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FACULDADE DE ODONTOLOGIA DE PIRACICABA

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AVALIAÇÃO CLÍNICA DO METRONIDAZOL EM FORMULAÇÃO GEL E COMPRIMIDO EM FUMANTES COM PERIODONTITE CRÔNICA.

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, no Programa de Pós-Graduação em Odontologia para obtenção do título de Doutora em Odontologia - Área de Farmacologia, Anestesiologia e Terapêutica.

Orientador: Prof. Dr. Francisco Carlos Groppo

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RESUMO

Foram objetivos deste estudo: 1) comparar o efeito das formulações (gel e comprimido) de metronidazol (Mtz) sobre o debridamento periodontal (DP) em pacientes fumantes; 2) comparar as concentrações plasmáticas (CP) e salivares (CS) destas formulações; 3) determinar a farmacocinética do Mtz em comprimido; e 4) avaliar o efeito do fumo na biodisponibilidade do Mtz comprimido. Cada objetivo constituiu um capítulo do estudo. **Capítulo 1**: 30 fumantes com periodontite crônica foram aleatoriamente divididos em 3 grupos: DP associado com 3 g de gel placebo; DP associado com aplicação tópica diária de 3 g de gel de benzoato de Mtz e; DP associado com dose oral única diária de 750 mg de Mtz (Flagyl®). Foram avaliados: Índice de Placa (IP), Índice de Sangramento Gengival (ISG), Profundidade à Sondagem (PS) e Nível Clínico de Inserção relativo (NIC); nos tempos: pré-operatório, baseline, e após 30, 90 e 180 dias do tratamento periodontal. Nenhuma diferença significante foi observada entre os grupos para todos os parâmetros e tempos avaliados. Houve uma significante redução no ISG, PS e NIC em todos os tempos comparados ao baseline ($p<0,05$). **Capítulo 2:** 13 voluntários sadios receberam aleatoriamente dose oral única de 750mg de Mtz ou 3g de gel Mtz (15%). Amostras de plasma e saliva foram colhidas em diferentes tempos após administração. Cromatografia Líquida de Alta Eficiência (CLAE) foi usada para quantificar as CP e CS do Mtz. Os parâmetros farmacocinéticos concentração máxima (C_{max}), área sob a curva de zero ao infinito (ASC_{0-inf}), área sob a curva de zero a t (ASC_{0-t}), volume de distribuição (VD) e clearence renal (CL) foram determinados. As CP do Mtz foram maiores que as CS nos períodos de 6 a 24 horas para o comprimido ($p<0,05$). Os parâmetros ASC_{0-inf} e ASC_{0-t} foram maiores no plasma que na saliva ($p<0,05$). As concentrações plasmáticas e salivares foram similares para a formulação gel ($p>0,05$). **Capítulo 3:** 13 fumantes (F) e 13 não-fumantes (NF) receberam dose oral única de 750mg Mtz. Amostras de plasma e saliva foram colhidas em diferentes tempos após administração. A CLAE foi usada para quantificar as CP e CS do Mtz. Os parâmetros farmacocinéticos AUC, C_{max}, T_{max}, VD e CL foram determinados. Foram observadas redução significante nas concentrações plasmáticas no grupo F comparada ao NF em 1, 1,5 e 2 horas após a administração e na C_{max} plasmática ($p<0,05$). Nenhuma diferença foi observada entre os grupos na concentração e nos

parâmetros farmacocinéticos do Mtz em saliva (p>0,05). **Conclusão geral:** 1) Não houve vantagem clínica no uso do Mtz associado ao DP; 2) A formulação em gel produziu igual disponibilidade de Mtz no plasma e na saliva; 3) Alguns dos parâmetros farmacocinéticos do Mtz foram maiores no plasma que na saliva para o comprimido; 4) O fumo interferiu apenas na biodisponibilidade plasmática do Mtz.

***Palavras-chave**:* periodontite crônica, metronidazol, fumantes, farmacocinética, avaliação clínica, fumo e biodisponibilidade.

ABSTRACT

The aim of this study were 1) to compare the effect of metronidazole (Mtz) gel and tablet on debridement periodontal (DP) in smokers; 2) to compare Mtz gel and tablet concentrations in both blood plasma and saliva; 3) to determine the pharmacokinetic profile of Mtz tablet; and 4) to verify the effect of cigarette smoking on bioavailability of Mtz tablet. This study was divided in three chapters. **Chapter 1**: 30 patients smokers with chronic periodontitis were randomly assigned into 3 groups: PD combined with 3 g placebo gel; PD combined with daily topical application of 3 g Mtz benzoate gel (15%); and PD combined with a daily single dose of 750 mg Mtz (Flagyl®). Clinical parameters evaluated were visible plaque index (VPI), gingival bleeding index (GBI), probing pocket depth (PPD) and relative attachment level (RAL) which were assessed preoperatively, baseline, and after 1, 3 and 6 months after PD. No significant difference was observed among the groups, considering all parameters tested ($p>0.05$). In all groups was observed a significant reduction in GBI, PPD and RAL, at all times compared to baseline ($p<0.05$). **Chapter 2**: 13 volunteers randomly received 750 mg single oral dose FLagyl® and 3 g Mtz benzoate gel (15%). Blood and saliva samples were collected in different times after gel application and oral administration of Mtz. High-performance liquid chromatography (HPLC) was used to quantify plasmatic (PC) and salivary (SC) concentrations of Mtz. Pharmacokinetic parameters determined were: the highest concentration (C_{max}), the time at which C_{max} ocurred (T_{max}), the area under concentration-time curve from zero to infinity ($AUC_{0-\infty}$), the area under concentration-time curve from zero to t (AUC_{0-t}), distribution volume (VD) and renal clearance (CL). Plasma showed higher Mtz concentration from 6 to 24 hours after drug administration and the highest values concerning T_{max}, AUC_{0-48h} and $AUC_{0-\infty}$ than those obtained in saliva ($p<0.05$). No significant difference was observed between SC and PC for Mtz gel considering all periods tested ($p>0.05$). **Chapter 3:** 13 smokers (S) and 13 non-smokers (NS) received a single oral dose of 750 mg Mtz tablet. Blood and saliva samples were collected in different times after oral administration of Mtz. HPLC was used to quantify plasmatic and salivary Mtz concentrations. Pharmacokinetic parameters (ASC, C_{max}, T_{max}, VD and CL) were determined. A significant reduction in plasmatic Mtz concentration was observed in S compared to NS at 1, 1.5 and 2 hours after administration

and in C_{max} to plasma ($p<0.05$). No significant difference was observed in Mtz concentration and pharmacokinetic parameters in saliva ($p>0.05$). **Conclusions:** 1) Mtz did not improve the clinical outcomes provided by PD alone; 2) Gel and tablet formulations had similar Mtz bioavailability in plasma and saliva; 3) Some pharmacokinetic parameters were higher in plasma than in saliva concerning Mtz tablet. 4) Smoking interfered with the plasmatic Mtz bioavailability but not the salivary.

Key words: chronic periodontitis, metronidazole, smokers, pharmacokinetic, clinical evaluation, cigarette and bioavailability.

SUMÁRIO

1. INTRODUÇÃO

A etiologia da doença periodontal envolve principalmente dois fatores: um hospedeiro susceptível e a presença de bactérias patogênicas. O processo da doença se desenvolve quando há uma quebra do equilíbrio existente entre resposta do hospedeiro e o desafio microbiano (Quirynen *et al.*, 2001).

Os microrganismos que causam a periodontite são capazes de agir diretamente no tecido periodontal ou de modificar a resposta do hospedeiro (Liebana *et al.*, 2004). Três importantes passos têm sido definidos na patogênese da doença periodontal: 1) início do processo inflamatório por um biofilme microbiano; 2) resposta imuno-inflamatória ao biofilme e seus produtos e; 3) destruição do tecido conjuntivo e osso que constituem o periodonto (Novak *et al.*, 2008).

A estrutura dental é uma superfície sólida adequada para a colonização de um grande grupo de bactérias patogênicas e não-patogênicas. A doença periodontal é sítio-dependente e deixa uma gravação histórica do dano ocorrido no periodonto na forma de perda de inserção e de osso alveolar. O aumento na profundidade de sondagem e no número de sítios comprometidos indica tendência à perda de inserção subseqüente (Socransky & Haffajee, 1997).

O tratamento periodontal consiste na remoção do biofilme dental e do cálculo dental da superfície radicular, promovendo um quadro aceito como saúde periodontal, obtido por meio da adesão epitelial sobre a superfície radicular (Waerhaug, 1978; Mullally *et al.*, 2008).

Na terapia periodontal convencional, a instrumentação mecânica é realizada por sextantes ou quadrantes em intervalos de 1 a 2 semanas, de maneira que o tratamento é concluído dentro de 4 a 6 semanas (Koshy et al., 2005). A raspagem e o alisamento radicular (RAR) são mecanismos eficazes no tratamento da doença periodontal, entretanto, em alguns casos, não é capaz de devolver ou manter a saúde periodontal, o que pode ser explicado pela recolonização de microrganismos em áreas já tratadas (Drisko, 1998). Os sítios não tratados assim como a mucosa jugal, a língua e a saliva podem contribuir para a

re-infecção das bolsas periodontais e explicar, em alguns casos, a resposta insignificante da terapia antibacteriana (Wong *et al.,* 1999).

A desinfecção da boca toda em estágio único consiste na instrumentação mecânica em duas sessões dentro de 24 horas e aplicação de clorexidina em todos os nichos interbucais (Quirynen *et al.*, 2001). A vantagem deste tratamento está na prevenção da recolonização de áreas já tratadas e na redução, em curto período de tempo e de maneira eficiente, da quantidade de bactérias subgengivais e em outros nichos intrabucais (Quirynen *et al*., 1995; Quirynen *et al.*, 1999; Mongardini *et al.*, 1999).

Jervoe-Storm *et al*. (2006) não observaram diferenças entre o tratamento convencional e a desinfecção de boca toda sem o uso da clorexidina (2 sessões de 2 horas cada, dentro de um período de 24 horas). Kosh *et al.* (2005) verificaram que a redução no tempo da instrumentação da boca toda com ultra-som (sessão única de 2 horas) não apresentava prejuízos nos resultados clínicos comparados à terapia convencional. Estes estudos contribuíram para dar suporte a uma nova abordagem da terapia periodontal não-cirúrgica, o debridamento da superfície radicular.

O debridamento periodontal é uma instrumentação radicular leve, mais conservadora, feita em tempo reduzido. Não tem o objetivo de remover todo cálculo e cemento "contaminado" como na RAR, mas tornar a superfície radicular biocompatível para restabelecimento da inserção (Smart *et al.*, 1990).

Os trabalhos de Wennstrom *et al.* (2001), Wennstrom *et al.* (2005) e Zanatta *et al.* (2006) não observaram diferenças clínicas quando comparados os procedimentos de debridamento periodontal em sessão única (duração de no máximo 1 hora) e RAR, o que permitiu sugerir que a instrumentação subgengival simplificada é efetiva no tratamento da periodontite crônica.

Os agentes antimicrobianos administrados por via sistêmica ou tópica podem aumentar a erradicação dos patógenos subgengivais (Jorgensen & Slots, 2000). A terapia antimicrobiana visa reforçar o tratamento periodontal mecânico e dar suporte às defesas do hospedeiro, inibindo os microrganismos que permanecem após a terapia convencional.

Devido à dificuldade dos procedimentos em eliminar completamente os patógenos e a natureza infecciosa da periodontite, o uso de antimicrobianos têm sido

recomendado como adjunto ao tratamento dos diferentes tipos de doença periodontal (Slots *et al*., 1990; Hitzig *et al.*, 1994; Leiknes *et al.*, 2007). A instrumentação ainda trata a doença periodontal de maneira inespecífica e pode falhar em eliminar periodontopatógenos como *Actinomyces actinomycetemcomitans* e *Tanarella forsythia* (Darby *et al.*, 2001). Estes microrganismos podem escapar do tratamento mecânico devido à sua habilidade em invadir os tecidos periodontais ou residirem em estruturas dentais inacessíveis à instrumentação, como bolsas profundas e áreas de bifurcação (Rudney *et al.*, 2001).

A organização em biofilmes permite que as bactérias sejam menos susceptíveis aos antimicrobianos (Anwar *et al.*, 1992), o que poderia enfatizar a necessidade de associar a terapia mecânica periodontal ao uso dos antimicrobianos. Os agentes antimicrobianos podem ser mais efetivos quando o biofilme dental é inicialmente rompido pelo procedimento mecânico, expondo os patógenos periodontais a estas substâncias (Mullally *et al.*, 2007).

O uso dos agentes antimicrobianos por via sistêmica ou tópica como adjunto a terapia mecânica pode proporcionar benefícios ao tratamento periodontal em algumas situações específicas como na periodontite agressiva, na pobre resposta clínica à terapia mecânica, na doença sistêmica que possa afetar a resistência do hospedeiro (Greenstein *et al.*, 2000; Jorgensen *et al.*, 2005).

Dentre os principais antimicrobianos utilizados no tratamento da periodontite estão o metronidazol (Mtz), as tetraciclinas e a combinação de amoxicilina/Mtz, sendo que esta associação vem sendo utilizada devido o surgimento de cepas de *A. actinomycetemcomitans* resistentes à tetraciclina (Mullally *et al.*, 2007).

O Mtz, antimicrobiano do grupo dos nitroimidazóis, tem ação bactericida por interferir com a síntese do DNA bacteriano. É efetivo contra protozoários e anaeróbios gram-positivos e gram-negativos estritos. É completa e rapidamente absorvido por via oral atravessando barreiras teciduais em altas concentrações, as quais similares ou levemente menores àquelas encontradas no sangue (Spénard *et al.*, 2004). Possui meia-vida de eliminação de aproximadamente 8,5 horas e clearence renal de 1,3 mL/min/kg. É excretado na urina após metabolismo hepático, sendo que apenas 10% da dose absorvida é excretada na forma inalterada (Sprandel *et al.*, 2004).

Os efeitos colaterais ocorrem raramente e dificilmente são graves demais para causar descontinuidade da terapia (Lau *et al.*, 1992). Os mais comuns são: dor de cabeça, náusea, xerostomia, gosto metálico, vômitos, diarréia e dor abdominal. Seu uso em pacientes com doenças neurológicas é desaconselhável devido à possível neurotoxicidade. Embora possa ser utilizado em gestantes durante todos os estágios da gestação sem nenhum efeito adverso aparente, o uso no primeiro trimestre não é aconselhável. Um monitoramento mais cuidadoso deve ser feito em pacientes com doenças hepáticas, alcoólatras ou que fazem uso concomitante com outras drogas que tenham metabolismo essencialmente hepático.

