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Full Length Research Paper

Preparation, characterization and *in vitro* antimicrobial activity of compound sustained-release periodontal suppository of ornidazole and pefloxacin mesylate

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Local delivery of sustained-release drugs in periodontal pocket is an effective approach for the treatment of periodontitis. The present study aimed to optimize and characterize the sustained-release periodontal suppository of compound ornidazole and pefloxacin mesylate (O&P) *in vitro*. Before suppository was prepared, the combined effect of O&P against dominant anaerobe and facultative anaerobe in periodontitis was investigated and then the optimal ratio of each drug was determined. After the compound suppository of O&P was prepared by cold compression, the optimal content of the suppository was determined through evaluating the bacteriostatic effect and then the sustained-release level of optimized suppository was investigated. There was no incompatibility between ornidazole and pefloxacin mesylate. When being applied in combination, they could exert synergistic bacteriostatic effect against 'porphyromonas gingivalid' and 'fusobacterium nucleatum', and additive effect against 'prevotella intermedia' and 'peptostreptococcus spp' and inhibit anaerobe growth at a low concentration. The optimal ratio of O&P is 2:3 and the optimal content of suppository 20% *in vitro*; this suppository could release both drugs in a sustained manner for 12 h. The compound suppository of O&P can serve as one of the potential candidates for the treatment of periodontitis and has favorable sustained-release ability which can meet the requirement of clinical treatment of periodontitis.

Key words: Ornidazole, pefloxacin mesylate, periodontitis, sustained-release delivery system.

INTRODUCTION

Periodontitis is a group of dentoalveolar infections and remains one of the major causes of adult tooth loss. These infections involve a variety of bacteria in local periodontal tissues. Plaques and their products are the original cause of parodontopathy (Zambon, 1996). To date, numerous investigations have been confirmed that anaerobe is a dominant type of pathogenic bacteria, including *Porphyromonas gingivalid* (Pg), *Fusobacterium nucleatum* (Fn), *Prevotella intermedia* (Pi) and *Peptostreptococcus* spp (Ps). Additionally, these facultative anaerobe such as *Staphylococcus aureus* (Sa), *Staphylococcus epidermidis* (Se), etc (Takahashi, 1998; Paju et al., 2009), also have synergistic action in the process of pathogenesis. Although, the use of systemic antibiotic treatment of periodontitis has shown some benefits, multiple systemic doses of antibiotics have several drawbacks including inadequate antibiotic concentration (subtherapeutic level) at the site of periodontal pocket and high plasma concentration. Inadequate antibiotic concentration is not able to continuously inhibit or kill the pathogenic microorganisms, and fail to control the periodontal inflammation which may facilitate the reconstruction of damaged tissues. High plasma concentration may be associated with bacterial resistance and occurrence of side effects (Bidault et al., 2007a, b). These disadvantages have evoked an interest in the development of novel local drug delivery systems for the treatment of periodontal diseases (Greenstein, 2006; Hussein et al., 2007).

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With the development of modern pharmacy, sustained-release drug has been used in the treatment of parodontopathy (Vandekerckhove et al., 1997; Vyas et al., 2000). If a therapeutic effective concentration can be maintained in the periodontal pocket for a desired period, local sustained-release drug may significantly improve the therapeutic efficacy of periodontitis. Moreover, side-effects can also be greatly decreased due to avoidance of high plasma concentration (Vyas et al., 2000). Ornidazole is a nitroimidazole antiprotozoal agent and has better anti-anaerobic activity than quinolones (Quirynen et al., 2002). Although, anaerobe is the dominant population involving in periodontitis, facultative anaerobe also plays a part role in the periodontal destruction. Pefloxacin mesylate is a quinolone antibiotic and has better activity against facultative anaerobes than nitroimidazoles (Wang et al., 2007). In the present study, the optimal ratio and optimal content of ornidazole (O) and pefloxacin mesylate (P) were determined to prepare the compound O&P sustained-release periodontal suppository for the treatment of periodontitis.

MATERIALS AND METHODS

Dominant pathogenic bacteria of periodontitis

Pg, Fn, Pi, Ps, Mutans streptococcu (Ms), Sa and Se are standard strain purchased from the R&D Department of P&G company.

Instruments for experiments

Agar medium (Shanghai Li Chen Biotechnology Co., Ltd.), drug susceptibility papers (Shanghai Wufeng Scientific Instruments Co., Ltd.), high performance liquid chromatograph (HPLC) (Shanghai Wufeng Scientific Instruments Co., Ltd.), O&P standard substance and chromatography-pure methanol (Shanghai Shengke Biotechnology Co., Ltd.) were used. SPSS13.0 was used for statistical analysis.

