UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

SINVALDO BAGLIE

FARMACÊUTICO

CONCENTRAÇÕES PLASMÁTICAS E SALIVARES E EFEITO SOBRE A MICROBIOTA ORAL DE DUAS FORMULAÇÕES DE AMOXICILINA. ESTUDO EM VOLUNTÁRIOS SADIOS.

Tese apresentada à Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, para obtenção do título de Doutor em Odontologia, Área de Farmacologia, Anestesiologia e Terapêutica.

PIRACICABA – SP
2005

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

SINVALDO BAGLIE

FARMACÊUTICO

CONCENTRAÇÕES PLASMÁTICAS E SALIVARES E EFEITO SOBRE A MICROBIOTA ORAL DE DUAS FORMULAÇÕES DE AMOXICILINA. ESTUDO EM VOLUNTÁRIOS SADIOS.

Tese apresentada à Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, para obtenção do título de Doutor em Odontologia, Área de Farmacologia, Anestesiologia e Terapêutica.

Orientador:
Prof. Dr. Francisco Carlos Groppo

Co-orientador:
Prof. Dr. Pedro Luiz Rosalen

Banca Examinadora:
Prof. Dr. Carlos Eduardo Pulz Araújo
Prof. Dr. Eduardo Dias de Andrade
Prof. Dr. Fernando de Sá Del Fiol
Prof. Dr. Francisco Carlos Groppo
Prof. Dr. Vitoldo Antonio Kozlowski Jr

PIRACICABA – SP
2005

Ficha Catalográfica

Baglie, Sinvaldo.
B146c Concentrações plasmáticas e salivares e efeito sobre a microbiota oral de duas formulações de amoxicilina. Estudo em voluntários sadios. / Sinvaldo Baglie. -- Piracicaba, SP : [s.n.], 2005.

Orientadores : Prof. Dr. Francisco Carlos Groppo, Prof. Dr. Pedro Luiz Rosalen.
Tese (Doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Amoxicilina. 2. Equivalência terapêutica. 3. Disponibilidade biológica. 4. Farmacocinética. 5. Saliva. I. Groppo, Francisco Carlos. II. Rosalen, Pedro Luiz. III. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. IV. Título.
(mg/fop)

Palavras-chave em inglês (*Keywords*): Amoxicillin; Therapeutic equivalency; Biological availability; Pharmacokinetics; Saliva
Área de concentração: Farmacologia, Anestesiologia e Terapêutica
Titulação: Doutor em Odontologia
Banca examinadora: Prof. Dr. Francisco Carlos Groppo; Prof. Dr. Carlos Eduardo Pulz Araújo; Prof. Dr. Eduardo Dias de Andrade; Prof. Dr. Fernando de Sá Del Fiol; Prof. Dr. Vitoldo Antonio Kozlowski Jr.
Data da defesa: 16/02/2005

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

A Comissão Julgadora dos trabalhos de Defesa de Tese de DOUTORADO, em sessão pública realizada em 16 de Fevereiro de 2005, considerou o candidato SINVALDO BAGLIE aprovado.

PROF. DR. FRANCISCO CARLOS GROPPO

PROF. DR. FERNANDO DE SÁ DEL FIOL

PROF. DR. VITOLDO ANTONIO KOZLOWSKI JUNIOR

PROF. DR. CARLOS EDUARDO PULZ ARAÚJO

PROF. DR. EDUARDO DIAS DE ANDRADE

DEDICATÓRIA

A **DEUS**, Pai todo poderoso e misericordioso, que guia e ilumina nossos caminhos para a felicidade eterna.

À **ROBERTA CRISTIANE CATELLI BAGLIE**, minha esposa amada e querida, pelo amor incondicional e por acreditar em meus sonhos, participar e ajudar a realizá-los.

Ao meu querido pai **GERALDO** e minha querida mãe **NEUSA**, que pelo amor, carinho, simplicidade, humildade e exemplos de vida me fazem enxergar o verdadeiro valor da vida.

Aos meus irmãos **MARILENE**, **JOSÉ GERALDO** e **LUCILENE** e a todos os meus familiares pelo constante amor e incentivo.

Com amor dedico este trabalho.

AGRADECIMENTO ESPECIAL

Ao Prof. Dr. **FRANCISCO CARLOS GROPPO**, meu orientador, mestre solicito em todos os momentos, amigo e incentivador, a quem tenho grande respeito e eterna gratidão. A convivência com a sua pessoa é uma honra.

AGRADECIMENTOS ESPECIAIS

Ao Prof. Dr. **PEDRO LUIZ ROSALEN**, meu co-orientador, pela oportunidade de trabalharmos juntos, pelo exemplo de profissionalismo e dedicação, meu muito obrigado.

Ao Prof. **LUIZ MADALENO FRANCO**, profissional exemplar que sempre admirei, pela honra de trabalharmos juntos.

A **ROGÉRIO HELÁDIO LOPES MOTTA**, amigo-irmão, companheiro de todas as horas, pela sua ajuda fundamental e a quem um futuro muito promissor o espera.

À **ROBERTA CRISTIANE CATELLI BAGLIE**, **ANA PAULA DEL BORTOLO RUENIS** e **GILSON CESAR NOBRE FRANCO** pela excelentíssima cumplicidade que tivemos nos trabalhos e pela amizade.

AGRADECIMENTOS

À Universidade Estadual de Campinas, UNICAMP, na pessoa do Magnífico Reitor, **Prof. Dr. Carlos Henrique de Brito Cruz** e à Faculdade de Odontologia de Piracicaba, FOP, por meio do Diretor **Prof. Dr. Thales Rocha de Mattos Filho**.