Embora muitos trabalhos tenham demonstrado benefício clínico adicional do Mtz quando adjunto a terapia mecânica no tratamento da doença periodontal (Lopez & Gamonal, 1998; Griffiths *et al.*, 2000; López *et al.*, 2000; Pavia *et al.*, 2004; Carvalho *et al.*, 2005; Moeintaghavi *et al.*, 2007; Novak *et al.*, 2008; Haffajee *et al.*, 2008), pouca atenção tem sido dada à dose ou à duração do tratamento, gerando dúvidas quanto à posologia ideal deste agente. Elter *et al.* (1997) mostraram alguns estudos empregando Mtz, nos quais pôde ser observada a variabilidade na duração do tratamento (entre um e 14 dias) e na dose total (entre 2 e 33,6 g).

A chave para o entendimento da relação antimicrobiano-hospedeiro-bactéria reside no conhecimento de como as propriedades farmacocinéticas e dinâmicas podem afetar a posologia e o uso clínico dos fármacos. Uma vez que a efetividade de um agente antimicrobiano depende da sua concentração e permanência no sítio de ação, é importante correlacionar a concentração do Mtz com sua eficácia terapêutica, com a finalidade de estabelecer a melhor dosagem para o tratamento odontológico (Jansson, 2006).

É conhecido que os fumantes têm maior risco em desenvolver a doença periodontal que os não-fumantes (Thomson *et al.*, 2007) e normalmente respondem menos favoravelmente ao tratamento periodontal (Heasman *et al.* 2006; Johnson & Guthmiller, 2007). Isto ocorre uma vez que o fumo, particularmente o cigarro, provoca alterações na resposta imuno-inflamatória do tecido do hospedeiro e na microvascularização gengival, supressão da função dos fibroblastos e osteoblastos, redução do conteúdo mineral ósseo e do fluído gengival, ações que favorecem a progressão da doença periodontal nestes

pacientes (Rosa *et al.*, 2008). Os metabólitos do cigarro em doses elevadas prejudicam a fagocitose e alteram o padrão de liberação das enzimas neutrofílicas e em baixas concentrações estimulam a quimiotaxia de neutrófilos. Tais alterações da função neutrofílica resultam em prejuízos no controle da infecção periodontal e/ou danos aos tecidos periodontais adjacentes já que são fortemente associados à atividade de reabsorção óssea (Salvi *et al.*, 1997).

Um dos objetivos do presente estudo foi verificar o efeito do Mtz, nas formulações gel e comprimido, sobre o debridamento periodontal em fumantes com periodontite crônica. Este estudo justifica-se devido serem escassos os trabalhos da literatura que avaliaram o efeito do Mtz no tratamento da periodontite crônica em fumantes e não há nenhum estudo na literatura que tenha avaliado o uso deste antimicrobiano na formulação gel ou em dose oral única de Mtz comprimido, particularmente na concentração de 750mg.

No segundo estudo foram determinadas as concentrações plasmáticas e salivares obtidas com o gel e comprimido de Mtz, uma vez que o gel de Mtz, por se tratar de uma nova formulação, não tem informações sobre suas concentrações plasmáticas e salivares. Além disso, informações sobre a farmacocinética destas formulações poderiam ajudar a compreender os resultados obtidos com o estudo clínico anterior.

A literatura mostrou que o fumo pode promover diminuição na biodisponibilidade de alguns medicamentos como irbesartam, fenilbutazona, vecurônio, codeína, morfina, teofilina, clozapina e olanzapina (Zhang *et al.*, 2005; Kroon, 2007; Sweeney & Grayling, 2009). No entanto, não foi encontrado nenhum estudo que tenha avaliado a possível influência do fumo na farmacocinética do Mtz, a qual poderia ser relevante, dada a relação entre fumo e aumento da gravidade da doença periodontal. Diante disso, o terceiro estudo verificou a possível alteração na farmacicinética do Mtz em plasma e saliva quando administrado em fumantes.

2. CAPÍTULOS

Essa tese está baseada na Informação CCPG/002/06/Unicamp que regulamenta o formato alternativo para dissertação e tese e permite a inserção de artigos científicos de autoria ou co-autoria do candidato.

Desta forma, esta tese é composta de 3 artigos, os quais serão ou foram submetidos à publicação, conforme descrito abaixo:

2.1. – Capítulo 1 - Clinical evaluation of metronidazole gel and tablet on one-session full-mouth debridement in smokers with chronic periodontitis.
Será submetido ao Journal of Clinical Periodontology.

2.2. – Capítulo 2 - Determination of plasmatic and salivary concentrations of metronidazole in gel and tablet.
Será submetido ao Journal of Clinical Pharmacy and Therapeutics.

2.3. – Capítulo 3 - Effect of smoking on bioavailability of metronidazole tablet in plasma and saliva.
Foi submetido ao Journal of Periodontology.

Capítulo 1

Clinical evaluation of metronidazole gel and tablet on one-session full-mouth debridement in smokers with chronic periodontitis.

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Abstract

Objective: The aim of this study was to compare the effect of two metronidazole (Mtz) formulations (gel and tablet) on full-mouth periodontal debridement (PD) in smokers with chronic periodontitis. **Methods:** This double-blind, randomized clinical trial involved 30 patients, having at least five teeth with a probing pocket depth (PPD) of ≥ 5 mm. Individuals were randomly assigned into three groups: 1) PD combined with 3 g placebo gel; 2) PD combined with daily topical application (applied by using a dental tray with gel overnight, during 7 days) of 3 g of 15% Mtz benzoate gel; and 3) PD combined with a daily single dose of 750 mg Mtz (Flagyl®), during 7 days. Clinical parameters evaluated was visible plaque index (VPI), gingival bleeding index (GBI), probing pocket depth (PPD) and relative attachment level (RAL) which were assessed preoperatively, after gently supragingival calculus scaling (baseline), and after 1, 3 and 6 months after PD. Kruskal-Wallis test was used to observe intra- and intergroup differences concerning the parameters. **Results:** No statistically significant difference was observed among the groups, considering all parameters tested. A significant reduction was observed for GBI at 3 and 6 months when compared to baseline ($p<0.05$); and for PPD and RAL at 1, 3 and 6 months when compared to baseline ($p<0.05$), for all groups. The average PPD reduction at 6 months were 1.8 (1.5-2.3) mm, 1.8 (1.5-2.4) mm and 2.4 (1.1-2.9) mm; and RAL gain at 6 months were 2.0 (1.7-2.8) mm, 1.9 (1.0-3.0) mm and 1.8 (1.0-3.8) for groups 1, 2 and 3, respectively. **Conclusion:** Mtz (gel and tablet) did not improve the clinical outcomes provided by PD alone in smokers with chronic periodontitis.

Key words: chronic periodontitis, smokers, antimicrobial agents, periodontal debridement.

Capítulo 1

Introduction

The presence of specific microorganisms is a crucial factor in inflammatory periodontal desease; however, it has become apparent that pathogens are necessary, but not sufficient, for disease activity to occur. The progression of the disease is related to host-based risk factors (Kinane & Bartold, 2007).

Previous studies, investigating the relationship between smoking and periodontal destruction, have reported smoking as a risk factor for periodontitis (Calsina et al., 2002; Lorentz et al., 2009). Smokers with periodontitis are known to be more susceptible to bone attachment loss than nonsmokers (Stoltenberg et al., 1993); moreover, a significant reduction in the extent of clinical improvements following periodontal treatment has been found in smokers which could have a less favourable response to mechanical therapies (Mascarenhas et al. 2005; Heasman et al., 2006; Pahkla et al., 2006, Grossi et al., 2007).

Several studies have reported that antimicrobial agents associated with the periodontal therapy might result in better clinical outcomes in smokers (Loesche et al., 1992; Kinane & Radvar, 1997; Söder et al., 1999; Winkel et al., 2001, Preshaw et al., 2005; Pahkla et al., 2006; Grossi et al., 2007; Matarazzo et al., 2008). Such supplemental antimicrobial therapy can be justified by the evidence that smoking and tobacco products directly alter the subgingival microbiota, favoring colonization with periodontal pathogens (Grossi et al., 1997). In addition, antimicrobial agents have the potential to reduce the number of periodontal pathogens in saliva and gingival crevicular fluid (GCF), as well as in sites of difficult mechanical instrumentation access.

Periodontal treatment includes surgical and regenerative techniques, and mechanical removal of supra- and subgingival microbial plaques combined or not with locally or systemically administered antimicrobial agents (Lopez et al., 2006). The main objective of periodontal therapy is to reduce probing pocket depth and bleeding on probing and suppuration, as well as to improve the attachment level (Socransky & Haffajee, 2005; Teles et al., 2006).

Previous studies, comparing full-mouth periodontal debridement (PD) to scaling and root planning (SRP) for sextant, showed similar clinical and microbiological results concerning probing depth, gingival inflammation reduction, and clinical attachment gain in

patients with chronic periodontitis (Greenstein, 2000; Tomasi et al., 2006; Koshy et al., 2005; Del Peloso Ribeiro et al., 2008)**.** Furthermore, PD might prevent bacterial re-infection when compared to SRP, a procedure which could result in a re-infection of the treated areas by pathogens residing in non-treated sites or other intraoral niches (Bollen et al., 1998).

Subgingival microbial plaque is an extremely complex biofilm, protecting the bacteria from antimicrobial attack. Antimicrobial agents can only be effective if the oral biofilm is initially disrupted by mechanical procedures, thus exposing the periodontal pathogens (Mullally et al., 2007).

Metronidazole (Mtz) is commonly prescribed for patients with chronic periodontitis because of its narrow spectrum, mainly against anaerobic microorganisms responsible for the periodontal diseases (Haffajee et al., 2008). Previous reports suggest that this antimicrobial agent, systematically and locally administered, has clinical and microbiological benefits, when compared to periodontal therapies using mechanical procedures alone, in patients with chronic periodontitis (Griffiths et al., 2000; López et al., 2000; Pavia et al., 2004; Carvalho et al., 2005; Haffajee et al., 2007; Moeintaghavi et al., 2007; Haffajee et al., 2008). However, few studies have investigated its antimicrobial effect on periodontal pathogens in smokers. Furthermore, there is no study that evaluated its antimicrobial effects using a new gel formulation as purposed in the present study.

The aim of the present study was to evaluate the effect of Mtz in two formulations (gel and tablet) on PD in smokers with chronic periodontitis.

Materials and methods

Study Design

This parallel, randomized, double-blind study, involving a 6-month follow up, was designed to evaluate the effect of a placebo gel (3 g), 15% Mtz benzoate gel (3 g), and 750 mg Mtz (tablet - Flagyl®) on PD. This study was approved by the Ethical Committee of Piracicaba Dental School - University of Campinas, São Paulo, Brazil (#085/2006). All participants gave written informed consent and were informed of the nature of the study in detail.

Study population

A detailed intra-oral examination was used to select 30 individuals from the periodontics clinic of Piracicaba Dental School, University of Campinas (UNICAMP), considering their medical and dental history. Examinations involved full-mouth periodontal probing and radiographic evaluation.

Inclusion criteria were: 1) diagnosis of chronic periodontitis established by the presence of periodontal pockets with a clinical attachment loss of ≥5 mm, bleeding on probing (BOP), and radiographic bone loss (Flemmig, 1999); 2) at least five teeth with a probing pocket depth (PPD) of ≥5 mm and bleeding on probing; 3) at least 20 teeth in their mouth (third molars excluded); and 4) smoking habits (at least 10 cigarettes per day for the past 4 years).

Exclusion criteria were: 1) periapical alterations in qualifying teeth; 2) medical disorders requiring prophylactic antibiotic therapy or interfering with the treatment; 3) periodontal treatment in the past 6 months; 4) use of drugs known to affect periodontal status (antibiotic, anti-inflammatory, anticonvulsant, immunosuppressant and calcium channel blocker) within the past 6 months; 5) orthodontic therapy; 6) pregnancy and lactation; 7) allergy to Mtz; and 8) systemic diseases (e.g.: diabetes and immunological disorders) that could intensify the natural progress of the periodontal disease.

Alcoholic drinks were refrained during the nine-day treatment (Hersh, 1999). Subjects were instructed to avoid systemic antimicrobial therapy, chlorhexidine mouthrinse, and toothpaste containing antimicrobial agents. Volunteers received the same toothpaste, which they were told to use during the study (Colgate Triple Action, Colgate-Palmolive Ind. Com. Ltda, São Bernardo do Campo, SP, Brazil).

Drugs

Mtz benzoate gel (lot#MB/0607/0046A, Amoli Organics, India), 250 mg MTZ tablet (Flagyl® - Aventis, lot #700277, São Paulo, Brazil), and a placebo gel were purchased in a Farmavip drugstore, São Paulo, Brazil.

The placebo gel (semi-solid suspension) contained carboxymethylcellulose, nipagin, deionized water, sacarine, glycerine and liquid sorbitol. For the Mtz gel, 15% Mtz benzoate was added to the placebo gel. To maximize the contact between the gel formulations and

gingiva, dental trays (Whiteness, Dentscare Ltda, Joinville, Brazil), which are usually used for home dental bleaching, were used to apply the gel in the lower and upper jaws. Trays were extended 4 mm above the gingival margin. The gel was stored in syringe at 8 °C

Clinical Protocol

Patients received detailed information on the etiology of periodontal disease and instructions for self-performed plaque control, including inter-dental flossing and inter-dental tooth brushing. At the first session, patients had removed the retentive factors (caries, excess of restorations, and supragingival calculus) for plaque. Toothpaste (Colgate Triple Action, Colgate-Palmolive Ind. Com. Ltda, Sao Bernardo do Campo, SP, Brazil) was provided for all volunteers during the study period. Baseline measurements were made 30 days after these initial procedures.

Patients were randomly assigned to one of the following treatments groups (n=10):

- G 1: Full-mouth debridement + placebo gel;
- G 2: Full-mouth debridement + Mtz benzoate gel;
- G 3: Full-mouth debridement + Mtz tablet

Full-mouth debridement (PD) in all the subjects was performed by the same periodontist using an ultrasonic device (Profi III, Dabi Atlante, Ribeirao Preto, Sao Paulo, Brazil) in a single session for approximately 1 hour. Subjects were told to use the gel or tablets starting at night on the same day of debridement. In addition, they should wear the trays with gel (G1 and G2) overnight (approximately 7 hours) or take the single oral dose of 750 mg tablets (G3) 2 hours after dinner. All the treatments were kept during seven days. The Mtz or placebo gels were applied in both upper (1.5 g) and lower (1.5 g) dental arches.