In vitro study on the anti-microbic characteristic of O&P

To investigate whether O&P have synergic anti-microbic action, the Kirby-Bauer test was applied. Round arid anti-microbic filter papers measuring 6 mm in diameter were prepared and soaked with 5 μg of ornidazole or pefloxacin mesylate, respectively. Then, these papers were put onto the agar plate containing Pg, Fn, Pi, Ps, Sa or Se. There were one ornidazole paper and one pefloxacin mesylate paper on each agar plate. Then, the synergic action was preliminarily estimated by the shape of intersecting angles between two inhibition rings. The interaction was further assessed by using agar dilution method and the effects evaluated with fractional inhibitory concentration (FIC) and minimun inhibitory concentration (MIC). In brief, Pg, Fn, Pi, Pa, Sa, Se (2 × 107 ml) were inoculated into a Mueller-Hinton broth and dispensed at 0.1 ml/well in 96-well plates. MICs were determined by a serial twofold dilution of ornidazole and/or pefloxacin mesylate from 2 to 1/32 of MIC alone.

After 24 h of incubation at 37°C, the minimal compound concentration at which the ornidazole/pefloxacin mesylate prevented the growth of a given organism was determined and defined as the MIC of the compound. The MIC was determined in three independent assays.

Screen on the optimal ratio of O&P

In the agar plates of Pg, Fn, Pi, Ps and Fn, the ditch plate method of agar diffusion was employed to detect anti-anaerobic effects of O&P at different ratios. L36 (62) orthogonal table was designed to select the optimal ratio of O&P on the 4 strains of anaerobes. In the orthogonal table, O&P are two factors and each factor is classified into 6 levels. In the orthogonal experiment, there were 36 groups in which the ratio of O&P varied from 1:6 to 6:1 and only 23 groups were finally subjected to analysis because sever groups were integrated into one (for example ratios at 1:2, 2:4 and 3:6 were the same to 1:2) (Table 1). According to findings in the orthogonal experiment, the range of each factor was calculated: the larger the range, the higher the antibacterial effect. Single-factor analysis of variance was used to identify the critical point of ratio for each anaerobe.

In order to get the optimal ratio, the MIC and MIC50 of dominant periodontal pathogenic bacteria were determined according to each critical point.

Preparation of compound O&P sustained-release periodontal suppository

A special mold (Figure 1) of suppository was designed according to anatomic shape and the depths of different periodontal pockets. Cold compression was employed for the preparation of suppository. The carrier which can reduce the application of antibacterial agents was composed of ethyl cellulose (EC) and hydroxypropyl methyl cellulose (HPMC). Firstly, EC was swollen with 95% alcohol, and then with sufficient distilled water. The glycerine, pefloxacin mesylate and ornidazole were added in a proper order. When all these materials were mixed sufficiently, HPMC was then added to the mixture. After agitation, alcohol was volatilized, the mixture was swollen to doughing time and then it was taken into the mold.

Screening optimal content of compound O&P periodontal suppository

Different contents (1, 2, 5, 10 and 20%) of O&P periodontal suppository were prepared. In every content group, three suppositories with same content were completely dissolved in 3 ml of purified water. After being filtrated, 5 μl of drug-filtrate was put into the drug -sensitive paper plates measuring 6 mm in diameter. So, in the paper plate of each group, the drug content was 0.5, 1, 2.5, 5 and 10 μg; which corresponded to the contents of suppositories. Then, Kirby-Bauer method was used to estimate the bacteriostatic effect on the 4 strains of anaerobic bacteria (Pg, Fn, Pi and Ps), and 3 strains of facultative anaerobe (Ms, Sa and Se). The optimized content of suppository was determined by comparing the size of inhibition rings.

Medicine release level of compound sustained-release periodontal suppository *in vitro*

Cumulative release ratio of drugs was detected through HPLC. The conditions for HPLC were chromatographic column: C18 (250 × 4.6 mm, 5 μm); mobile phase: 0.1 mol/L monopotassium phosphate solution-methanol (60:40); detecting wavelength: 277 nm; flow velocity: 1.0 ml/min; sampling size: 20 μl; column temperature: 30°C. Firstly, the standard curves of O&P were delineated and the peak areas were corresponding to the drug concentration. Then, the suppositories with 20% drug were soaked into a beaker containing 5 ml of purified water and stirred every 10 min (n = 5). At the designed time points (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 18 and 24 h), 200 μl of fluid were collected for the detection and

Table 1. Ratio of ornidazole and pefloxacin mesylate for testing.

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Ornidazole	6	5	4	3	5	2	5	3	4	5	6	1	5	4	3	2	3	1	2	1	1	1	1
Pefloxacin	1	1	1	1	2	1	3	2	3	4	5	1	6	5	4	3	5	2	5	3	4	5	6

