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Evaluation of saliva levels and therapeutic effects of morinidazole, a new 5-nitroimidazole derivative, in patients with periodontal diseases

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The aim of this study was to evaluate and compare the saliva and gingival crevice fluid concentrations, as well as therapeutic effects of morinidazole (MNZ) and ornidazole (ONZ) in patients with periodontitis after intravenous (i.v.) infusion administration. 142 outpatient subjects (80 women and 62 men) were enrolled in the phase II clinical trial, and the patients were randomly assigned to receive intravenous infusions of either Ornidazole or Morinidazole, twice daily for 5 days (500 mg each time, infusion period was 50 min). A saliva was collected prior to and again after finishing the first i.v. dose at intervals of 15, 30, 45, 60, 90 min on day 1. Bacteriological studies were undertaken to determine the effectiveness of the MNZ or ONZ. Reverse phase high performance liquid chromatography (RP-HPLC) method with UV detection at 318 nm was carried out, using a mixture of KH_2PO_4, acetonitrile and methanol (55:15:30, v/v/v) as a mobile phase, with a flow rate of 1.0 ml/min. There was no significant difference between the MNZ and ONZ groups. The cure rate of morinidazole was found to be slightly higher than that of ONZ, just like the result of concentration in saliva, but no significant differences between the two agents were apparent. During our trial, we found that the clinical resistance of anaerobes to morinidazole was proven to be extremely rare.

Key words: Periodontitis, morinidazole, ornidazole, tinidazole, saliva.

INTRODUCTION

Periodontal disease are generally considered to cause major oral health problems around the world. Specific anaerobic bacteria within the periodontal pocket are thought to be responsible for periodontal disease and as infection takes hold, a cascade of tissue- destructive pathways ensues, fuelled by inflammatory mediators (Ha et al., 2012)

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et al., 2012). Over 400 species of bacteria have been isolated from plaques (biofilms formed by bacteria), but only a few species of bacteria release toxins and induce a host inflammatory response resulting in the destruction of alveolar bone and connective tissues that support the dentition.

There is increasing evidence of an association between poor oral health and a number of clinically important medical conditions, such as cardiovascular disease, type 2 diabetes, respiratory disease, kidney disease and adverse pregnancy outcomes (Newnham et al., 2009; Shub et al., 2009; Ford et al., 2010). The therapeutic goal is to remove bacteria that is responsible for the infection by both mechanical cleaning and the application of antimicrobial agents, such as tetracycline, 5-nitroimidazole derivatives, clindamycin, ofloxacin, etc (Perioli et al., 2004). Tetracyclines, in particular doxycycline, are used extensively in the treatment of periodontal disease, but the development of bacterial resistance has led to a preference for the use of 5-nitroimidazole derivatives which are very selective agents against anaerobic bacteria (Patel et al., 2008).

Metronidazole (MEZ) is the first generation member of 5-nitroimidazole derivative with bactericidal activity against most anaerobic bacteria and protozoa. It is now used as the principal treatment for *Helicobacter pylori* infections, amebiasis, giardiasis, trichomoniasis, bacterial vaginosis, Crohn's disease and as a prophylactic antibiotic in surgical interventions. It is also a widely used antibacterial compound in the treatment of some types of periodontal diseases. MEZ, one of the most globally used drugs, has been used for over 43 years. It is among the top 100 most frequently prescribed drugs in the US and among the top 10 during pregnancy (Akay et al., 2003).

Tinidazole (TNZ) and Ornidazole (ONZ) are both second and third generation members of the 5-nitroimidazole groups with selective activity against anaerobic bacteria and protozoa, such as *Tichomonas vaginalis*, *Entamoeba histolytica* and *Gardia lamblia*. They can also be used effectively against metronidazole-resistant strains of *T. vaginalis* and recurrent periodontitis (Mombelli et al., 2005; Zhang et al., 2006). Tinidazole has been widely used in Europe and developing countries for over two decades with established efficacy and acceptable tolerability (Fung and Doan, 2005). Tinidazole is also effective in the treatment of respiratory tract infections, intra-abdominal sepsis, obstetrical and gynecological infections, colonic and abdominal surgery, emergency appendectomy and amebic liver abscess, etc (Austin et al., 2006; Bakshi and Singh, 2004).

With the development of bacterial resistance, there is an increasing need for R&D of new 5-nitroimidazole derivatives as very selective agents against anaerobic bacteria. Morinidazole (MNZ) (Figure 1) is a new synthetic bacterial infections, such as periodontal diseases and gynecological diseases. It is currently in Phase III Clinical Trials (Lv, 2010). The aim of the present study is to evaluate and compare the saliva concentrations and therapeutic effects of MNZ to ONZ in patients with periodontitis after i.v. infusion administration.