One clinician (a calibrated examiner) was responsible for performing the treatment and recording clinical parameters, and a second clinician for providing the subjects with the drugs. The first operator was blinded to the treatment.

Re-assessment examination

All subjects were reassessed by means of an oral maintenance programme comprising supragingival plaque control and reinforcement of oral hygiene instructions, after 1, 3, and 6 months of the periodontal procedure. At the sixth month, sites having PPD higher than 5 mm and bleeding on probing were re-instrumented. All subjects were asked

to report any possible side effect resulting from the treatments in a self-filled questionnaire provided seven days after PD.

Clinical measurement

Supragingival biofilm and gingival inflammation, concerning all teeth, were evaluated using the visible plaque index (VPI) and the gingival bleeding index (GBI), considering four different tooth surfaces: mesial, distal, buccal and lingual (Ainamo & Bay, 1975). An individual occlusal stent made of self-curing clear resin was used to standardize the location and angulation of periodontal probes. The relative attachment level (RAL) was measured from the stent to the bottom of periodontal pocket. All these measurements were taken by using a periodontal probe (Probe UNC 15 Hu-Friedy, Chicago, IL, USA). The clinical parameters were taken at preoperative (first appointment), baseline (30 days after supragingival scaling) and 1, 3 and 6 months after PD.

Drug use compliance

To measure drug use compliance, saliva (5 mL) was collected from patients (G2 and G3) on the eighth day after PD. A previously adapted method (Cássia-Bergamaschi et al., 2006) was used to analyze Mtz in saliva. The samples were submitted to protein extraction using a protein-precipitation solution, resulting in a supernatant with the pH adjusted to 5.4. The salivary drug concentration was then detected by means of high performance liquid chromatography (HPLC) using a mobile phase (elution solution) consisting of an aqueous solution (0.01 M) of sodium monophosphate (pH: 4.7) and acetonitrile (85:15). The chromatographic separations were carried out using a Lichrospher column 100 RP18 (125mm x 4mm x 5µm, Merck, Darnstadt, Germany). The flow rate was 1.0 mL/min and the wavelength was set at 320 nm. A standard stock solution of Mtz was diluted in drug-free saliva (5µg/mL to 0.01µg/mL) to establish the calibration curve and measure the salivary drug concentration. . A randomly accessed salivary concentration was detected in eight day after PD in all subjects evaluated in concentration from 3.19 to 0.26 µg/mL.

Subject's study compliance

Forty-six patients were evaluated and 30 patients who attended the preoperative phase completed the 6-month study. A flow diagram of the study is shown in the Figure 1. Patients were recruited between March 2007 and January 2008. All the 6-month follow-up

visits were completed in august 2008. The data entry of all information and statistical analysis were performed by the same time.

Statistical analyses

A power calculation was performed before the study and indicated that 10 subjects in each group would provide a power higher than 80% to detect a 1 mm difference in clinical attachment level between the groups, considering a 5% of significance level.

Data were submitted to Levene's and Shapiro-Wilk's tests to verify similarity of variances and normal distribution, respectively. Analysis of Variance (ANOVA) was used to compare the demographic factors among the groups. Neither normal distribution nor similar variance was observed; thus, Kruskal-Wallis and Friedmann tests were used to observe inter- and intra group differences concerning GBI, VPI, PPD, and RAL. Chi-Square test was used to verify the proportion of sites presenting RAL gain or PPD improved (≥ 2mm). The analysis was carried out using Software BioEstat 5.0, Mamirauá Institute, Belem, PA, Brazil. The level of significance was set at 0.05.

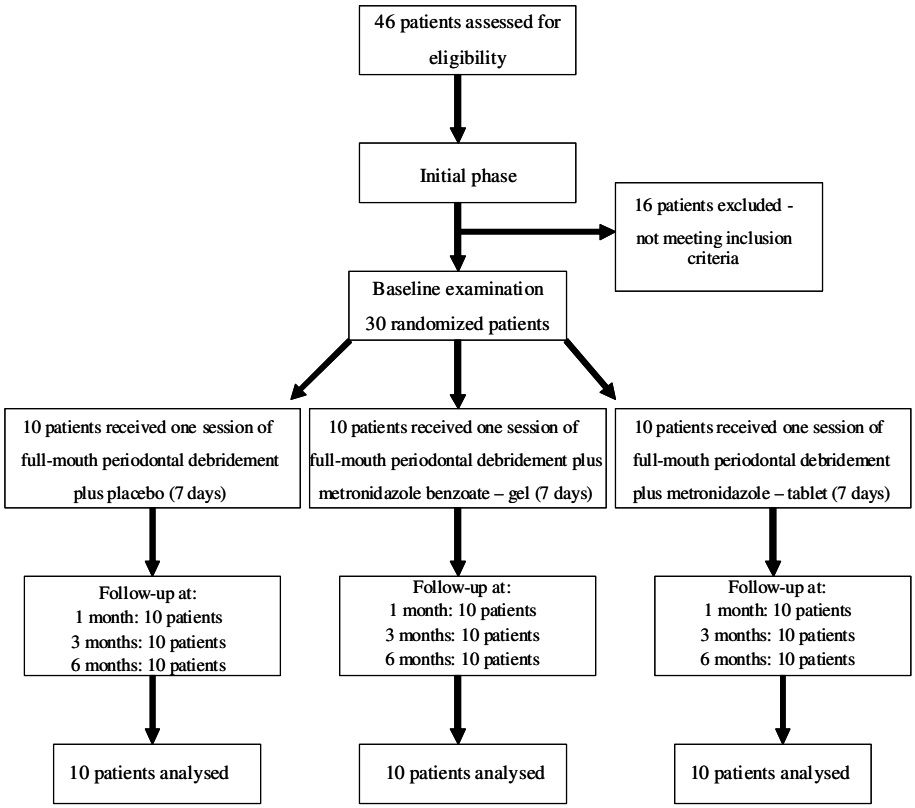

Figure 1. Flowchart of the study.

Results

The demographic data of the patients and full-mouth average values of GBI and VPI at baseline for the treatment groups are presented in Table 1. No statistically significant differences were observed among groups for any parameter listed in Table 1 ($p>0.05$).

Table 1. Mean (±SD) of demographic patient date and clinical parameters at baseline.

Variables	PD + Placebo Gel (n=10)	PD + Mtz Gel (n=10)	PD + Mtz Tablet (n=10)
Gender (male/female)	4/6	4/6	4/6
Age (years)	45.0 ± 5.70	42.7 ± 5.98	45.7 ± 8.30
Number of smoking/day	16.7 ± 4.47	13.8 ± 5.14	18.1 ± 5.66
% sites PPD ≤ 4 mm	74.7 ± 12.34	77.1 ±16.69	71.6 ± 21.25
% sites PPD 5 to 6 mm	18.56 ± 7.51	24.71 ± 21.42	17.08 ± 9.29
% sites PPD ≥ 7 mm	7.3 ± 5.94	5.8 ± 5.07	9.6 ± 9.51
%VPI at baseline	51.7 ± 24.64	45.8 ± 24.45	44.8 ± 20.13
% GBI at baseline	36.9 ± 19.04	30.3 ± 14.92	34.9 ± 18.37

No significant difference (ANOVA) was observed considering age, number of smoking/day, % PPD.

No significant difference (Kruskal-Wallis) was observed considering GBI and VPI.

Values of VPI and GBI refer to means of the whole mouth.

Periodontal procedure (PD), metronidazole (Mtz), visible plaque index (VPI), gingival bleeding index (GBI) and probing pocket depth (PPD)

Clinical results

VPI and GBI

Changes in GBI and VPI among periods of time and groups are presented in Figure 2. No statistically significant differences among the groups were observed for both GBI and VPI ($p>0.05$). A significant reduction was observed for all groups in mean GBI at 3 and 6 months to baseline ($p<0.05$). At the 6-month, the GBI score was reduced from 36.9% to

25.5% (PD plus tablet), from 30.3% to 22.4% (PD plus Mtz gel) and from 34.9% to 27.9% (PD).

No significant reduction was observed for all groups in mean VPI at 3 and 6 months to baseline ($p>0.05$), however this standard of oral hygiene was maintained, or even slightly improved during the study period.

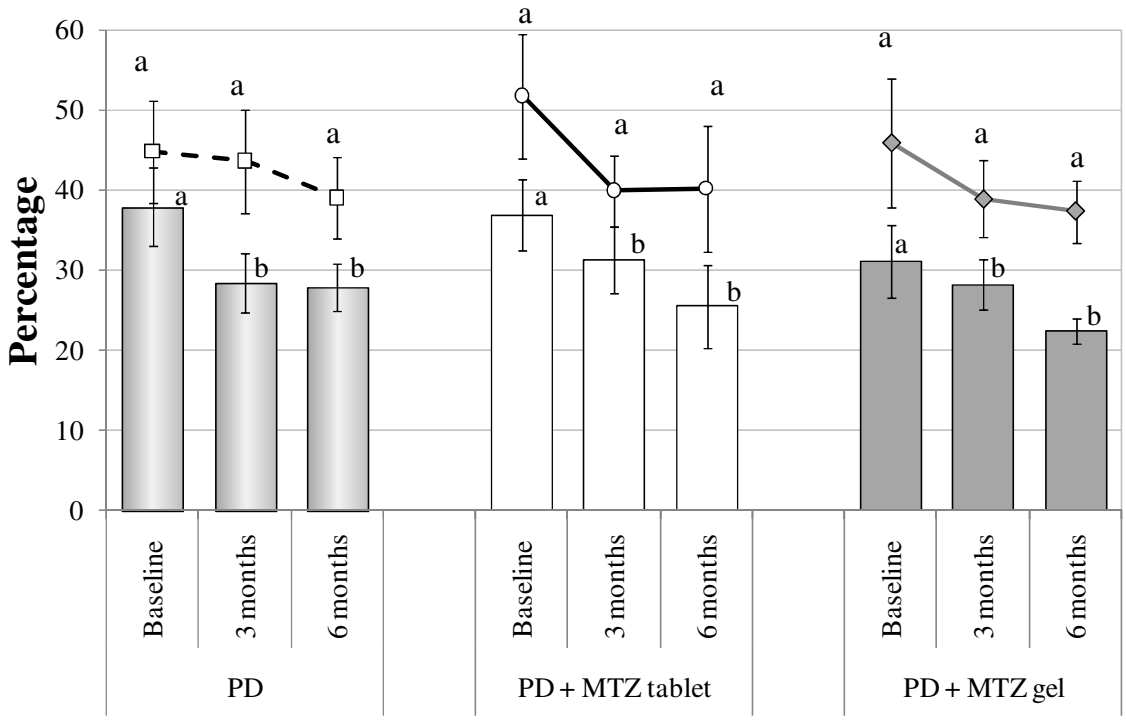

Figure 2. Lines represent visible plaque index – VPI values (mean ± SEM) and bars represent gingival bleeding index – GBI values (mean ± SEM) at the three time intervals for each treatment groups.

Probing Assessments: PPd and RAL of the qualified sites

Figure 3 shows the probing assessments (median – interquartile range) PPD and RAL. PPD and RAL were improved ($p<0.05$) in all the groups, starting from the first month until the last period evaluated. However, no statistically significant differences ($p>0.05$) were observed among the groups considering each period separately.

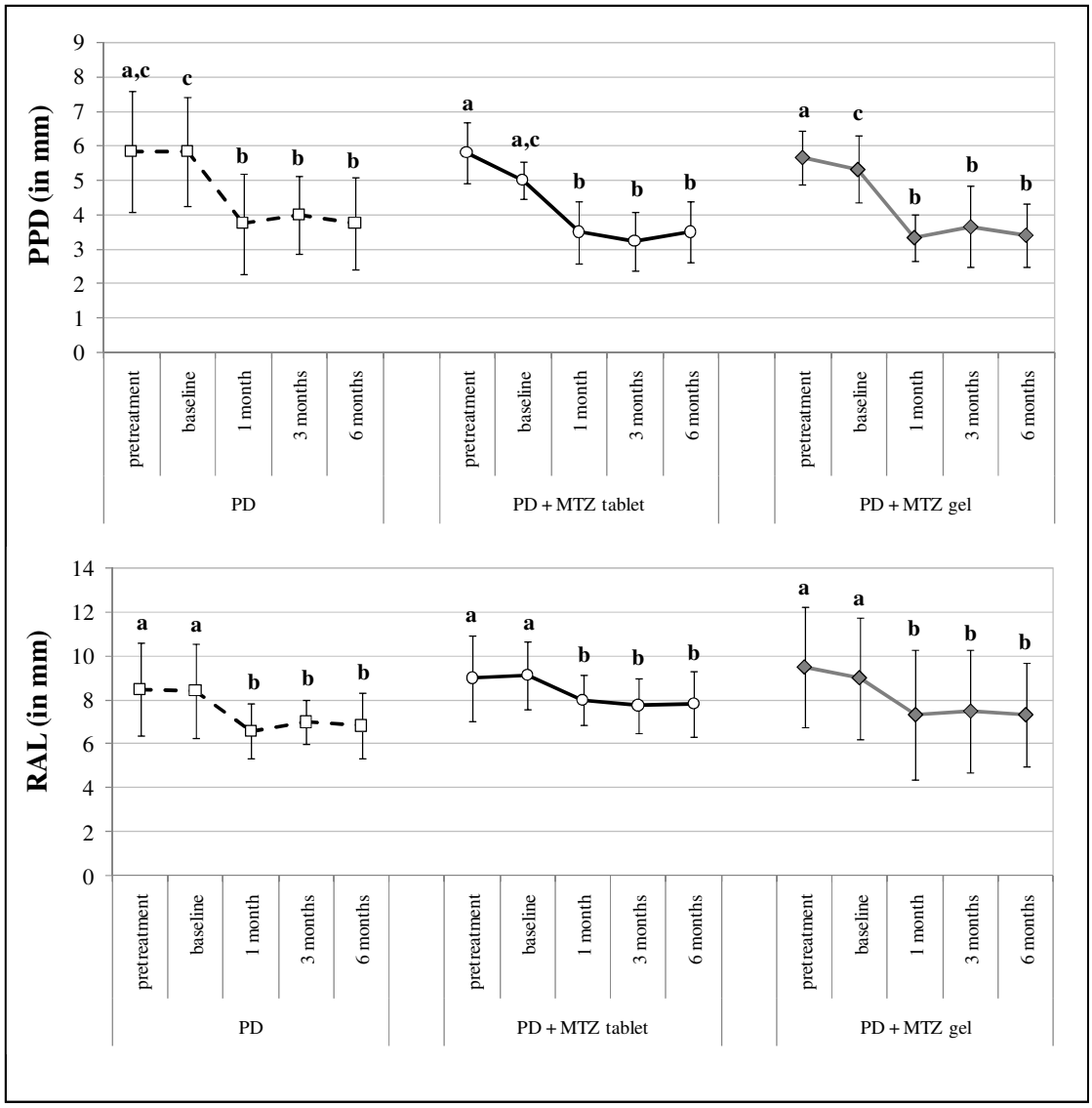

Figure 3. Median values (interquartile range) of PPD and RAL of all groups according to the time periods. Different letters mean statistically significant differences ($p<0.05$) among time intervals and groups.