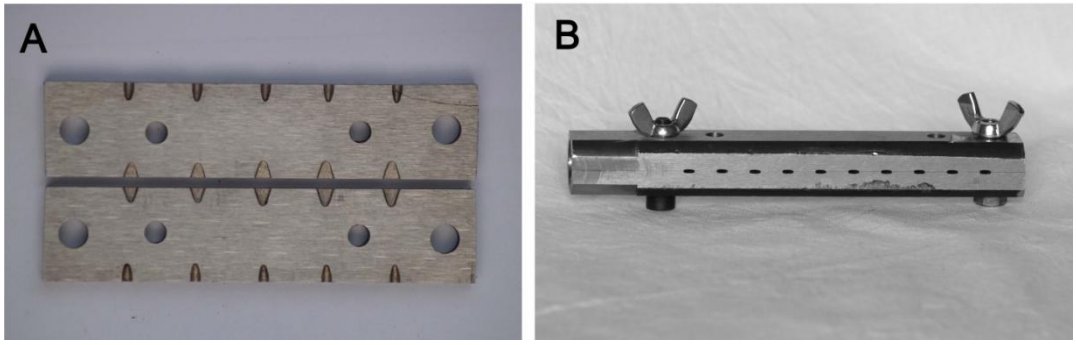

Figure 1. Mold for sustained-release periodontal suppository. The mould is opened (A) and the mould is closed (B). The mold of suppository is made of stainless steel. According to anatomic shape and the depth of different periodontal pockets, the die hole of anterior teeth is 3 ~ 4 mm in length, 2 mm in width and 350 to 500 μm in thickness. The die hole of posterior teeth is 3 to 4 mm in length, 4 mm in width, 350 to 500 μm in thickness. All the die holes are shuttle-shaped with obtuse margin.

additional 200 μl of purified water was added to the beaker. The peak areas at different time points were measured by HPLC. According to the standard curve, average concentrations at different time points were calculated. Based on the average concentrations, the discharge amounts at different time points were obtained.

Finally, the release curves of two drugs were delineated according to the time and corresponding release rate.

RESULTS

Anti-microbic characteristic of O&P

On the anaerobe flat plate, the intersecting angles of two inhibition rings of two drugs displayed straightened or even evaginated which suggests they may have synergistic action against anaerobe (Figure 2A). On the facultative anaerobe flat plate, there was no inhibition rings of ornidazole (Figure 2B); which indicates that they have independent effect against facultative anaerobe and only pefloxacin mesylate exerts anti-anaerobic effect. FIC of two drugs against anaerobe were lower than 1, further demonstrating that both drugs can confer synergetic or additive effect against anaerobes when being used together; and they can inhibit the growth of anaerobes at a low concentration (Table 2). For the facultative anaerobe, when two drugs were used together, the concentration of pefloxacin was equal to or higher than its own MIC and the FIC was 1.0625, which suggests that, the activities of two drugs are independent and the bactriostatic effect depends on pefloxacin mesylate alone (Table 2).

Optimization on the ratio of O&P

The results of orthogonal test are shown in Table 3. Analysis showing the range of ornidazole and pefloxacin could be calculated (Table 4). The range of ornidazole was larger than that of pefloxacin which provides the evidence that ornidazole has high anti-anaerobic activity than pefloxacin mesylate. In each group, the length of bacteriostatic area at each ratio was analyzed by single-factor analysis of variance. For the Pg, Fn, Pi and Ps, the critical point of the ratio of two drugs was 1:2, 2:3, 2:3 and 1:1, respectively. According to the 3 critical points, the MIC and MIC50 of dominant periodontal pathogenic bacteria were determined (Table 5). The MIC of ornidazole against Pg, Fn, Pi, Ps was 0.062, 0.062, 0.125 and 0.25 mg/L, respectively; showing that all these 4 kinds of bacteria are very sensitive to ornidazole. The MIC of pefloxacin mesylate against these bacteria was 0.25, 0.25, 0.5 and 4 mg/L, respectively; showing that pefloxacin mesylate has bacteriostatic effect on all the 4 kinds of bacteria. The MIC of pefloxacin mesylate against Sa and Se was 0.062 and 0.125 mg/L, respectively; also showing a bacteriostatic effect.

When ornidazole was combined with pefloxacin mesylate, the MIC of ornidazole or pefloxacin mesylate against anaerobes was lower than their own MIC. The results demonstrated that two drugs have synergic action