Ethical approval

Our study was approved by the Ethics Committee of the first affiliated Hospital with Nanjing Medical University, Nanjing, China.

MATERIALS AND METHODS

Study population and study groups

The study population consisted of 142 outpatient subjects (80 women and 62 men) attending the Department of Periodontics in the first affiliated hospital with Nanjing Medical University and the first affiliated hospital with Shanghai Jiao Tong University, China. Written consent was obtained from those who agreed to participate voluntarily and ethical clearance was obtained from the Hospital's ethical committee (Table 1). Periodontal disease was defined as the presence of periodontal pockets ≥ 4 mm in depth at 12 or more probing sites in fully erupted teeth (typically excluding wisdom teeth) (Hart et al., 2012). Patients with a habit of smoking, gross oral pathology, heart diseases, respiratory diseases, tumors or any other systemic disease, which can alter the course of periodontal disease, were excluded. Furthermore patients on any medication like phenytoin, cyclosporins, anti-inflammatory, antibiotics, or who had received periodontal therapy in the preceding 2 weeks, as well as pregnant and lactating females, were excluded from the study. Each subject underwent a full mouth periodontal probing and charting, along with the periapical radiographs.

Experimental protocol

The study was conducted in a double-blind fashion in which patients were randomly assigned to one of the two antimicrobial treatment groups. Certain rules were defined at the outset, and these were followed throughout the study. No patient would receive other systemic or local antimicrobial treatments about individual teeth. After the completion of the necessary scaling and root planning, the patients would receive intravenous infusions of either Ornidazole or Morinidazole, twice daily for 5 days. The dosage was both 500 mg each time, and the infusion period was 50 min. The packaging and labeling of the various medications were performed by persons who were not involved in the treatment or clinical decision-making. These medications were provided in coded fashion to the clinical team so as to maintain the double-blind nature of the study.

Collection of saliva

Saliva (1.0 ml) was collected from the gingival crevice fluid by aspiration through a capillary micropipette into 1.5 ml tubes prior to and again after finishing the first i.v. dose of Ornidazole or Morinidazole at intervals of 15, 30, 45, 60, 90 min on day 1. After collection, the saliva samples were placed immediately on ice and aliquoted before freezing at -70°C until analyzed.

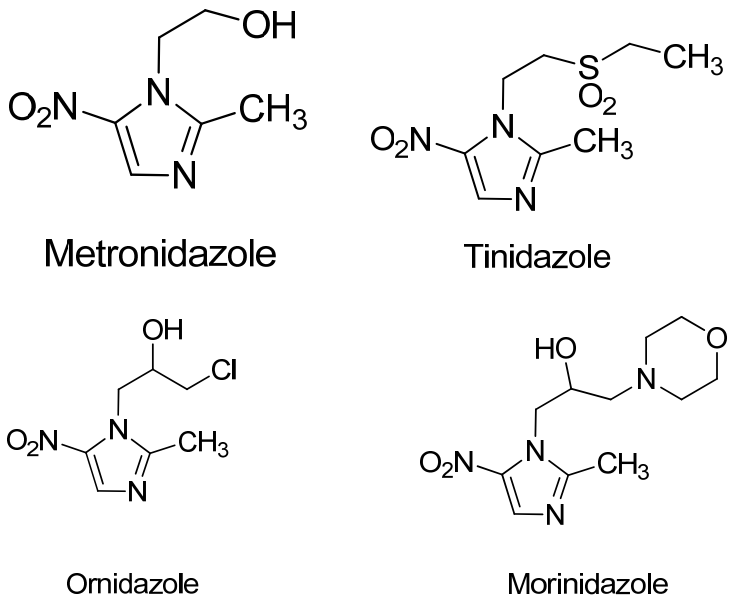

Figure 1. Chemical structure of metronidazole (MEZ, a), tinidazole (TNZ, b), ornidazole (ONZ, c) and morinidazole (MNZ, d).

Table 1. Demographic characteristic of treatment groups.