Table 2 summarizes RAL gain and PPD reduction considering all groups after 1, 3 and 6 months from baseline measurement. Both PPD reduction and RAL gain ($p>0.05$) did not differ among groups or among periods of time. Changes in RAL for each subject at 6 months from the baseline showed that there are no differences among groups. These results are presented in Figure 4.

Table 2. PPD reduction and RAL gain values of all groups after 1, 3 and 6 months from baseline measurement.

	PPD reduction (in mm) Median (1st – 3rd quartiles)			RAL gain (in mm) Median (1st – 3rd quartiles)		
Groups	**1 month**	**3 months**	**6 months**	**1 month**	**3 months**	**6 months**
PD	2 (1.7 – 2.0)	1.8 (1.4 – 2.0)	1.8 (1.5 – 2.3)	1.8 (1.3 – 3.6)	2 (1.2 – 2.8)	2 (1.7 – 2.8)
PD + Mtz Tablet	1.6 (1.5 – 2.8)	1.8 (1.1 – 3.3)	2.4 (1.1 – 2.9)	1.8 (1.4 – 3.3)	1.6 (1.0 – 3.8)	1.8 (1.0 – 3.8)
PD + Mtz Gel	1.6 (1.1 – 1.8)	1.9 (1.7 – 2.6)	1.8 (1.5 – 2.4)	1.6 (1.0 – 3.0)	1.8 (1.0 – 2.8)	1.9 (1.0 – 3.0)

No significant differences were observed intergroup ($p>0.05$) and intragroup ($p>0.05$) to PPD reduction and RAL gain. (periodontal debridement - PD, metronidazole - Mtz, probing pocket depth - PPD and relative attachment level – RAL).

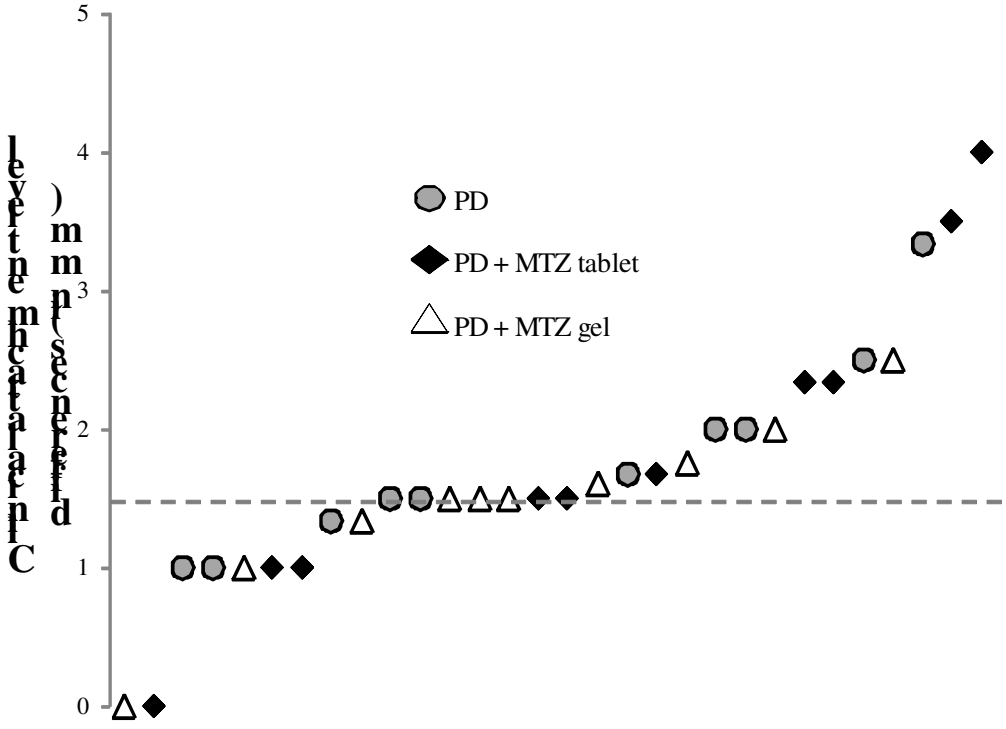

Figure 4. Changes in RAL (in mm) for each subject at 6 months from the baseline.

The probing assessments were further analyzed with respect to proportions of sites showing ≥2mm change PPD and RAL (Table 3). There was no difference between the treatments approaches regarding the proportions of sites presenting a RAL gain or PPD improved (≥ 2mm) at any experimental time (p>0.05).

Treatment Discomfort

All subjects completed the study in good health conditions. No effect adverse was related due to the periodontal procedure. Gastrointestinal intolerance was observed in one subject right after using Mtz tablet (10%). An increase in salivation was observed in eight subjects using gels (40%), and five subjects reported bitter flavor during the use of the Mtz gel (50%). These effects did not interfere in or cause interruption of the treatment, and all subjects reported full adherence to the treatments.

Table 3. Number (percentage) of sites that showing RAL gain (≥2 mm) and PPD reduction (≥2 mm) to groups according to the time intervals.

	PPD Improved (≥ 2 mm)			**RAL Gain (≥ 2 mm)**		
Groups	**1 month**	**3 months**	**6 months**	**1 month**	**3 months**	**6 months**
PD (n=26)	19 (73.1%)	15 (57.7%)	17 (65.40%)	11 (42.3%)	10 (38.5%)	10 (38.5%)
PD + Mtz gel (n=29)	15 (51.7%)	22 (75.9%)	18 (62.1%)	10 (34.5%	11 (37.9%)	10 (34.5%)
PD + Mtz tablet (n= 23)	16 (69.6%)	14 (60.1%)	15 (65.2%)	13 (56.5%)	12 (52.2%)	13 (56.5%)
p values	0.2407	0.0669	0.9698	0.607	0.6585	0.5455

No significant differences were observed (Chi-Square test, p>0.05) towards PPD improvement (≥2 mm) and RAL gain (≥2 mm). (periodontal debridement - PD, metronidazole - Mtz, probing pocket depth – PPD and relative attachment level – RAL).

Discussion

Smokers have been reported to respond to the mechanical periodontal treatment less favorably than non-smokers (Winkel et al. 2001, Darby et al. 2005, Mascarenhas et al. 2005, Heasman et al. 2006, Hughes et al. 2006, Pahkla et al. 2006, Grossi et al. 2007). Also, a higher recurrence of periodontal disease has been associated with cigarette smoke (MacFarlane et al., 1992; Loesche et al., 2002; Quirynen et al., 2006).

In the present study, the choice of Mtz, systemically and topically administered, was based on clinical and microbiological benefits reported in previous studies aimed at the treatment for chronic periodontitis in smokers (Loesche et al., 1992; Soder et al., 1999, Griffiths et al., 2000; Winkel et al., 2001; Pahkla et al., 2006; Matarazzo et al., 2008;) and non-smokers (Elter et al., 1997; Lopez et al., 2000; Pavia et al., 2004; Carvalho et al., 2005, Ehmke et al., 2005; Loesche et al., 2005; Moeintaghavi et al., 2007; Haffajee et al., 2007; 2008).

The literature shows different local antimicrobial delivery systems for treating periodontal pockets using some gel formulations as follows: 5% Mtz with collagen gel (Hitzig et al., 1994); 25% Mtz benzoate with monoglyceride triglyceride gel (Stoltze and Stellfeld, 1992); 15% Mtz benzoate with chitosan gel (Akıncıbay et al., 2006); among others. To our knowledge, the present study is the first to evaluate the effect of 15% Mtz benzoate gel applied by using a custom dental tray system. This tray system was chosen to keep Mtz very close to the gingival and periodontal tissues. In addition, it could maintain a relative concentration for an extended period of time.

Despite the flow rate of the gingival crevice fluid (GCF), which was estimated by Goodson (1989) to be replaced 40 times during one hour in a pocket, the dental tray system could allow a high antimicrobial concentration at the diseased sites. Drugs applied directly into periodontal pockets are easily eliminated due to the crevicular fluid flow, but the contact of gel used in the present study could be maintained during overnight allowing a continuous replacement of the antimicrobial agent into the pocket. Furthermore, a less systemic absorption of Mtz could be achieved. However, the Mtz benzoate, chosen in the present study as the main active compound of the gel, could not have the ideal properties to delivery predictable Mtz concentration into the periodontal pocket.

Mtz benzoate, a benzoic acid ester of Mtz, is generally preferred in oral formulations due to its less bitter taste when compared to Mtz (Baeyens et al., 1998). In the present study, the Mtz benzoate and placebo gel formulations were considered safe for periodontal therapies since no side effect was reported by subjects.

Several studies have investigated systemically Mtz in different oral dosages: 250 mg (t.i.d.) (Loesche et al., 1991); 400 mg (t.i.d.) (Soder et al., 1999; López et al., 2000; Feres et al., 2001; Guerrero et al., 2005; López et al., 2006; Xajigeorgiou et al., 2006); and 500 mg (t.i.d.) (Carvalho et al., 2004; 2005; D'avila et al., 2005). However, this is the first study evaluating the effect of a single oral dose of 750 mg Mtz in the periodontal treatment.

The results of the current study revealed that antimicrobial therapy had similar clinical improvement with that achieved by the full mouth periodontal debridement (PD) alone, considering all parameters evaluated. The magnitude of the clinical improvement to the treatments was similar to the previously published data. Wilkell et al. (2001) reported a PPD reduction of 1.4 mm after 3-months treatment and relative attachment gain ≥2 mm of 25.1% in smokers treated with systemic Mtz (250 mg) plus amoxicillin (375 mg) (t.i.d/8days) after PD. Matarazzo et al. (2008) observed that use of 400 mg (t.i.d.) Mtz tablets for 14 days combined with scaling and root planning (SRP) improved PPD (1.3 mm) and RAL (1.4 mm) after 3-months of periodontal treatment, showing similar values with the ones obtained in the present study.

The traditional approach of quadrant-wise SRP is considered as an effective treatment for periodontal disease (Leiknes et al., 2007). However, a concern regarding microbial recolonization and the level of instrumentation required for periodontal healing exists (Kinane, 2005). During the PD procedure, all teeth are instrumented during a single session for a restricted period of time. Several studies verified that PD resulted in a similar clinical and microbiological outcome compared to SRP (Wennstrom et al., 2005; Koshy et al., 2005; Zanatta et al., 2006; Tomasi et al., 2006; Del Peloso et al., 2008).

Tomasi et al. (2006) and Wennstrom et al. (2005) reported clinical results similar to observed in the present study. Tomasi et al. (2006) reported a similar PPD improved at 6-months after treatment. Wennstrom et al. (2005) verified a mean PPD reduction of 1.80 mm

and RAL gain of 1.2-1.3mm at 3-months after PD in smokers. Similar results too were obtained to PPD improved (≥2mm) and RAL gain (≥2mm).

As observed in the present study, Palmer et al. (1999) also found no additional benefits in the systemic use of Mtz associated wit SRP (200 mg, t.i.d., for 7 days) or in the subgingival application of 25% Elyzol® (Mtz gel) in smokers with moderate or severe chronic periodontitis. Van Winkelhoff et al. (1999) related that the low antimicrobial dose, as used in tablet formulations, could be responsible for the lack of clinical efficacy. In the present study, the dose used (750mg in tablets - or 3g in the 15% benzoate gel) or the duration of the treatments (7 days), could not reached the ideal concentration and time for the bacterial elimination and consequently to obtain additional clinical improvement. PD could be also quite effective in producing clinically improvements, since it was performed by an experienced periodontist, masking the antimicrobial effect.

Previous studies reported that the periodontal treatment associated with Mtz was more effective in deep pockets than shallow pockets (Jenkins et al., 1989; Loesche et al. 1992; 2002; Eisenberg et al., 1991; Noyan et al., 1997; Feres et al., 2001; Carvalho et al., 2004). The volunteers of the present study showed a few percentages of sites with deep pockets ≥ 7mm (5.8 to 9.6%), which could also masked any additional clinical benefit of the antimicrobial therapy.

Smokers that received Mtz associated with periodontal procedure showed no additional clinical benefit of this antimicrobial agent (Leiknes et al., 2007; Jansson et al., 2003; Lie et al., 1998; Palmer et al., 1999). Jansson et al. (2003) observed no difference comparing 25% Mtz gel combined with SRP and SRP alone. Like others, MacFarlane et al., 1992 and Loesche et al., 2002 related highest incidence of the disease recurrence in smokers. The lower reduction of subgingival microbial load following pocket instrumentation (Van Winkelhoff et al., 2001; Van der Velden et al., 2003) and the impaired host response (Labriola et al., 2005; Palmer et al., 2005) are pointed as possible explanation to the reduced effectiveness of periodontal treatment in smokers.

This paper updates the previous 6-months report on the efficacy of topical and systemic Mtz for the treatment of chronic periodontitis. The data showed to be favorable for use PD alone in smokers with chronic periodontitis, since the use adjunct of Mtz tablet

and gel showed the same clinical outcome.

Conclusion

The association of metronidazole (gel or tablet) with periodontal debridment resulted in no improvement in the clinical outcome in smokers with chronic periodontitis, compared to periodontal debridment alone.

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Capítulo 2

Determination of plasmatic and salivary concentrations of metronidazole in gel and tablet.

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Abstract

Objectives: To compare Mtz gel and tablet concentrations in both blood plasma and saliva. In addition, the pharmacokinetic profile of Mtz tablet was determined. **Methods:** In this randomized crossover study with a one-week washout period, 13 volunteers randomly received 1) a single oral dose of 750 mg Mtz (FLagyl® - tablet) and 2) 3 g of 15% Mtz benzoate gel (applied by using a dental tray, overnight). Blood and saliva samples were collected at baseline and 30 min, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 hours after Mtz tablet administration and at 8, 9, 12, 13, 15, 19, 32, and 56 hours after gel application. High-performance liquid chromatography (HPLC) with UV detection was used to quantify plasmatic and salivary concentrations of Mtz. Pharmacokinetic parameters (AUC, C_{max}, T_{max}, VD and CL) of Mtz tablet were determined. Paired Student's t test was used to compare the pharmacokinetic parameters and the plasmatic and salivary concentrations of Mtz. **Results:** The values obtained for AUC_{0-48}, C_{max} and T_{max} regarding Mtz tablet were: 197.8 µg.h/ml (± 47.88); 11.3 µg/ml (± 2.06); 1.96 h (± 0.72) for plasma; and 115.1 µg.h/ml (± 59.36); 11.8 µg/ml (± 7.9); 1.35 h (± 0.55) for saliva. Plasma showed higher Mtz concentration from 6 to 24 hours after drug administration and the highest values concerning T_{max}, AUC_{0-48h} and $AUC_{0-\infty}$ than those obtained in saliva ($p<0.05$). No significant differences were observed between salivary and plasmatic concentrations for Mtz gel considering all periods tested ($p>0.05$). **Conclusions:** Gel formulation had similar Mtz bioavailability in plasma and saliva and the bioavailability of Mtz tablet was higher in plasma than in saliva.