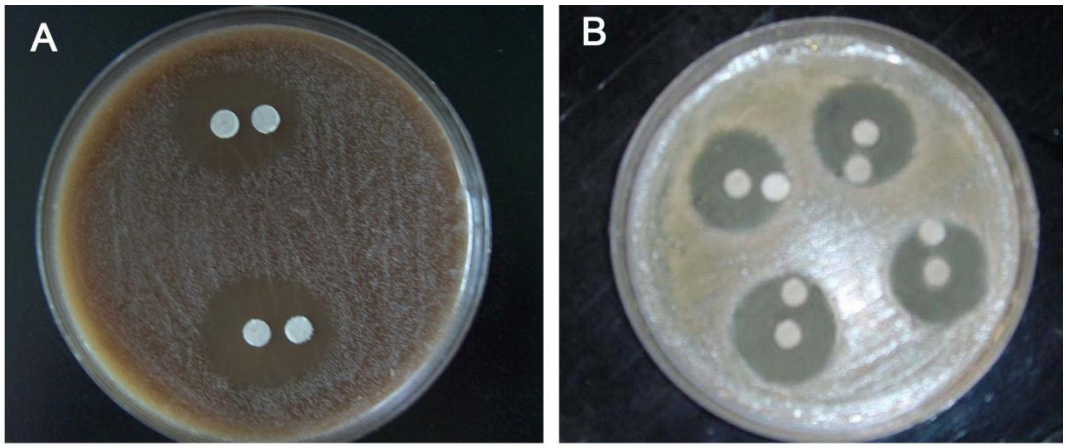

Figure 2. Compound synergic anti-microbic characteristic of ornidazole and pefloxacin mesylate with Kirby-Bauer method. The synergic anti-microbic characteristic of ornidazole and pefloxacin mesylate (A). On the anaerobe flat plate, the intersecting angles of two inhibition rings of two drugs displayed straightened or even evaginated which suggests they have synergistic action against anaerobe (B). The indenpedent anti-microbic characteristic of ornidazole and pefloxacin mesylate. On the facultative anaerobe flat plate, there was no inhibition rings of ornidazole, which suggests that two drugs have indenpedent actions against facultative anaerobe and the bactriostasis depends on pefloxacin mesylate alone.

Table 2. FIC of compound ornidazole and pefloxacin mesylate.

Bacterium		Ornidazole (MIC)	Pefloxacin mesylate (MIC)	FIC
Anaerobe	*Porphyromonas gingivalid*	1/4	1/16	0.3125
	Fusobacterium nucleatum	1/4	1/8	0.375
	Prevotella intermedia	1/4	1/16	0.5625
	Peptostreptococcus spp	1/4	1/4	0.75
Facultative anaerobe	*Staphylococcus aureus*	1/16	1	1.0625
	Staphylococcus epiermidis	1/16	1	1.0625

FIC = ($MIC_{Drug\ A\ in\ combination}/MIC_{Drug\ A\ alone}$) + ($MIC_{Drug\ B\ in\ combination}/MIC_{Drug\ B\ alone}$). FIC ≤ 0.5, 0.5 < FIC≤1, 1 < FIC ≤ 2, and > 2 were defined as synergistic, additive, independent, and antagonism, respectively. All FICs of two drugs against anaerobes were less than 1. For *Pg* and *Fn*, the FIC was less than 0.5 which suggests two drugs have synergistic action. For *Pi* and *Ps*, the FIC was between 0.5 and 1 which suggests two drugs have additive action. All FICs of two drugs against facultative anaerobes were higher than 1 but lower than 2. For *Sa* and *Se*, the FIC was 1.0625, which suggests two drugs have independent action and the bactriostatic effect depends on pefloxacin mesylate alone.

against the anaerobic bacteria. Especially, MIC against Pg was kept constantly at 0.062 mg/L. The MIC at 3 different ratios of O&P showed no significant difference against 4 kinds of anaerobic bacteria. The antiblastic effect was not significantly diminished when reducing the ratio of ornidazole or increasing that of pefloxacin.

Optimal content of each drug in the compound O&P periodontal suppository

The inhibition rings were examined at different drug contents ranging from 1 to 20% and results showed that the anti-bacterial effects of both drugs were dose-dependent against the 7 major pathogenic bacteria (Figure 3A). The 20% drug had the strongest anti-bacterial effect against Sa (inhibition ring was 27 mm in diameter) and 1% drug had the weakest anti-bacterial effect against Ms (inhibition rings was 7.9 mm in diameter). The sizes of bacterial inhibition rings against the 7 different bacteria decreased in the following order: Ms, Ps, Pi, Fn, Pg, Se and Sa (Figure 3B).

In vitro release of sustained-release periodontal suppository containing 20% drug

HPLC showed the retaining times of ornidazole (Bodyguard Pharmaceutical Co. Ltd., China) and pefloxacin mesylate standard substance (North China

Table 3. Anti-anaerobic effect of different ratios of ornidazole and pefloxacin mesylate.

