers and the dentists to work together for the development of new dosage forms and thus possibly extend the benefit of the drugs to patients in the near future. In addition, we also wish to draw the attention of scientists of the dentist and pharmaceutical community to generate more pharmacokinetic and pharmacodynamic data on larger patient populations and establish effectiveness of 5-nitroimidazole group of medicines.

Declaration of interest

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