Key words: antimicrobial agents; gel; tablet; pharmacokinetic; plasma; saliva.

Introduction

Metronidazole (Mtz), belongs to the nitroimidazole family, it is active against a wide variety of anaerobic protozoa parasites and anaerobic bacteria (Dilger et al., 2007). It is one of the most widely used antimicrobials in the treatment for periodontal diseases that do not react favorably to the conventional treatment (Pahkla et al., 2005).

Usually, drug dosage schemes are based on empirical data rather than on drug pharmacokinetics for specific purposes (Slots & Ting, 2002). Systemic use of Mtz for periodontal treatment has been evaluated in different oral doses: 250 mg - 3 times/day (Loesche, 1991); 400 mg - 3 times/day (López & Gamonal, 1998, Soder et al., 1999; Feres et al., 2001; Guerrero et al., 2005; López et al., 2006; Xajigeorgiou et al., 2006); and 500 mg - 3 times/day (Carvalho et al., 2004; 2005; D'avila et al., 2005).

Mtz results in excellent oral absorption (Spénard et al., 2004) and oral bioavailability (>90%) is generally reported (Lau et al., 1992; Lamp et al., 1999), with tissue concentrations generally similar to or slightly lower than those measured in the serum (Sprandel et al., 2004). The blood serum protein binding is 10 to 15% (Lamp et al., 1999). This drug undergoes hepatic metabolism resulting in two main oxidative products: hydroxy and acetic acid metabolites (Jessa et al., 1996). Mtz and its metabolites are mostly excreted via renal excretion and up to 77% of the dose is recovered in the urine as unchanged drug (10%) or metabolites (90%) (Sprandel et al., 2004).

The plasmatic and salivary Mtz concentrations were determined by using High-performance liquid chromatography (HPLC) according to techniques previously described (Van Oosten et al., 1986; Jessa et al., 1996; Spénard et al., 2004; Pahkla et al., 2005; Dilger et al., 2007). Knowledge of pharmacokinetic properties of drugs might be useful in understanding how pharmacokinetic parameters can affect the drug regimen and its clinical use (Patsalos, 2004).

The benzoate, chemically a benzoyl ester, is Mtz prodrug (Lahiani-Skiba et al., 2006). Benzoate is generally preferred in oral formulations due to the bitter taste of the Mtz (Baeyens et al., 1998). The use of Mtz benzoate gel, in different formulations, was described in previous studies investigating periodontal diseases (Stoltze and Stellfeld, 1992; El-Kamel et al., 2007; Akncbay et al., 2007). However, these studies observed the Mtz gel

effect applied inside the periodontal pocket. In the present study was described the plasmatic and salivary concentrations of the new formulation of Mtz benzoate gel (15%) to the treatment of chronic periodontitis, that was applied by using a custom dental tray system.

The aim of present study was to compare a new Mtz gel formulation with Mtz tablet, in both plasmatic and salivary concentrations. In addition, the pharmacokinetic profile of Mtz tablet was determined.

Materials and methods

Study Design

This study was conducted in an open, randomized, two-period, crossover balanced design with a one-week washout period between doses. This study was approved by the Ethical Committee of Piracicaba Dental School - University of Campinas, São Paulo, Brazil (#086/2006). All participants gave written informed consent and were informed of the nature of the study in detail.

Subject Population

This study consisted of thirteen healthy male volunteers, aged 18 to 30 years-old (22 ± 1.5 years), weighing 65 to 90 kg (74.5 ± 7.9 kg), and 1.69 to 1.90 m (1.8 ± 0.1 m) in height. The index of body mass was 21.3 to 27.8 kg/m^2 (23.6 ± 2.3 kg/m^2). Volunteers were free from cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, hematological diseases, psychiatric disorders, diabetes, glaucoma and allergy to antimicrobials.

Subjects were instructed to avoid any kind of drug therapy for at least 4 weeks prior to study and up to its completion. They were also refrained from cigarette, and xanthine-containing foods as well as beverages such as tea, coffee, and cola, 48 hours prior to each dosing and until the last blood and saliva sample was collected (Kim et al., 2001). They were also refrained from alcoholic drinks up to a period of 48 hours before and after the last sampling due to risk of drug interaction, as Mtz has the ability to produce disulfiram-like reactions in alcohol consumers (Hersh, 1999).

Drugs

Mtz benzoate gel (lot#MB/0607/0046A, Amoli Organics, India) and Mtz tablet (250 mg, Flagyl® - Aventis, lot #700277, São Paulo, Brazil) were obtained in a Farmavip drugstore, São Paulo, Brazil.

The gel comprised a semi-solid suspension containing 15% Mtz benzoate, carboxymethylcellulose, nipagin, deionized water, sacarine, glycerine and liquid sorbitol. Dental trays (Whiteness, Dentscare Ltda, Joinville, Brazil), which are usually used for home dental bleaching, were used to apply the gel in the lower and upper jaws. Trays were extended 4 mm above the gingival margin. The gel was stored in syringe at 8 °C.

Chemicals

Mtz and the tinidazole (internal standard) were provided by Sigma (St Louis, MO, USA). All reagents were of analytical grade. Acetonitrile were obtained from J.T. Baker Co. (Phillipsburg, NJ, USA). Sodium hydroxide 1N and perchloric acid (70%, w/v) were obtained from Merck (Darmstat, Germany). Distilled water was purified in a Millipore system Milli Q.

Clinical Protocol

Blood and saliva collections were carried out at the ambulatory of Piracicaba Dental School (UNICAMP, SP, Brazil). The volunteers were observed and instructed to inform of any adverse event during the study.

After a 12-hour fasting period, the subjects received a single oral dose of 750 mg Mtz with water (200 mL). Two hours after drug administration, they had a standard breakfast (a cup of peach juice and a 50-g bread loaf with margarine) and were allowed to consume water *ad libitium*. A complete standardized meal (lunch) was allowed four hours after drug administration. Blood samples (5 mL) from antecubital vein via heparinized cannula and saliva samples (3 mL) were collected at pre-dose (0), 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 hours after oral administration of Mtz tablets.

Subjects were told to apply 3 g (1.5 g each jaw) of 15% Mtz benzoate gel (Mtz gel) during the night for 7 hours (midnight to seven am). Blood (5 mL) and saliva (not

stimulated - 3 mL) samples were collected at 8, 9, 12, 13, 15, 19, 32, and 56 hours after gel application. The first collection (8 h) was performed at eight o'clock in the morning.

The blood sample was collected by direct venepuncture into sterile tubes containing K_3EDTA solution. The heparinized saline solution (0.9% NaCl and heparine, 9.8:0.2) injection (0.2 mL) was flushed into the cannula to prevent blood clotting, after each blood sampling. The 24 and 48 hours (Mtz tablet) and 32 and 56 hours (Mtz gel) sampling were obtained using sterile syringe and needle. The saliva samples (3mL) were placed into sterile tubes (15mL). Immediately after each blood and saliva collection, the samples were centrifuged at 3000g for 15min., the plasma and saliva supernatant were placed into microtubes (Eppendorf, Hamburg, Germany) and stored at -70 °C until analysis.

Chromatographic Analysis

Plasmatic and salivary Mtz concentrations were measured by high performance liquid chromatography (HPLC), consisting of a Varian pump (Model 9012), a Varian detector (ProStar 335 DAD), a Galaxie software and a Varian autosampler (ProStar 410). The chromatographic separations were carried out using a Lichrospher column 100 RP18 (125mm x 4mm x 5µm, Merck, Darnstadt, Germany). A previously adapted and validated method (De Cássia-Bergamaschi et al., 2006) was used to analyze Mtz in saliva and plasma.

The saliva samples (500 µL) were submitted to extraction by using 250 µL of a protein-precipitation solution (10% perchloric acid and phosphate-citrate buffer, pH 5.4). The plasma samples (500 µL) were submitted to extraction by using 250 µL of a protein-precipitation solution (20% perchloric acid and phosphate-citrate buffer, pH 5.4).

The samples were vortexed by 30 s, kept at room temperature for 10 min and centrifuged (15.000 g) for 15 min at 4°C. Subsequently, 225 µL of pH-adjusting solution (sodium hydroxide 1 M and buffer phosphate-citrate, pH= 5.4) was added to supernatant (450 µL).

Tinidazole (20 µL of 100 µg/mL), used as an internal standard, was added to samples, which were then eluted with a mobile phase consisting of a 0.01-M aqueous solution of sodium monophosphate (pH= 4.7) and acetonitrile (85:15). The flow-rate was

1.0 mL/min and the wavelength was set at 320 nm. Retention time was approximately 2 and 4 minutes for Mtz and tinidazole, respectively. Standard stock solutions from Mtz concentrations were diluted in drug-free plasma and saliva (50 µg/mL to 0.01 µg/mL, n=9) to achieve the calibration curves. Sample concentrations were calculated by using the regression equation of the straight-line y = ax + b, where 'y' refers to the peak area ratio, 'a' the slope, 'b' the intercept and 'x' the unknown drug concentration.

Statistical and Pharmacokinetic analyses

Computer software (PK Solutions, Non-compartmental Pharmacokinetics Data analysis, Excel Template, 2001, Summit Research Services, Montrose, CO, USA) was used to obtain the following parameters: $\mathbf{C_{max}}$ - the highest concentration observed during the 48-hour study period; $\mathbf{T_{max}}$ - the time at which $\mathbf{C_{max}}$ occurred; $\mathbf{AUC_{0-48}}$ - the area under the plasma concentration-time curve from baseline to 48 h; $\mathbf{AUC_{0-\infty}}$ - the area under the plasma concentration-time curve from baseline to infinity; **CL** - renal clearance; and **VD** - distribution volume.

A power calculation based on the plasmatic and salivary $\mathbf{AUC_{0-48}}$ values (mean and standard deviation) showed that 13 subjects had a power of 95%, considering a significance level of 5%. Paired Student's t test was used to compare the pharmacokinetic parameters of Mtz tablet and the plasmatic and salivary concentrations of Mtz to tablet and gel, at a significance level of 5%. Data analysis was carried out using computer Software (BioEstat 5.0, Mamiraua Institute, Belém, PA, Brazil).

Results

All volunteers completed the study in good health conditions. Metronidazole (Mtz) tablet and gel were well tolerated, since no adverse effects were reported or observed.

The HPLC was sensitive in quantifying Mtz in plasma and saliva (limit of quantification=0.01µg/mL). The method was linear over concentration range 50 µg/ml to 0.01 µg/ml to saliva and plasma. No interfering peaks were registered at the retention times of the drug and internal standard. The assay was validated using seven calibrations curves for plasma and saliva that showed good linearity, since the values of linear regression curve (R^2) were above 0.99.

Table 1 shows mean values for each pharmacokinetic parameter (C_{max}, $T_{max,}$ AUC_{0-48h}, $AUC_{0-\infty}$, VD and CL) concerning plasmatic and (C_{max}, $T_{max,}$ AUC_{0-48h} and $AUC_{0-\infty}$) salivary concentrations involving 13 volunteers, after single oral administration of Mtz. Plasma analysis revealed the highest values concerning AUC_{0-48h} and $AUC_{0-\infty.}$

Mtz was detected in both plasma and saliva 30 minutes after oral administration. Plasmatic Mtz concentration values were higher than those observed for saliva at the period between 6 and 24 hours after drug tablet administration ($p<0.05$) (Figure 1A). There were no statistically significant differences between salivary and plasmatic concentrations of Mtz considering all periods tested for Mtz gel (Figure 1B).

Table 1. Mean (±SD) pharmacokinetic parameters after oral administration of Mtz tablet.

Pharmacokinetic parameters	**Body Fluid**	**Mean arithimetic**	**Confidence intervals (95%)**	***p***
C_{max}	Plasma	11.3 (± 2.06)	9.66 to 12.33	*0.367*
(µg/ml)	Saliva	11.8 (± 7.9)	7.01 to 16.59	
T_{max}	Plasma	1.77 (± 0.72)	1.46 to 3.07	*0.091*
(h)	Saliva	1.50 (± 0.55)	1.01 to 1.68	
$AUC_{(0-48)}$	Plasma	197.8 (± 47.88)	178.89 to 227.41	***0.0001**
(µg.h/mL)	Saliva	115.1 (± 59.36)	79.20 to 150.95	
$AUC_{0-\infty}$	Plasma	210.2 (± 45.69)	187.1 to 239.32	***0.0002**
(µg.h/mL)	Saliva	124.2 (± 64.10)	85.42 to 162.90	
VD	Plasma	625.3 (± 194.65)	511.10 to 798.32	—
(mL/Kg)	Saliva	—		
CL	Plasma	62.5 (± 15.54)	52.30 to 70.39	—
(mL/h)	Saliva	—		

*statistically significant differences (paired t test, $p<0.05$), (-) data not calculated

(C_{max} - the highest concentration observed, T_{max} - the time at which C_{max}; AUC_{0-48} - the area under the concentration-time curve from baseline to 48 h, $AUC_{0-\infty}$ - the area under the concentration-time curve from baseline to infinity, CL - renal clearance, and VD - distribution volume).