Factor			Anti-anaerobic effects (mm)			
No.	O	P	*Pg*	*Fn*	*Pi*	*Ps*
y_1	6	1	31.2±1.37	31.0±1.17	28.3±1.06	26.4±1.28
y_2	6	2	30.5±1.07	30.9±1.22	28.3±0.87	26.3±0.91
y_3	6	3	30.8±1.05	30.83±1.35	27.91±0.95	25.72±0.87
y_4	6	4	30.5±1.61	30.6±0.93	27.3±0.74	25.1±1.16
y_5	6	5	30.4 ±1.21	30.6±1.07	27.0±1.13	24.8±1.13
y_6	6	6	30.0±0.94	30.4±1.02	26.9±1.02	24.5±0.74
y_7	5	1	31.4±1.53	31.1±1.45	28.3±0.87	26.0±.1.03
y_8	5	2	31.0± 1.32	30.7±1.10	28.1±0.89	26.1±1.01
y_9	5	3	30.2±1.32	30.7±1.01	27.6±0.86	25.1±0.99
y_{10}	5	4	30.5±1.34	30.4±0.95	27.1±0.81	25.1±1.15
y_{11}	5	5	30.0±0.94	30.4±1.02	26.9±1.02	24.5±0.74
y_{12}	5	6	29.9±1.32	30.0±1.55	26.8±1.11	23.4±0.85
y_{13}	4	1	31.2± 1.36	31.1±1.42	28.2±0.94	26.4±0.96
y_{14}	4	2	30.86±1.05	30.83±1.35	27.91±0.95	25.72±0.87
y_{15}	4	3	29.9 ±1.26	30.5±1.09	27.3±0.97	25.0±0.99
y_{16}	4	4	30.0±0.94	30.4±1.02	26.9±1.02	24.5±0.74
y_{17}	4	5	29.9±1.33	29.8±1.07	26.8±1.22	23.2±0.67
y_{18}	4	6	30.1±1.05	29.5±1.06	26.7±1.01	22.8±0.99
y_{19}	3	1	30.5±1.07	30.9±1.22	28.3±0.87	26.3±0.91
y_{20}	3	2	30.5±1.61	30.6±0.93	27.3±0.74	25.1±1.16
y_{21}	3	3	30.0±0.94	30.4±1.02	26.9±1.02	24.5±0.74
y_{22}	3	4	30.2±1.50	30.0±1.02	26.9±0.99	22.9±0.87
y_{23}	3	5	29.3±1.33	29.0±0.94	26.5±0.74	22.8±1.02
y_{24}	3	6	29.2±1.52	28.6±0.72	25.9±1.00	22.6±1.20
y_{25}	2	1	30.4±1.29	30.8±1.37	27.7±1.13	25.5±1.35
y_{26}	2	2	30.0±0.94	30.4±1.02	26.9±1.02	24.5±0.74
y_{27}	2	3	30.1±1.05	29.5±1.06	26.7±1.01	22.8±0.99
y_{28}	2	4	29.2±1.52	28.6±0.72	25.9±1.00	22.6±1.20
y_{29}	2	5	28.3±0.95	27.6±0.96	24.2±1.16	22.4±1.29
y_{30}	2	6	27.1±1.12	26.5±1.16	23.6±1.01	21.8±0.94
y_{31}	1	1	30.0±0.94	30.4±1.02	26.9±1.02	24.5±0.74
y_{32}	1	2	29.2±1.52	28.6±0.72	25.9±1.00	22.6±1.20
y_{33}	1	3	27.1±1.12	26.5±1.16	23.6±1.01	21.8±0.94
y_{34}	1	4	26.7±1.17	26.0±0.81	22.7±0.91	21.5±1.02
y_{35}	1	5	26.0±1.13	25.5±0.89	22.1±1.15	20.2±0.98
y_{36}	1	6	26.0± 0.78	25.1±0.80	21.3±1.59	19.8±0.89

Pharmaceutical Group Co. Ltd., China) were 10.1 and 13.7 min, respectively (Figure 4A). The regression equation of ornidazole was y = 5.7507x + 1.0714, r = 0.9993 (Figure 4B) and that of pefloxacin mesylate was y = 1.1279x + 0.9904, r = 0.9999 (Figure 4C). The peak areas, average concentrations and release amounts of O&P at different time points are shown in Tables 6 and 7. Ornidazole was released by 9.08% at 30 min, 59.50% at 5 h and 98.30% at 13 h, while pefloxacin mesylate was released by 15.26% at 30 min, 45.00% at 5 h and 96.01% at 13 h (Figure 4D).

DISCUSSION

The appearance of periodontal pocket is one of the important signs of periodontitis (Liu et al., 2003). The periodontal pocket provides a natural space for the local delivery system (Mundargi et al., 2007). Meanwhile, the gingival crevicular fluid provides a liquid medium for the release of drug from a carrier and for its distribution throughout the periodontium (Jain et al., 2008). Therefore, a therapeutic effective concentration of an anti-microbial agent could be maintained in the periodontal

Table 4. Antibacterial index of ornidazole and pefloxacin mesylate with range analysis.

Bacterium	Ornidazole	Pefloxacin mesylate
Porphyromonas gingivalid	$R^{(1)} = 7.5955$	$R^{(2)} = 2.6142$
Fusobacterium nucleatum	$R^{(1)} = 3.3219$	$R^{(2)} = 1.8027$
Prevotella intermedia	$R^{(1)} = 8.3327$	$R^{(2)} = 2.7861$
Peptostreptococcus spp	$R^{(1)} = 10.8025$	$R^{(2)} = 4.7611$