Characteristic		Morinidazole (n=71)	Ornidazole (n=71)
Age (years)		36.1±12.2 (20-61)	34.9±11.6 (23-65)
Gender	Men	30	32
	Women	41	39
Height (cm)		166.9±7.4 (155-183)	165.8±8.1 (154-181)
Body Weight (kg)		59.0±10.0 (46-85)	58.6±10.5 (47-81)
Body mass index (BMI)		21.1±2.7 (17.3-27.5)	22.1±2.5 (18.0-28.7)

Determination of ornidazole or morinidazole in saliva samples

Morinidazole or ornidazole saliva levels were measured by high-performance liquid chromatography (HPLC) with UV detection. Morinidazole (Purity 99.6%, Lot 20050805) was supplied by Hansoh Pharmaceutical Group, China. TNZ was used as the internal marker for each sample (ONZ and TNZ were supplied by the National Institute for the Control of Pharmaceutical and Biological Products, China). The assay was performed as follows: A 100 µl aliquot of human saliva sample was transferred to a 1.5 ml test tube into which 400 µl methanol was spiked. After vortex for 2 min and then centrifuge at 15,000 rpm for 10 min, the supernatant was transferred to an autosampler vial and 20 µl was injected into the HPLC system (LC-2010-CTH; Shimadzu, Kyoto, Japan) equipped with a Kromasil 100-5 C18 column (250 × 4.6 mm, 5 µm, Sweden). The mobile phase consisted of potassium dihydrogen phosphate (0.02 mol/l), acetonitrile and methanol (55:15:30, v/v/v, pH4.51). The flow rate was 1.0 ml/min, and the detector wave was set at 318 nm. Retention time was approximately 4.0, 4.5 and 5.6 min for TNZ, MNZ and ONZ, respectively. The peaks of interest were well separated and free from interference of endogenous substances. Calibration curves were plotted over the concentration range of MNZ (25.4 to 5080.0 ng/ml) and ONZ (25.0 to 5000.0 ng/ml). Both of the intra- and inter-batch relative standard deviations (RSD) were less than 10.0%.

Bacteriological studies

Bacteriological studies were undertaken to determine the effectiveness of MNZ or ONZ *in vitro*, which was performed as follows: *Peptostreptococcus anaerobius*, *Veillonella parvula*, *Actinomyces dentocariosus*, *Porphyromonas gingivalis*, *Bacteroides thetaiotaomicron* and *Prevotella melaninogenica* were maintained under anaerobic conditions at 37°C for 48 h. Five to 10 well-isolated colonies were removed from brain heart infusion (BHI) plates, transferred to 5 ml of BHI broth, vortex, and incubated at 37°C for 48 h under anaerobic conditions (80% nitrogen, 10% hydrogen, and 10% carbon dioxide). Then, the cell suspension was standardized to match the turbidity of the 1.0 McFarland Standards by using a

Table 2. Minimum inhibitory concentration (MIC) of MNZ and ONZ to several anaerobic bacteria.

Species of anaerobic bacterium	Morinidazole (mg/L)	Onidazole (mg/L)
Peptostreptococcus anaerobius (n=2)	0.25-1.0	0.25-1.0
Veillonella parvula (n=4)	0.31±0.13 (0.25-0.5)	0.31±0.13(0.25-0.5)
Actinomyces dentocariosus (n=1)	0.25	0.25
Porphyromonas gingivalis (n=1)	2.00	2.00
Bacteroides thetaiotaomicron (n=1)	4.00	4.00
Prevotella melaninogenica (n=1)	0.5	0.5

Table 3. The concentration of MNZ and ONZ in saliva after finishing a 50-min period i.v. infusion administration at the following time point of 15, 30, 45 and 60 min.

Time (min)	Concentration (ng/ml)	
	MNZ (n=34)	ONZ (n=29)
15	4267.5±2307.2	4108.1±2388.1
30	4199.1±2224.9	3759.8±2492.9
45	3972.3±2155.1	3654.4±2203.7
60	3842.0±2421.3	3428.7±2398.7

microscan turbidity meter (LeCorn et al., 2007). The resulting suspensions contained approximately 3 × 10^8 colony forming units/ml per sample. Four-millimeter thick Brucella agar plates supplemented with 5% defibrinated sheep blood, 1% vitamin K, and 0.5% hemin were inoculated by streaking a cotton swab, previously submerged in the bacterial suspension, over the entire agar surface. This procedure was repeated twice, rotating the plate 60° each time to ensure even distribution of the inoculum. Lastly, the rim of the agar was swabbed. After 10 min, antimicrobial test strips were positioned in the center of the plates. The plates were inverted and incubated anaerobically at 37°C for 48 to 72 h. The MIC was measured by two independent investigators.

Statistical analysis

All the data was analyzed using a software program (SPSS version 10.5, SPSS Inc., USA). The differences before and after each treatment regimen were also compared across the two treatment groups by one-factor analysis of variance (ANOVA). Paired t-test was used to compare MNZ or ONZ concentrations in saliva, which pair differ significantly at 5% level of significance.