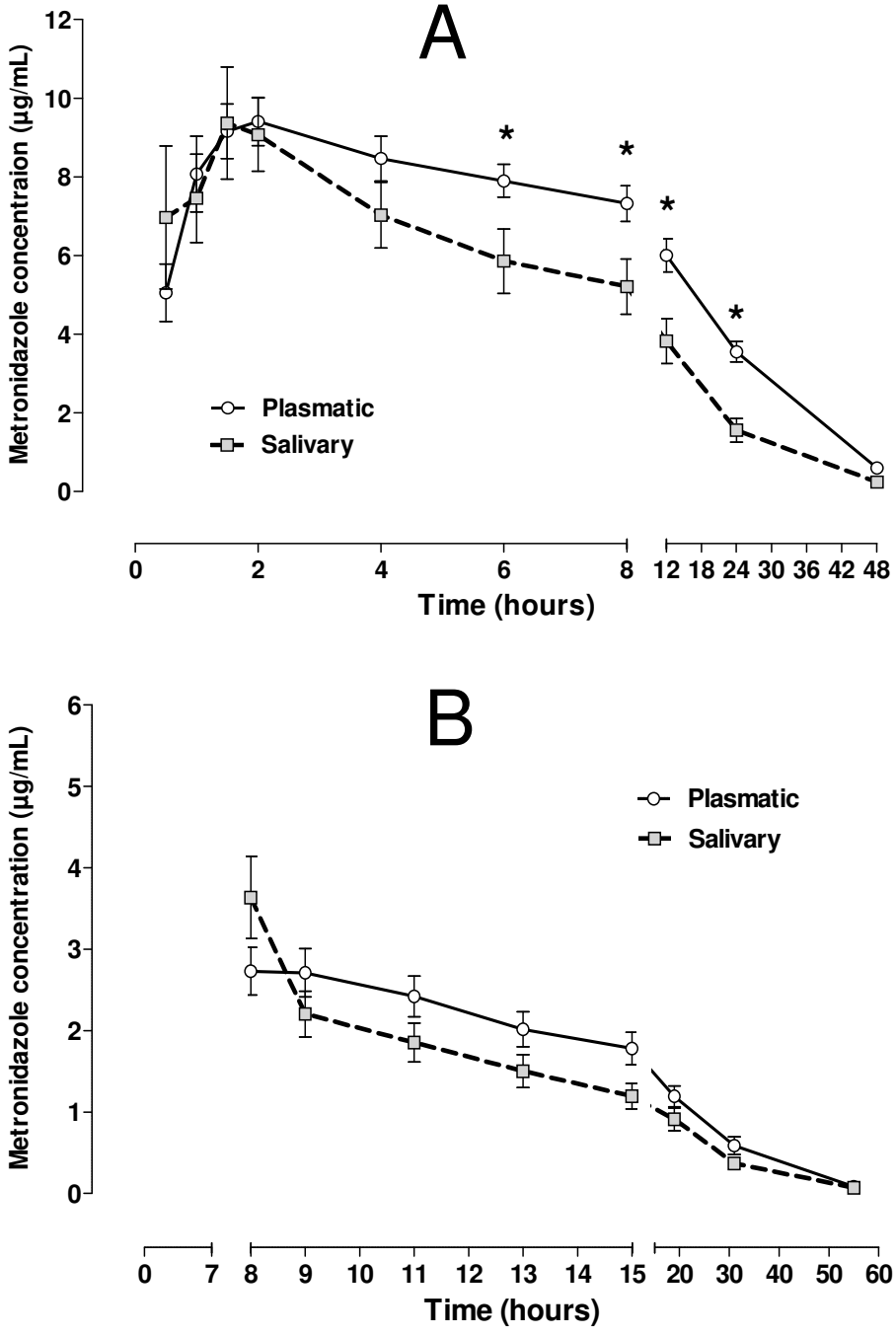

Figure 1. Mean (±SEM) values for plasmatic and salivary concentrations of both Mtz tablet (A) and Mtz gel (B). *Statistically significant differences (paired t test, $p<0.05$).

Discussion

The present study used a simple, reproducible and selective chromatography method which allows quantification of metronidazole (Mtz) in both plasma and saliva. This method requires 5 minutes for analysis and it uses a low volume of clinical sample (500 µL). The limit of quantification (0.01 µg/mL) is agreement with previous findings indicating the HPLC as a sensitive and reliable method to quantify Mtz in biological fluids (Mustofa & Santoso, 1991; Jessa et al., 1996; Spénard et al., 2005; Pahkla et al., 2005).

Mtz pharmacokinetic has been investigated in different single oral doses of 125 mg (Spénard et al., 2004), 500 mg (Mattila et al., 1983; Mustofa & Santoso, 1991), 1000 mg (Sprandel et al., 2004), and 1500 mg (Dilger et al., 2007). One study (Van Oosten et al., 1986) was related in literature measuring Mtz concentrations in plasma, saliva and gingival crevice fluid (GCF), after administration of a single oral dose Mtz (750 mg). However, their study included only four subjects, which did not observe fasting. Food is known to interfere with the bioavailability of Mtz, mainly in the absorption, reducing the C_{max} and AUC parameters (Spénard et al., 2005).

Previous studies using chromatography analyses reported C_{max} mean values in plasma of 6.9 µg/mL after 400 mg (Jensen & Gugler, 1983), 9.0 µg/mL after 500 mg (Matila et al., 1983), 13.6 µg/mL after 750 mg (Van Oosten et al., 1986), 16.3 and 19.6 mg/L after 1000 mg (Amon et al., 1978; Bergan & Arnold, 1980) and 40.6 mg/L after 2000 mg (Amon et al., 1978) of Mtz (single oral dose). These results suggest a linear relationship between increased antimicrobial dose and increased plasmatic concentration of Mtz. AUC values were also found to rise as dosage increased. Daneshmend et al. (1982) observed values of 132.2 µg/mL/h for AUC0-24 and Mustofa et al. (1991) of 153.56 µg/mL/h for $AUC0_{-48}$, after oral use of 500 mg Mtz.

The T_{max} values observed in the present study to plasma and saliva were similar to those reported by Wood & Monro (1975), Daneshmend et al. (1982) and Jessa et al. (1997), using single oral doses of 2000, 400 and 500 mg Mtz, respectively. It is well established that T_{max} is not influenced by dosage; however, these values were reported varied according to methodologies used to measure the drug concentration (Wood & Monro, 1975).

The high salivary concentration values observed in the present study indicate the high Mtz diffusion from blood to saliva. This drug has low molecular weight and it has the ability to permeate all tissues and body fluids. Furthermore, Mtz is a weak base with minimal plasmatic protein binding (Mustofa et al., 1991). Most drugs, even those highly bounded to plasmatic protein, are known no to be bound to salivary proteins (Matin et al., 1974).

Although the salivary concentration values, in some periods evaluated in the present study, were similar to those obtained for plasma, statistically significant difference was observed between $AUC_{0\text{-}48h}$ and $AUC_{0\text{-}\infty}$. This result occurred mainly due to the higher decay of salivary concentration during the elimination phase of drug. Similar results were observed by Jessa et al. (1997). However, Mustofa et al. (1991) found similar plasmatic and salivary concentrations and suggested replacing plasma with saliva to measure Mtz concentration.

Van Oosten et al. (1986) and Rotzetter et al. (1994) showed that Mtz concentrations in GCF were similar to those obtained in saliva and plasma after systemic administration. Van Oosten et al. (1986) reported concentrations in GCF varying from 8.7 μg/mL to 13.8 μg/mL after a single 750 mg Mtz oral dose. Pahkla et al. (2005) verified a GCF concentration approximately 13 μg/mL after 500mg Mtz (2 or 3 times/day).

A single dose of 160 mg Mtz benzoate corresponds to 100 mg Mtz (Mishal & Sober, 2005), since the Mtz benzoate is Mtz prodrug. Homeida et al. (1986) related that oral bioavailability of Mtz derived from Mtz benzoate is less than 80%. The quantity of Mtz benzoate in 3 g of Mtz benzoate gel (15%), which was used in the present study, corresponds to 450 mg Mtz benzoate. Considering that the available quantity of Mtz in plasma could be at least 20% inferior to that of Mtz benzoate, the initial dose of Mtz in the gel formulation tested was approximately 360 mg of Mtz. This lower dose, when compared to that used for the tablet, can explain the lower plasmatic and salivary concentrations found for Mtz gel between 8 and 48 hours.

In conclusion, gel formulation had similar Mtz bioavailability in plasma and saliva. The bioavailability of Mtz tablet was higher in plasma than in saliva.

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Capítulo 3

The effect of smoking on the bioavailability of metronidazole tablet in plasma and saliva.

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ABSTRACT

Background: Smoking has been widely reported as a significant risk factor for periodontal diseases. Metronidazole (Mtz) is a widely prescribed antibiotic in periodontics and the effect of smoking on the Mtz pharmacokinetics is not known. The aim of this study was to evaluate the effect of cigarette smoking on the bioavailability of Mtz. **Methods:** 13 smokers (SM) and 13 nonsmokers (NSM) received a single oral dose of 750 mg Mtz. Blood and saliva samples were collected at baseline and 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 hours after Mtz administration. Plasmatic and salivary Mtz concentrations were quantified by HPLC-UV and its pharmacokinetic parameters, such as the highest concentration (C_{max}), C_{max} time (T_{max}), the area under concentration-time curve from zero to infinity ($AUC_{0-\infty}$), the area under concentration-time curve from zero to t (AUC_{0-t}), distribution volume (VD) and renal clearance (CL), were determined. Non-paired t-test and Mann-Whitney test were used to compare the plasmatic and salivary concentrations and the pharmacokinetic parameters, respectively (α=5%). **Results:** A significant reduction ($p<0.05$) in plasmatic Mtz concentration was observed in SM at 1 hour (SM=6.6±0.58µg/ml and NSM=9.3±0.85µg/ml), 1.5 hours (SM=8.2±0.59µg/ml and NSM=10.7±0.49µg/ml) and 2 hours (SM=8.7±0.74µg/ml and NSM=11.2±0.51µg/ml). A significant reduction in plasmatic C_{max} was observed in SM (9.7±2.92µg/ml) compared to NSM (11.9±1.95µg/ml) ($p<0.05$). No statistically significant difference was observed between SM and NSM considering both salivary concentration and salivary pharmacokinetic parameters of Mtz ($p>0.05$). **Conclusion:** Smoking reduced the Mtz concentration in plasma; however, it had no interference with both salivary and plasmatic pharmacokinetic parameters. Further studies are needed to assess the clinical significance of these results.

Keywords: bioavailability; metronidazole; smoking; plasma; saliva.

Capítulo 3

INTRODUCTION

Periodontal disease is an infection occurring in the tooth-supporting tissues. The cause and progression of periodontal diseases remain rather unclear; however, bacterial plaque at the dentogingival junction and in periodontal pockets is known to be the major etiologic factor of such diseases.[1]

Previous studies, investigating the relationship between smoking and periodontal destruction, have reported smoking as a risk factor for periodontitis.[2] Cigarette smokers are known to be more susceptible to periodontal diseases [3] and to respond less favorably to the periodontal treatment compared to non-smokers.[4] They also have a higher risk for developing periodontitis which might be due to the length of tobacco use and dose.[5]

It has been suggested that 40% of the cases of chronic periodontitis might be attributed to smoking.[6] Smoking and tobacco products can alter the subgingival microbiota, favoring colonization with periodontal pathogens [7] and the immune-inflamatory host response.[8]

Periodontitis treatment includes mechanical removal of supra and subgingival microbial biofilm, as well as surgical techniques combined or not with local and systemic antimicrobial agents.[9] Metronidazol (Mtz) is one of the most commonly used antimicrobial agents to treat chronic and aggressive periodontitis, neither of which responds favorably to any mechanical treatment.[10]

Mtz is active against a wide variety of anaerobic protozoal parasites and anaerobic bacteria.[11] Its strict spectrum of activity against anaerobes and its few side effects make it suitable for treating periodontitis.[12] The treatment for periodontitis by using Mtz has been reported as a 5-to-14-day therapy, at doses varying from 250 to 500 mg, two to three times a day.[13-18]

Previous studies showed that cigarette smoking interfere with the bioavailability of some drugs, such as antineoplasic (erlotinib),[19] anti-hipertensive (irbesartan),[20] nonsteroidal anti-inflammatory (phenylbutazone),[21] opioids (codeine and morphine),[22,23] neuromuscular blocking agents (vecuronium),[24] anti-asthmatic (theophylline)[24] and antipsychotics (clozapine, olanzapine).[25,26] However, none study evaluating the effect of cigarette on the

pharmacokinetic of Mtz was found. Thus, the aim of this study was to evaluate the effect of cigarette smoking on the bioavailability of Mtz in both plasma and saliva.

MATERIALS AND METHODS

Subject Population

This study was approved by the Ethical Committee of São Leopoldo Mandic Dental School, São Paulo, Brazil (#06/456). All participants gave written informed consent and were informed of the nature and details of the study.

This study included twenty-six male subjects, assigned to two groups: G1 – individuals smoking 12 to 20 (15.5 ± 3.44) cigarettes a day during the study period (n=13) and G2 – nonsmokers (n=13). Smokers were 18–24 years old (22 ± 1.67 years), weighed 60–95 kg (74.6 ± 9.92 kg), were 1.69–1.84 m (1.78 ± 0.046 m) tall and had a body mass index of 20.1–27.8 kg/m^2 (23.8 ± 2.3 kg/m^2). Nonsmokers were 20–24 years old (22.4 ± 1.35 years), weighed 65–82 kg (73.0 ± 6.82 kg), were 1.68–1.85 m (1.77 ± 0.07 m) tall and had body mass index of 20.3–26.1 kg/m^2 (23.23 ± 2.07 kg/m^2). To be included, smokers should have been puffing on at least 12 cigarettes a day for the past 4 years, and nonsmokers should never have smoked. All the volunteers were free of cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, and hematological diseases, as well as psychiatric disorders, diabetes, glaucoma and allergy to antimicrobials.

Subjects were instructed to avoid any kind of drug therapy for at least 4 weeks prior to study and up to its completion. They were also refrained from xanthine-containing foods as well as beverages such as tea, coffee, and cola, 48 hours prior to each dosing and until the last blood/saliva sample was collected.[27] They were also refrained from alcoholic drinks up to a period of 48 hours before and after the last sampling due to risk of drug interaction, as Mtz has the ability to produce disulfiram-like reactions in alcohol consumers.[28]

Drugs and chemicals

Mtz tablet (250 mg Flagyl® - Aventis, lot #700277, São Paulo, Brazil) was obtained in a drugstore. Mtz and tinidazole (internal standard) were provided by Sigma (St Louis, MO, USA). All reagents were of analytical grade. Acetonitrile was obtained from J.T.

Baker Co. (Phillipsburg, NJ, USA). Sodium hydroxide 1N and perchloric acid (70%, w/v) were obtained from Merck (Darmstat, Germany). Distilled water was purified using a Millipore system (Milli Q., Millipore).

Clinical Protocol

Blood and saliva samples were collected in the ambulatory of São Leopoldo Dental School (Campinas, SP, Brazil). The volunteers were instructed to inform of any adverse effect they could have during the study.