The range of ornidazole was larger than that of pefloxacin which provides the evidence that ornidazole has stronger sensitivity than pefloxacin mesylate against anaerobes. The range can be calculated from Table 3. $R^{(1)} = [\bar{k}_1^{(1)} - \mu]^2 + [\bar{k}_2^{(1)} - \mu]^2 + [\bar{k}_3^{(1)} - \mu]^2 + [\bar{k}_4^{(1)} - \mu]^2 + [\bar{k}_5^{(1)} - \mu]^2 + [\bar{k}_6^{(1)} - \mu]^2$; $R^{(2)} = [\bar{k}_1^{(2)} - \mu]^2 + [\bar{k}_2^{(2)} - \mu]^2 + [\bar{k}_3^{(2)} - \mu]^2 + [\bar{k}_4^{(2)} - \mu]^2 + [\bar{k}_5^{(2)} - \mu]^2 + [\bar{k}_6^{(2)} - \mu]^2$; $\mu = (y_1 + y_2 + y_3 + y_4 \ldots\ldots\ldots + y_{36}) \times 1/36$; $k_1^{(1)} = y_1 + y_2 + y_3 + y_4 + y_5 + y_6$: $\bar{k}_1^{(1)} = 1/6\ k_1^{(1)}$: $k_2^{(1)} = y_7 + y_8 + y_9 + y_{10} + y_{11} + y_{12}$: $\bar{k}_2^{(1)} = 1/6\ k_2^{(1)}$; $k_1^{(2)} = y_1 + y_7 + y_{13} + y_{19} + y_{25} + y_{31}$ $\bar{k}_1^{(2)} = 1/6\ k_1^{(2)}$: $k_2^{(2)} = y_2 + y_8 + y_{14} + y_{20} + y_{26} + y_{32}$: $\bar{k}_2^{(2)} = 1/6\ k_2^{(2)}$ and so on.

Table 5. MIC in 4 strains of anaerobes and 3 strains of facultative anaerobes with 3 different critical points in ratio.

Bacterium (strain)	Ornidazole	Pefloxacin mesylate	1:1	2:3	1:2
	MIC/MIC_{50}	MIC/MIC_{50}	MIC/MIC_{50}	MIC/MIC_{50}	MIC/MIC_{50}
Pg	0.062	0.25	0.062	0.062	0.062
Fn	0.062	0.25	0.062	0.062	0.125
Pi	0.125	0.5	0.125	0.25	0.25
Ps	0.25	4	0.25	0.5	1
Sa	>256	0.06~8/0.5	0.125~32/1	0.06~16/0.5	0.06~8/0.5
Se	>256	0.125~16/1	0.25~32/2	0.25~32/1	0.25~16/1
Ms	>256	0.125~64/4	0.25~64/8	0.25~64/4	0.125~64/4

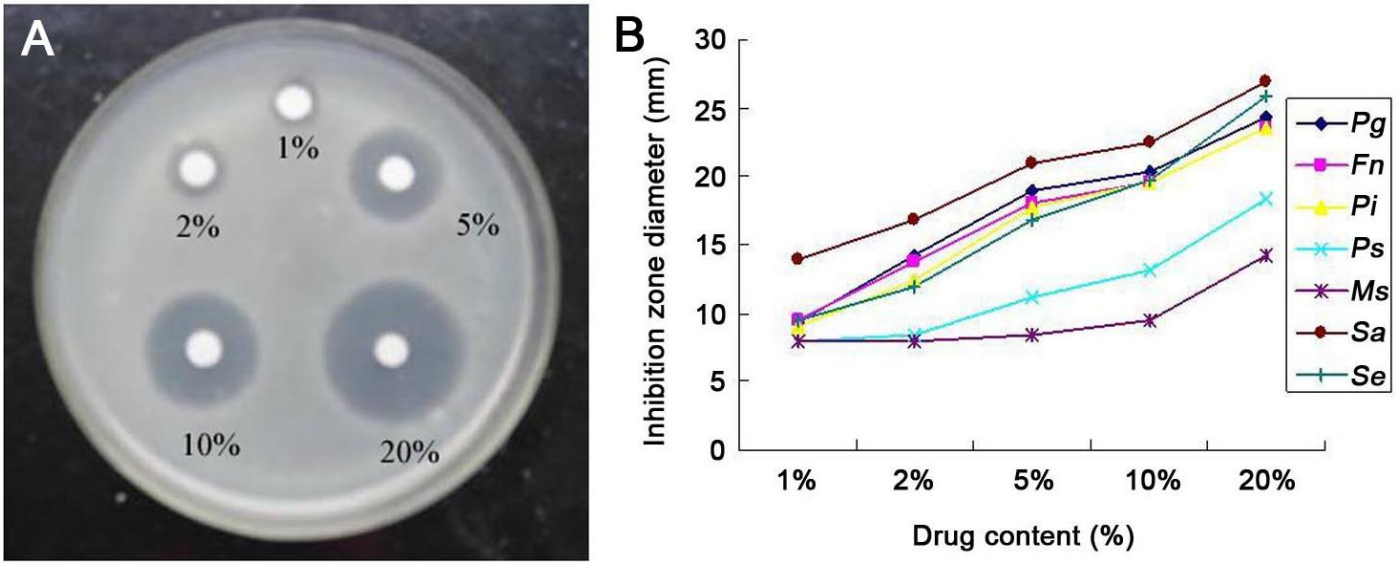

Figure 3. *In vitro* bacteriostatic effect of ornidazole and pefloxacin mesylate periodontal suppository with five different drug contents. Diameters of inhibition zones at five different drug contents against one bacterium (A); bacteriostatic effect against different bacteria at different drug contents (B). An obvious dose-dependent manner was observed in the anti-bacterial effect against the 7 major suspected pathogenic bacteria.