RESULTS

Clinical trial results

After administration of the drugs, the main index of the clinical cure rate in full analysis set (FAS) of MNZ and ONZ groups was 92.9 and 91.5%, respectively. Total anaerobic bacteria clearance of MNZ and ONZ was 100.0 and 91.3%, respectively. The full recovery rate of MNZ and ONZ was just the same as that of total anaerobic bacteria clearance. The minimum inhibitory concentration (MIC) of MNZ and ONZ to several anaerobic bacteria is shown in Table 2. There is no significant difference between MNZ and ONZ in clinical cure rate, as well as total anaerobic bacteria clearance and also the full recovery rate. After administration of the drugs, the main index of the clinical cure rate in per protocol set (PPS) of MNZ and ONZ groups were both 100.0%. Total anaerobic bacteria clearance of MNZ and ONZ were 100.0 and 91.3%, respectively. The full recovery rate of MNZ and ONZ were both 100%. The result of PPS was kept in accordance with that of FAS.

Concentration of MNZ and ONZ in saliva

After finishing a 50 min period i.v. infusion of MNZ and ONZ for 15 min, both of them reached almost the same saliva level at more than 4100 ng/ml, then the concentration dropped moderately. The mean saliva concentration of MNZ was a litter higher than that of ONZ, but there was no significant statistical difference between them (Table 3).

DISCUSSION

Periodontal diseases are groups of infections and inflammatory conditions, including gingivitis and periodontitis that affect teeth-supporting structures. These diseases occur when bacteria from dental plaque invade

the surrounding tissues and from the accumulation of plaque at the gingival margin which induces an inflammatory response (Hart et al., 2012). Periodontal diseases are one of the many potential risk factors for systemic conditions and can affect their onset and progression by various mechanisms (Ebersole, 2003; Katancik et al., 2005). It has proved that there is a moderate association between cardiovascular diseases and periodontal diseases (Beck and Offenbacher, 2005). The risk factors for systemic diseases and the initiation or progression of these medical conditions can be reduced by the use of general medicine. The use of antimicrobials in the treatment of periodontal infections should be based on clinical symptoms and subsequent bacteriologic diagnosis. The systemic antimicrobial agents are used only when the clinical condition indicates that access surgery is a likely treatment procedure (Pähkla et al., 2005). Morinidazole has a broad spectrum for anaerobic bacteria and penetrates well into gingival crevice fluid, which makes it the drug of choice for anaerobic periodontal infections. The present findings confirm that morinidazole shows a satisfactory cure rate after 5 days of treatment. The concentration of MNZ in crevice fluid is about 38.7% to the peak plasma concentrations after been given by same i.v dosage infusion for 50 min, reaching a saliva level of more than 4100 ng/ml, which can inhibit or kill the *Bacteroides thetaiotaomicron* (the MIC of *B. thetaiotaomicron* was 4000 ng/ml). We find that many anaerobic bacteria are sensitive to the MNZ or ONZ, such as *Peptostreptococcus anaerobius*, *Veillonella parvula* and *Actinomyces dentocariosus*. All of them have the same MIC of 250 ng/ml, which is far below the peak saliva concentration of MNZ and ONZ. Clinical resistance of anaerobes to morinidazole is extremely rare.

In this study, the concentration of MNZ and ONZ in saliva reached peak levels and steady for about 45 min after finishing a 50 min period i.v. infusion and the variability of the concentrations is very large. The big variability of MNZ or ONZ concentration in saliva within the patients can be attributed to the different secretion status of individual patients at the time of collection of saliva sample. The cure rate of morinidazole is slightly higher than that of ONZ, as the result of concentration in saliva, but no significant differences between the two agents are apparent.

An antimicrobial dose of MNZ at 500 mg has been found to be both safe and effective when given twice daily over 5 days in randomized controlled clinical trials. The incidence rate of adverse events occurring in MNZ or ONZ groups is 5.6 and 2.8%, respectively. All the adverse events are mild, and all subjects complete the trial. The main adverse effects relate to the drugs are dizziness, drowsiness and nausea, etc.

The system used to deliver the drug should maintain the concentration of antibiotic for sufficient duration to reduce the anaerobic population in the subgingival ecosystem. Reduction in the Gram-negative anaerobic populations is frequently accompanied by an increase in aerobic or microaerophilic species such as *Streptococci* and also *Actinomyces* species. It is important that we should pay more attention to this phenomenon during the next clinical trials and clinical application. By greater integration of medicine and dentistry, dentists can take more responsibility for the management of their patients' systemic health and conversely the physicians can assume a more active role in their patients' oral health (Pradeep et al., 2009).

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Conflict of interest

The authors have no conflicts of interest to disclose.

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