After a 12-hour fasting period, subjects received a single oral dose of 750 mg Mtz with water (200 mL). Two hours after drug administration, they had a standard breakfast (a cup of peach juice and a 50-g loaf of bread with margarine) and were then allowed to consume water *ad libitium*. A complete standardized meal (lunch) was allowed four hours after drug administration. Blood (5mL) from antecubital vein via heparinized cannula and saliva (not stimulated - 3 mL) were collected at pre-dose (0), 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 hours after Mtz oral administration.

The blood samples were collected by direct venipuncture into sterile tubes containing K_3EDTA solution. The heparinized saline solution (0.9% NaCl and heparine, 9.8:0.2) injection (0.2 mL) was flushed into the cannula to prevent blood clotting, after each blood sampling. The 24-and-48-hour plasma (5 mL) samples were collected by using sterile syringes and needles and placed into sterile vials. Immediately after each blood and saliva collection, the samples were centrifuged at 3000 g for 15 min and the supernatant of plasma and saliva was placed into microtubes (Eppendorf, Hamburg, Germany) and stored at -70°C.

Chromatographic Analysis

Plasmatic and salivary Mtz concentrations were measured by high performance liquid chromatography (HPLC), which consists of a pump (Varian, Model 9012), a diode array detector (Varian, ProStar 335, DAD), software (Varian, Galaxie) and an autosampler (Varian, ProStar 410). Chromatographic separations were carried out by using a Lichrospher column 100 RP18 (125mm x 4mm x 5µm, Merck). A previously adapted and validated method[29] was used to analyze salivary and plasmatic Mtz concentrations.

The saliva (500 µL) and plasma (500 µL) samples were submitted to drug extraction

by using 250 µL of a protein-precipitation solution (10% perchloric acid and phosphate-citrate buffer, pH 5.4, for saliva and 20% perchloric acid and phosphate-citrate buffer, pH 5.4, for plasma). The samples were vortexed by 30 s, kept at room temperature for 10 min and centrifuged (15.000 g) for 15 min at 4 °C. Subsequently, 225 µL of a pH-adjusting solution (sodium hydroxide 1 M and buffer phosphate-citrate, pH= 5.4) was added to the supernatant (450 µL).

Tinidazole (20 µL out of 100 µg/mL), used as an internal standard, was then added to the salivary and plasmatic samples, which were then eluted with a mobile phase — a 0.01-M aqueous solution of sodium monophosphate (pH= 4.7) and acetonitrile (85:15). The flow rate was set at 1.0 mL/min, with a wavelength of 320 nm. The retention time was approximately 2 and 4 min for Mtz and tinidazole, respectively.

Standard stock solutions from Mtz concentrations were diluted in drug-free plasma and saliva (50µg/mL to 0.01µg/mL, n=9) to obtain the calibration curves. Sample concentrations were calculated by using the regression equation of the straight-line (y = ax + b), where 'y' refers to the peak area ratio, 'a' the slope, 'b' the interception and 'x' the unknown drug concentration.

Statistical and Pharmacokinetic analyses

Computer software (PK Solutions, Non-compartmental Pharmacokinetics Data analysis, Excel Template, 2001, Summit Research Services, Montrose, CO, USA) was used to obtain the following parameters: $\mathbf{C_{max}}$ - the highest concentration observed during the 48-hour study period; $\mathbf{T_{max}}$ - the time at which $\mathbf{C_{max}}$ occurred; $\mathbf{AUC_{0-t}}$ - the area under the plasma concentration-time curve from baseline to 24 and 48 h; $\mathbf{AUC_{0-\infty}}$ - the area under the plasma concentration-time curve from baseline to infinity; **CL** - renal clearance; and **VD** - distribution volume.

A power calculation based on C_{max} plasmatic values (mean and standard deviation) showed that 13 subjects in each group had a power of 70% towards detecting difference in pharmacokinetic parameters, at a significance level of 5%. Non-paired t test and Mann Whitney test were used to compare concentration and pharmacokinetic parameters of Mtz in plasma and saliva, at a significance level of 5%. Data analysis was carried out using computer Software (BioEstat 5.0, Mamirauá Institute, Belém, PA, Brazil).

RESULTS

No significant difference was observed between smokers and nonsmokers, regarding their physical characteristics such as age, weight, height and body mass index (Non-paired t test, $p>0.05$). All volunteers completed the study and no adverse effect was reported or observed.

The chromatography method was sensitive in quantifying Mtz in plasma and saliva (limit of quantification=0.01µg/mL) and showed good linearity, with the linear regression (R^2) being higher than 0.99.

A significant reduction (Non-paired t test, $p<0.05$) in plasmatic Mtz concentration was observed for smokers after 1, 1.5 and 2 hours after Mtz administration. However, no significant difference was observed between smokers and nonsmokers, concerning salivary Mtz concentration, in any of the periods evaluated (Non-paired t test, $p>0.05$). The plasmatic and salivary concentrations regarding smokers and nonsmokers are shown in Figure 1.

There were no statistically significant differences between smokers and nonsmokers concerning all pharmacokinetic parameters, except C_{max}, in plasma (Mann-Whitney, $p<0.05$). Table 1 shows mean values for plasmatic pharmacokinetic parameters (C_{max}, T_{max}, ASC_{0-48}, ASC_{0-inf}, VD and CL). No statistically significant difference was observed between smokers and nonsmokers concerning pharmacokinetic parameters in saliva (Mann-Whitney, $p>0.05$). Table 2 shows mean values obtained for salivary pharmacokinetic parameters (C_{max}, T_{max}, ASC_{0-24} and ASC_{0-inf}).

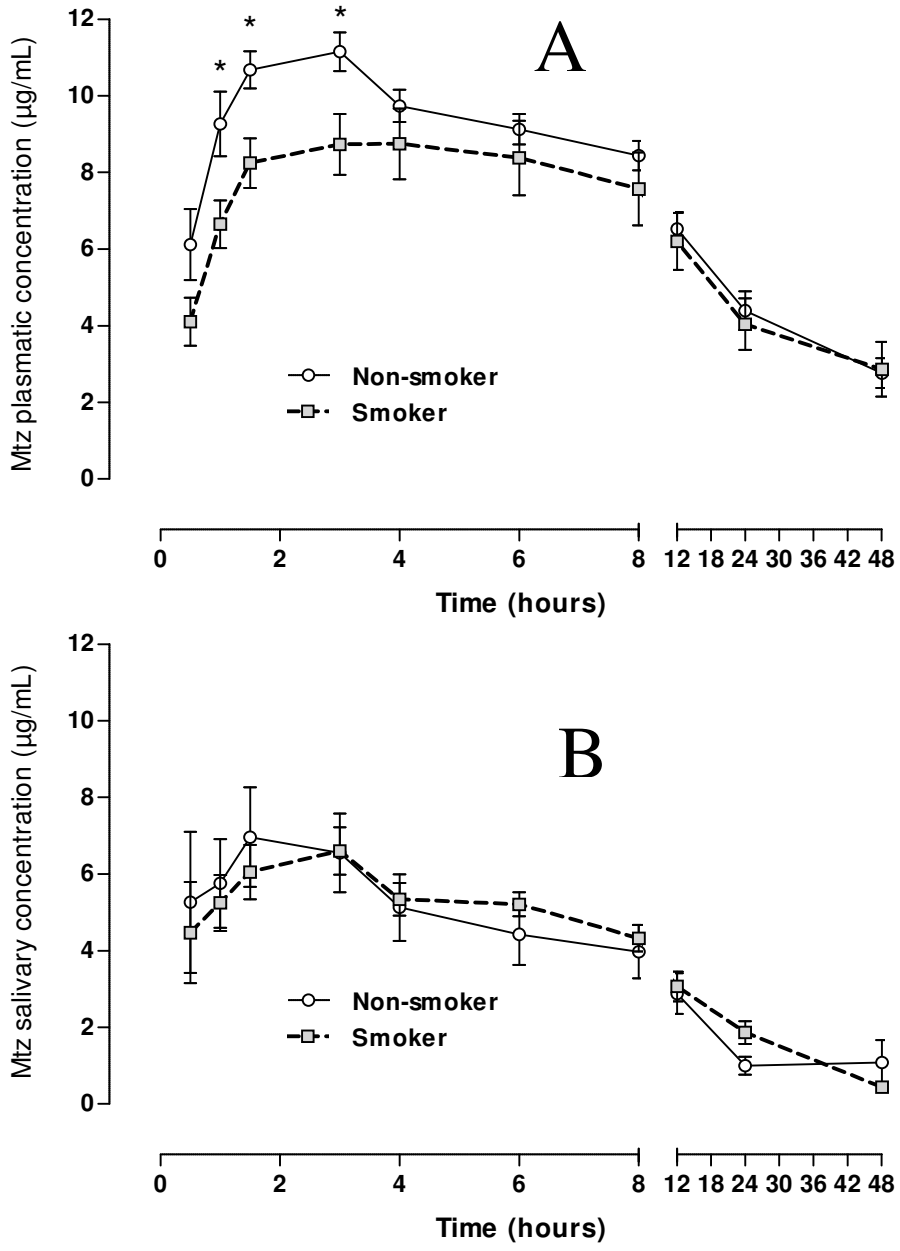

Figure 1. Mean values (± SEM) for plasmatic (A) and salivary (B) Mtz concentration in smokers and nonsmokers.

Table 1. Mean values (± SD) for plasmatic pharmacokinetic parameters in smokers and nonsmokers.

Parameters	Subjects	Mean ± SD	Interval of Confidence	p-values
C_{max} (µg/ml)	smoker	9.7 ± 2.92	7.85 to 11.26	*0.0230**
	nonsmoker	11.9 ± 1.95	10.69 to 13.05	
T_{max} (h)	smoker	2.9 ± 1.80	1.87 to 4.06	*0.1910*
	nonsmoker	1.8 ± 0.88	1.22 to 2.30	
AUC $_{(0-48)}$ (µg.hr/ml)	smoker	194.9 ± 90.10	140.40 to 249.07	*0.5964*
	nonsmoker	210.9 ± 59.48	174.99 to 246.88	
AUC $_{(0-\infty)}$ (µg.hr/ml)	smoker	297.2 ± 228.38	159.54 to 435.46	*0.4464*
	nonsmoker	290.3 ± 113.98	217.68 to 362.65	
VD (mL/Kg)	smoker	1071.4 ± 561.11	670.04 to 1472.70	*0.2899*
	nonsmoker	809.7 ± 263.28	621.03 to 998.07	
CL (mL/h)	smoker	55.1 ± 33.77	30.86 to 79.13	*0.4497*
	nonsmoker	42.0 ± 14.94	31.29 to 52.69	

*statistically significant differences (Mann-Whitney, $p<0.05$).

C_{max} - the highest concentration detected, T_{max} - the time at which C_{max} occured; AUC_{0-48} - the area under the concentration-time curve from 0 to 48 h, $AUC_{0-\infty}$ - the area under the concentration-time curve from 0 to infinity, CL - renal clearance, and VD - distribution volume.

Table 2. Mean values (± SD) for salivary pharmacokinetic parameters in smokers and nonsmokers.

Parameters	Subjects	Mean ± SD	Interval of Confidence	p-values
C_{max} (µg/ml)	smoker	8.9 ± 4.16	6.32 to 11.34	*0.3551*
	nonsmoker	8.3 ± 5.87	4.54 to 12.00	
T_{max} (h)	smoker	1.9 ±1.36	1.02 to 0.27	*0.5239*
	nonsmoker	1.5 ± 0.4 9	1.10 to 1.80	
AUC $_{(0-24)}$ (µg.hr/ml)	smoker	81.9 ± 24.47	67.13 to 97.68	*0.7373*
	nonsmoker	76.7 ± 47.29	46.67 to 106.78	
AUC $_{(0-\infty)}$ (µg.hr/ml)	smoker	109.9 ± 56.31	75.93 to 143.98	*0.3907*
	nonsmoker	89.3 ± 24.86	49.72 to 128.90	

C_{max} - the highest concentration detected, T_{max} - the time at which C_{max} occured; AUC_{0-24} - the area under the concentration-time curve from 0 to 24 h, $AUC_{0-\infty}$ - the area under the concentration-time curve from 0 to infinity.

DISCUSSION

The possible interaction between smoking and Mtz was investigated since smoking is considered a risk factor for periodontal diseases and Mtz seems to be one of the most commonly used antibiotics to treat periodontitis. Several studies reported clinical and microbiological benefits for the use of Mtz (alone or in combination with amoxicillin) to treat chronic [16, 30-35] and aggressive periodontitis [15, 18, 36-40], when used in association with mechanical periodontal therapy.

The method used in the present study to quantify Mtz in plasmatic and saliva samples was considered sensitive (limit of quantification=0.01µg/mL) and reliable (linearity=0.999).

Van Oosten et al.[41] reported 13.6 µg/mL as C_{max} in plasma after a single oral dose of 750 mg Mtz. Daneshmend et al.[42] observed 132.2 µg/mL/h for $AUC_{0-24,}$ while Mustofa et al.[43] found 153.56 µg/mL/h for AUC_{0-48} after a single oral dose of 500 mg Mtz. In the present study, AUC_{0-48} values were observed to be slightly higher than those reported by Mustofa et al.,[43] suggesting that AUC values might rise as dosage increases. In addition, T_{max} values previously reported by Wood & Monro[44] and Jessa *et al.*[45] (2 h and 1.90 h, respectively) were similar to those observed in the present study.

Mtz MIC values against anaerobic bacteria, known to cause periodontal diseases, were found to range from 0.1 to 8 µg/mL.[46] The mean Mtz plasmatic concentrations ranged from 2.2 to 8.7 µg/mL for smokers and from 2.4 to 11.2 µg/mL for nonsmokers at periods between 0.5 and 48 hours. Mtz concentration in saliva (ranging from 1.3 to 6.8 µg/mL for smokers and from 1.5 to 7.4 µg/mL for nonsmokers) was in the interval of MIC values reported by Leiknes et al.[1] in all periods. However, it is necessary to consider that these concentrations were observed after a single dose of Mtz. Ralph et al.[46] reported higher values of Mtz pharmacokinetic parameters and concentrations in plasma for multiple oral doses, when compared to single oral doses.

The number of cigarettes that an individual smokes per day was reported to interfere with the pharmacokinetic of drugs in plasma. Wu et al.[26] verified that smoking more than five cigarettes a day could lead to an enzymatic induction of the olanzapine metabolism.

In the present study, the mean use of 15 cigarettes/day was found to interfere with the Mtz concentration in plasma. Therefore, it is possible to suggest that the use of 20 or more cigarettes/day, the number consumed by heavy smokers,[47] could better evidence a possible influence of cigarette smoking on the pharmacokinetic parameters of Mtz in plasma and/or saliva.