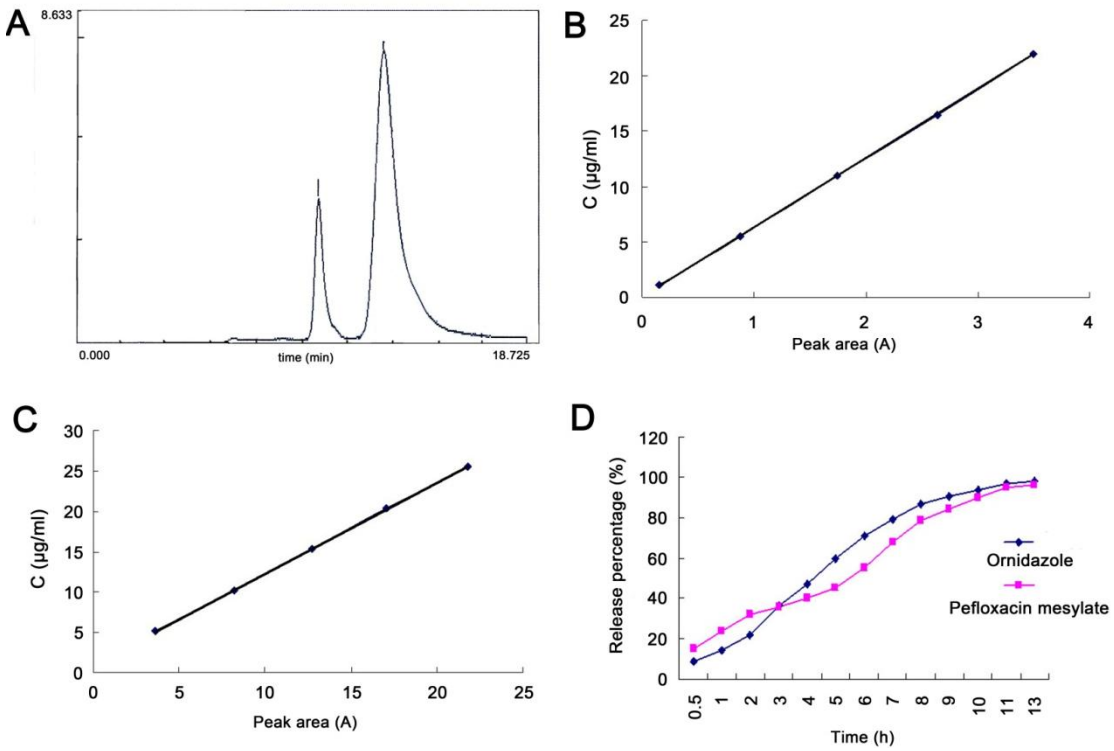

Figure 4. *In vitro* release of sustained-release suppository containing 20% drug. Chromatography of ornidazole and pefloxacin mesylate standard substance (A); A represents ornidazole T_R = 10.166 min and B pefloxacin T_R = 13.722 min; the standard curve of ornidazole (B); the standard curve of pefloxacin mesylate (C) and the release curve of ornidazole and pefloxacin mesylate *in vitro* (D).

Table 6. The peak area and delayed release of ornidazole in the periodontal suppository.

Time (h)	Peak area	Average concentration (mg/ml)	Release amount (mg)	Accumulative release degrees (%)
0.5	5.757	0.036	0.18	9.08
1	8.781	0.055	0.10	14.63
2	12.793	0.080	0.14	22.01
3	20.629	0.129	0.26	36.25
4	26.227	0.164	0.20	47.20
5	32.623	0.204	0.23	59.50
6	37.900	0.237	0.21	70.86
7	41.579	0.260	0.16	79.47
8	44.137	0.276	0.13	86.55
9	47.816	0.299	0.07	90.18
10	47.816	0.299	0.06	93.34
11	48.775	0.305	0.03	96.79
13	47.655	0.298	0.03	98.30

pocket for a desired period, which is the basis of; and ensure the successful treatment of periodontitis. A variety of potentials of sustained-release preparations have been displayed in this treatment (Chen et al., 2006). In this

Table 7. The peak area and delayed release of pefloxacin mesylate in the periodontal suppository.

Time (h)	Peak area	Average concentration (mg/ml)	Release amount (mg)	Accumulative release degrees (%)
0.5	101.470	0.084	0.42	15.26
1	155.154	0.128	0.24	24.10
2	199.423	0.164	0.22	32.11
3	215.410	0.177	0.10	35.80
4	236.315	0.194	0.12	40.23
5	258.449	0.212	0.13	45.00
6	319.935	0.262	0.29	55.45
7	388.110	0.317	0.33	67.62
8	444.136	0.363	0.29	78.33
9	467.500	0.382	0.17	84.48
10	483.486	0.395	0.14	89.64
11	498.243	0.407	0.14	94.57
13	488.405	0.399	0.04	96.01

study, in order to provide a high anti-microbial activity, we designed local compound O&P periodontal suppository which consisted of ornidazole, pefloxacin mesylate and carrier. The carrier was composed of EC and HPMC, materials widely used in controlled release system and its application reduced the content of antibacterial agents.

The Kirby-Bauer test and FIC detection indicated that there was no incompatibility between ornidazole and pefloxacinmesylate, and they could exert synergetic effect against anaerobe and inhibit the anaerobic growth at a low concentration when they were applied together. For facultative anaerobe, however, the anti-bacterial effect depended on pefloxacin mesylate alone. In this study, the ratio of two drugs was for the first time optimized. Orthogonal experiment was performed to determine the optimal ratio against 4 strains of anaerobes. Through single-factor analysis of variance, three critical points of ratio were identified: 1:2, 2:3 and 1:1. According to 3 critical points, the MIC and MIC50 of the dominant periodontal pathogenic bacteria were determined. Among the 3 different critical points, for the anaerobes, there were no significant differences in the anti-bacterial activities and their MIC. The present study also demonstrated that the antibacterial activities of the compound increased with the increase of pefloxacin when the anti--bacterial effect against facultative anaerobes was taken into account. Because two drugs have independent anti-bacterial effect against facultative anaerobe and the bactriostatic effect depends on pefloxacin mesylate alone; that is to say, the ratio of 1:2 is preferred. However, periodontal diseases are predominantly caused by anaerobic infection, and quinolones have been clinically applied for years, there is a possibility of drug resistance.

In addition, ornidazole has first expose effect and its anti-bacterial effect is concentration dependent (Kamma et al., 2000). Moreover, the anti-bacterial activity of pefloxacin mesylate against anaerobes *in vivo* should be further studied (Appelbaum, 1999). Taking the reasons earlier mentioned into account, we speculate that the ratio of 2:3 is a preferred ratio. O&P are freely soluble in gingival crevicular fluid, and hence selection of release-retarding excipient is necessary to achieve a constant input rate in the gingival crevicular (Paquette et al., 2008; Akncbay et al., 2007; Bosco et al., 2009). Because of its flexibility, HPMC (a hydrophilic polymer matrix) was used to obtain a desirable drug release profile and broad regulatory acceptance. HPMC has been well known to retard drug release by swelling in aqueous media (Li et al., 2005). However, for a water soluble drug, application of a hydrophilic matrix system alone is restricted because of rapid diffusion of dissolved drug through the hydrophilic gel network. In such circumstances, EC, one of hydrophobic polymers is required along with the HPMC for developing sustained-release delivery system (Barat et al., 2007).

Incorporation of a high concentration of EC controls the drug release in a better manner, which may be attributed to the decreased penetration of solvent molecules in the presence of hydrophobic polymer, leading to decreased drug diffusion from the matrix (Bromberg et al., 2001). Furthermore, because of the presence of EC which is generally responsible for the hardness of suppository, the suppository is not susceptible to being cracked when it is held by pliers. The sustained-release materials used in this study have certain drug saturation. When the drug content is higher than 20%, the precipitation of drug may occur. So, 5 different contents (1, 2, 5, 10 and 20%) were applied. The optimal content was selected by comparing the size of inhibition rings. Statistical analysis demonstrated that content at 20% has obvious advantages. The *in vitro* experiment on the sustained-release periodontal suppository containing 20% drug revealed that the average release rates of ornidazole and

pefloxacin mesylate were 0.14 and 0.20 mg/h, respectively; and the accumulated release percentages of ornidazole and pefloxacin mesylate at 13 h were 98.3 and 96.01%, respectively. Moreover, the initial burst release was not observed. All the parameters related to sustained-release behavior were within the limits proposed by the pharmacopeia.

The present study indicated that the compound periodontal suppository containing 20% drug had potent sustained-release bacteriostatic effect against the suspected pathogenic bacteria. Especially, for the sensitive bacteria, the sustained-release could be sustained for about 24 h. For the non-sensitive bacteria such as Ps and Ms, the sustained-release could be sustained for about 10 h. These features demonstrated that the suppository can meet the requirement of sustained-release. So, in the treatment of periodontitis, the suppository may be medicated every other day. This study affirmatively provides a better understanding of the synergistic effect of O&P which may be helpful for the periodontal therapies. Our results provide convincing evidence and useful information for future clinical application of sustained-release periodontal suppository containing 20% drug. In future study, we will further optimize the periodontal suppository. For example, the ratio of sustained-release materials should be optimized which may prolong the sustained-release time.

In addition, the therapeutic effect of periodontal suppository will be investigated in rats with ligature-induced periodontitis.

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