Drug interactions associated with cigarette smoking were reported to interfere with the hepatic biotransformation of drugs known as substrates of such enzymes as CYP1A1, CYP1A2, and CYP2E1, which might be induced by cigarette smoke constituents. Polycyclic aromatic hydrocarbons (PAHs) are the main substances known to induce hepatic microsomal enzymes and can lead to an increase in the rate of metabolism of certain drugs.[48] Another metabolic pathway (glucuronide conjugation) can also be induced by PAHs.[48] Other cigarette smoke substances may also interact with hepatic enzymes but their effects appear to be less relevant.[49]

Drugs, such as phenylbutazone,[21] morphine,[22] codeine,[23] vecuronium,[24] theophylline,[24] clozapine and olanzapine,[25] were reported to be metabolized by the enzymes mentioned above, and the drug interaction with cigarette smoking resulted in a significant reduction in their plasmatic concentrations. It is hypothesized that this same drug interaction mechanism might account for the reduction in the Mtz plasmatic concentration found in the present study.

In conclusion, cigarette smoking reduced the Mtz concentration in plasma; however, it had no interference with both salivary and plasmatic pharmacokinetic parameters. Further studies are needed to assess the clinical significance of these results.

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3. CONSIDERAÇÕES GERAIS

A literatura apresenta muitos estudos clínicos com o uso de antimicrobianos associados ao tratamento periodontal mecânico. No entanto, os achados apresentam resultados divergentes quanto à efetiva contribuição desses agentes ao tratamento periodontal. Segundo Haffajee *et al.* (2003), os dados da literatura são suficientes para sugerir que os antimicrobianos auxiliam no tratamento das periodontites, a dificuldade é estabelecer um protocolo de uso.

O presente estudo pretendeu verificar se o uso do gel de metronidazol (Mtz) (15%) em moldeira traria benefício clínico adicional quando associado ao procedimento mecânico em fumantes com periodontite crônica. Este tipo de aplicação poderia promover a reposição do antimicrobiano "perdido" para dentro da bolsa periodontal, uma vez que o alto fluxo do fluído gengival dificulta sua permanência neste local. Além disso, a alta concentração salivar do Mtz durante o uso do gel ajudaria a eliminar microrganismos presentes na saliva.

Entretanto, no presente estudo, não foi observado benefício clínico adicional ao tratamento periodontal com o uso do Mtz nas condições avaliadas. Na tentativa de explicar estes resultados, foi observado que o uso de Mtz foi mais efetivo em bolsas profundas que em rasas (Loesche *et al*., 2002; Feres *et al.,* 2001; Carvalho *et al.,* 2004). Também foi sugerido que a dose usada ou a duração do tratamento são fatores relevantes para avaliar a eficácia antimicrobiana deste agente no tratamento periodontal (Van Winkelhoff *et al.,* 1999; Herrera *et al*., 2008). Além disso, o fato de fumantes ter uma resposta menos favorável à terapia periodontal comparada à não-fumantes (Van der Velden *et al.,* 2003; Labriola *et al.*, 2005; Palmer *et al*., 2005) poderia interferir nos resultados observados.

No segundo estudo foi observada uma maior concentração plasmática e salivar com o uso do comprimido comparado ao gel de Mtz nos períodos de 8 a 48 horas; no entanto, ambas as formulações não demonstraram benefício clínico adicional ao procedimento periodontal sozinho, podendo-se sugerir que um aumento na concentração do gel e na dosagem do comprimido pudesse proporcionar esta melhora ao tratamento periodontal.

Não foi possível determinar as concentrações de Mtz na saliva durante o uso do gel devido ao viés que o horário de colheita poderia proporcionar; no entanto, é possível que as

concentrações salivares tenham sido maiores durante o uso do gel comparadas àquelas observadas nos períodos de colheita das amostras (8 a 48 horas após a aplicação do gel).

Deve-se levar em consideração à crescente preocupação com a prescrição de antimicrobianos no que se refere aos efeitos adversos e principalmente ao aumento da resistência bacteriana (Mombelli, 2005; Haffajee *et al.,* 2006), o que restringe o uso destas substâncias a certas condições periodontais definidas (doença refratária ou severa) e a determinados pacientes (como os fumantes) na qual a doença pode requerer um tratamento mais agressivo (Herrera *et al.,* 2002; Herrera *et al.,* 2008).

No terceiro estudo foi observada uma diminuição na concentração plasmática do Mtz que em contrapartida resultou apenas em diminuição do parâmetro C_{max} em plasma. Estes achados merecem melhor investigação, uma vez que o estudo foi realizado com o uso de dose oral única e os pacientes foram selecionados com base num mesmo padrão de consumo de cigarro. É possível que haja uma alteração mais significativa na farmacocinética do Mtz, quando modificados os parâmetros tempo de uso do antimicrobiano e consumo de cigarros. Embora exista a necessidade de melhor investigação, há evidências que comprovam diminuição na biodisponibilidade plasmática do Mtz em fumantes e, somando este achado aos resultados obtidos com o estudo clínico de pacientes fumantes com doença periodontal; pode-se concluir, dentro das limitações do presente estudo, que o Mtz não mostrou vantagem clínica adicional ao tratamento da periodontite crônica em fumantes, não havendo necessidade do seu uso associado ao tratamento mecânico.

4. CONCLUSÕES

As conclusões do presente estudo foram:

1) O metronidazol (Mtz) nas formulações gel e comprimido associados ao debridamento periodontal não demonstraram melhora clínica adicional, não havendo vantagem no uso destas formulações, dentro das condições avaliadas no presente estudo;
2) As concentrações plasmáticas e salivares obtidas com a formulação gel de Mtz foram similares;
3) Alguns dos parâmetros farmacocinéticos do Mtz em comprimido foram maiores no plasma que na saliva;
4) O cigarro reduziu a concentração plasmática do Mtz no plasma, mas não inteferiu nos parâmetros farmacocinéticos deste antimicrobiano em plasma e saliva.

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[1] De acordo com a norma da UNICAMP/FOP, baseadas na norma do Internacional Commitee of Medical Journal Editors – Grupo Vancouver. Abreviatura dos periódicos em conformidade com o Mediline.

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ANEXO 1

INFORMAÇÃO CCPG/002/06

Tendo em vista a necessidade de revisão da regulamentação das normas sobre o formato e a impressão das dissertações de mestrado e teses de doutorado e com base no entendimento exarado no Parecer PG nº 1985/96, que trata da possibilidade do formato alternativo ao já estabelecido, a CCPG resolve:

Artigo 1º - O formato padrão das dissertações e teses de mestrado e doutorado da UNICAMP deverão obrigatoriamente conter:

I. Capa com formato único ou em formato alternativo que deverá conter informações relativas ao nível (mestrado ou doutorado) e à Unidade de defesa, fazendo referência à Universidade Estadual de Campinas, sendo o projeto gráfico das capas definido pela PRPG.
II. Primeira folha interna dando visibilidade à Universidade, a Unidade de defesa, ao nome do autor, ao título do trabalho, ao número de volumes (quando houver mais de um), ao nível (mestrado ou doutorado), a área de concentração, ao nome do orientador e co-orientador, ao local (cidade) e ao ano de depósito. No seu verso deve constar a ficha catalográfica.
III. Folha de aprovação, dando visibilidade à Comissão Julgadora com as respectivas assinaturas.
IV. Resumo em português e em inglês (ambos com no máximo 500 palavras).
V. Sumário.
VI. Corpo da dissertação ou tese dividido em tópicos estruturados de modo característico à área de conhecimento.
VII. Referências, formatadas segundo normas de referenciamento definidas pela CPG da Unidade ou por critério do orientador.
VIII. Todas as páginas deverão, obrigatoriamente, ser numeradas, inclusive páginas iniciais, divisões de capítulos, encartes, anexos, etc... As páginas iniciais poderão ser numeradas utilizando-se algarismos romanos em sua forma minúscula.
IX. Todas as páginas com numeração "impar" serão impressas como "frente" e todas as páginas com numeração "par" serão impressas como "verso".

§ 1º - A critério do autor e do orientador poderão ser incluídos: dedicatória; agradecimento; epígrafe; lista de: ilustrações, tabelas, abreviaturas e siglas, símbolos; glossário; apêndice; anexos.

§ 2º - A dissertação ou tese deverá ser apresentada na língua portuguesa, com exceção da possibilidade permitida no artigo 2º desta Informação.

§ 3º - As dissertações e teses cujo conteúdo versar sobre pesquisa envolvendo seres humanos, animais ou biossegurança, deverão apresentar anexos os respectivos documentos de aprovação.

Artigo 2º - A critério do orientador e com aprovação da CPG da Unidade, os capítulos e os apêndices poderão conter cópias de artigos de autoria ou de co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, escritos no idioma exigido pelo veículo de divulgação.

§ único - O orientador e o candidato deverão verificar junto às editoras a possibilidade de inclusão dos artigos na dissertação ou tese, em atendimento à legislação que rege o direito autoral, obtendo, se necessária, a competente autorização, deverão assinar declaração de que não estão infringindo o direito autoral transferido à editora.

Artigo 3º - Dependendo da área do conhecimento, a critério do orientador e com aprovação da CPG da Unidade, a dissertação ou tese poderá ser apresentada em formato alternativo, desde que observados os incisos I, II, III, IV, V e VII do artigo 1º.

Artigo 4º - Para impressão, na gráfica da Unicamp, dos exemplares definitivos de dissertações e teses defendidas, deverão ser adotados os seguintes procedimentos:

§ 1º - A solicitação para impressão dos exemplares de dissertações e teses poderá ser encaminhada à gráfica da Unicamp pelas Unidades, que se responsabilizarão pelo pagamento correspondente.

§ 2º - Um original da dissertação ou tese, em versão definitiva, impresso em folha tamanho carta, em uma só face, deve ser encaminhado à gráfica da Unicamp acompanhado do formulário "Requisição de Serviços Gráficos", onde conste o número de exemplares solicitados.

§ 3º - A gráfica da Unicamp imprimirá os exemplares solicitados com capa padrão. Os exemplares solicitados serão retirados pelas Unidades em no máximo, cinco dias úteis para impressão preto e branco e 10 dias úteis para coloridas.

§ 4º - No formulário "Requisição de Serviços Gráficos" deverão estar indicadas as páginas cuja reprodução deva ser feita no padrão "cores" ou "foto", ficando entendido que as demais páginas devam ser reproduzidas no padrão preto/branco comum.

§ 5º - As dissertações e teses serão reproduzidas no padrão frente e verso, exceção feita às páginas iniciais e divisões de capítulos; dissertações e teses com até 100 páginas serão reproduzidas no padrão apenas frente, exceção feita à página que contém a ficha catalográfica.

§ 6º - As páginas fornecidas para inserção deverão ser impressas em sua forma definitiva, ou seja, apenas frente ou frente/verso.

§ 7º - O custo, em reais, de cada exemplar produzido pela gráfica será definido pela Administração Superior da Universidade.

Artigo 5º - É obrigatória a entrega de dois exemplares para homologação.

Artigo 6º - Esta Informação entrará em vigor na data de sua publicação, ficando revogadas as disposições em contrário, principalmente as Informações CCPG 001 e 002/98 e CCPG/001/00.

Campinas, 13 de setembro de 2006

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Avaliação clínica e microbiológica do metronidazol em formulação gel. Perspectivas do uso em periodontia**", protocolo nº **085/2006**, dos pesquisadores **FRANCISCO CARLOS GROPPO** e **CRISTIANE DE CÁSSIA BERGAMASCHI**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 18/07/2006.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Clinical and microbiological evaluation of gel metronidazole. Perspectives of use in periodontology**", register number **085/2006**, of **FRANCISCO CARLOS GROPPO and CRISTIANE DE CÁSSIA BERGAMASCHI**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 18/07/2006.

Profa. Cecília Gatti Guirado
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

a: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
ice: The title of the project appears as provided by the authors, without editing.

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Determinação plasmática e salivar do metronidazol por cromatografia líquida de alta eficiência**", protocolo nº **086/2006**, dos pesquisadores **FRANCISCO CARLOS GROPPO e CRISTIANE DE CÁSSIA BERGAMASCHI**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 18/07/2006.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Plasmatic and salivary determination of metronidazole by high performance liquid chromatography**", register number **086/2006**, of **FRANCISCO CARLOS GROPPO and CRISTIANE DE CÁSSIA BERGAMASCHI**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 18/07/2006.

Profa. Cecília Gatti Guirado
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

a: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
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SÃO LEOPOLDO MANDIC
FACULDADE DE ODONTOLOGIA
CENTRO DE PÓS-GRADUAÇÃO

Aprovado pelo CEP

Campinas, 01 de Dezembro de 2006.

Ao

C. D. Victor Ângelo Martins Montalli

Curso: Graduação

Prezado (a) Aluno (a):

O projeto de sua autoria: **"Comparação dos parâmetros farmacocinéticos do metronidazol em pacientes fumantes com não fumantes".**

Orientado pelo (a) Prof. (a) Dr. (a) Juliana Cama Ramacciato

Entregue na Secretaria de Pós-Graduação do CPO - São Leopoldo Mandic, no dia 27/11/2006, com número de protocolo **nº06/456** foi APROVADO pelo Comitê de Ética e Pesquisa, instituído nesta Universidade de acordo com a resolução 196 / 1.996 do CNS – Ministério da Saúde, em reunião realizada no dia 29/11/2006.

Cordialmente

Prof. Dr. Thomaz Wassall
Coordenador de Pós-Graduação

ANEXO 3

YAHOO! BRASIL MAIL Classic

Journal of Periodontology - JOP-09-0314 Has Been Submitted

Terça-feira, 2 de Junho de 2009 0:20

De: "julie@perio.org" <julie@perio.org>
Para: cristianebergamaschi@yahoo.com.br
* JOP-Conflict-of-Interest-and-Financial-Disclosure-Form.pdf (42 KB)

01-Jun-2009

Dear Mrs. Bergamaschi:

Thank you for submitting your manuscript, "The effect of smoking on the bioavailability of metronidazole tablet in plasma and saliva" (JOP-09-0314), to the Journal of Periodontology. Your manuscript will be forwarded to Dr. Kornman or an Associate Editor prior to peer review.

As a reminder, the Journal of Periodontology requires all authors to submit a conflict of interest and financial disclosure form. If you have not yet done this, please e-mail or fax these forms to Bethanne Wilson in the Editorial Office (e-mail: bethanne@perio.org; fax: 312.573.3225). A copy of the form is attached for your use.

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If you have any questions, or if I can provide further information, please do not hesitate to contact me at julie@perio.org. It will be most helpful if you would include both your manuscript title and reference number in any correspondence.

Sincerely,

Julie Daw
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