À Universidade Estadual de Ponta Grossa, UEPG, na pessoa do Magnífico Reitor, **Prof. Paulo Roberto Godoy** e aos professores do Departamento de Ciências Farmacêuticas da UEPG, pelo apoio para realização deste trabalho.

Ao **Prof. Dr. Pedro Luiz Rosalen**, coordenador dos Cursos de Pós-Graduação da Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas.

Ao **Prof. Dr. Francisco Carlos Groppo**, coordenador do Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas.

À **CAPES**, Centro de Aperfeiçoamento de Pessoal Especializado, pelo apoio financeiro.

Ao professor e amigo **Jorge Valério** pelo convívio e ensinamentos em Língua Inglesa.

AGRADECIMENTOS

Aos docentes do Programa de Pós-Graduação em Odontologia, Área de Farmacologia, Anestesiologia e Terapêutica da FOP/UNICAMP: **Prof. Dr. Eduardo Dias de Andrade**, **Prof. Dr. Francisco Carlos Groppo**, **Prof. Dr. José Ranali**, **Prof[a] Dra. Maria Cristina Volpato**, **Prof. Dr. Pedro Luiz Rosalen** e **Prof. Dr. Thales Rocha de Mattos Filho**, pelos ensinamentos e pela constante contribuição em minha formação profissional.

À **Roberta Pessoa Simões**, pelo incentivo e garra que ajudaram a viabilizar este trabalho.

A **Bioagri Laboratórios Ltda.** e ao **Hospital dos Fornecedores de Cana de Piracicaba** pela parceria na realização dos estudos.

Aos amigos contemporâneos de Pós-Graduação em Farmacologia: **Roberta**, **Ana Paula**, **Rogério**, **Cristiane**, **Gilson**, **Karina**, **Giovana**, **Juliana**, **Michelle**, **Patrícia**, **Fabiano**, **Marcelo**, **Vanessa**, **Rodrigo**, **Simone**, **Regiane**, **Ramiro**, **Carina Denny**, **Marcos**, **Alcides**, **Humberto** pela amizade e cumplicidade.

À **Profa. Yoko Oshima Franco**, a **Flávia Silva**, a **Carolina Nóbrega** e **Giovana Pecharki** pela imensa contribuição na fase experimental.

AGRADECIMENTOS

À Srta. **Maria Elisa dos Santos**, secretária eficientíssima da Área de Farmacologia, Anestesiologia e Terapêutica, pela competência, eficiência, dedicação, amizade e pela sua doce presença.

Aos amigos **Eliane Melo Franco** e **José Carlos Gregório** da Área de Farmacologia, Anestesiologia e Terapêutica, pela preciosa colaboração e disposição.

A **Márcia Eurich Belinski** da secretaria da Pró-Reitoria de Pesquisa e Pós-Graduação da Universidade Estadual de Ponta Grossa pela competência, dedicação e presteza em todos os momentos.

Aos voluntários que se dispuseram a participar deste trabalho, sem os quais seria impossível a realização do mesmo.

A todos os amigos, colegas e pessoas que, embora não citados, direta ou indiretamente contribuíram para a realização deste trabalho.

EPÍGRAFE

PARÁBOLA DO SEMEADOR

"O semeador, saiu a semear; e, enquanto semeava, uma parte da semente caiu ao longo do caminho, e vindo os pássaros do céu a comeram.

Outra caiu em lugares pedregosos, onde não havia muita terra; e logo nasceu porque a terra onde estava não tinha profundidade. Mas o Sol tendo se erguido, em seguida, a queimou; e, como não tinhas raízes, secou.

Outra caiu entre espinheiros, e os espinhos, vindo a crescer, a sufocaram.

Outra, enfim, caiu em terra boa, e deu frutos, alguns grãos rendendo cem, outros sessenta e outros trinta por um."

(São Mateus, cap. 13, v. de 3 a 8)

SUMÁRIO

RESUMO

As concentrações de amoxicilina (AMO) em plasma e saliva e seus efeitos na microbiota oral de voluntários sadios foram analisados, em um estudo aberto, de forma aleatória, cruzado com dois períodos. AMO 875 mg (Amoxicilina - EMS - medicamento teste e Amoxil® - medicamento referência) foi administrada para todos os voluntários com 1 semana de intervalo entre as doses. Amostras de plasma e saliva foram colhidas em um intervalo de até 12h após a administração. As concentrações plasmáticas de AMO foram obtidas por cromatografia líquida e espectrometria de massas com ionização por *electrospray* (LC-ESI-MS). As concentrações de amoxicilina em saliva foram analisadas por cromatografia líquida de alta eficiência (HPLC). As contagens dos microrganismos totais, anaeróbios e estreptococos foram obtidas em diferentes condições de cultura. ASC foi calculada pelo método de extrapolação dos trapezóides. C_{max} e T_{max} foram compilados dos dados de concentração-tempo. Análise de variância foi realizada usando dados logaritmicamente transformados para $ASC_{0\text{-}inf}$, $ASC_{0\text{-}12h}$, C_{max} e não transformados para T_{max}. As médias (±dp) para $ASC_{0\text{-}12h}$ ($\mu g.h.mL^{-1}$), $ASC_{0\text{-}inf}$ ($\mu g.h.mL^{-1}$), C_{max} ($\mu g.mL^{-1}$) and T_{max} (h), foram respectivamente: 55,42(±16,85), 55,42 (±16,85), 18,59 (±6,3), 2,04(±0,75) para o medicamento teste e 51,11 (±18,9), 51,29 (±19,12), 17,83 (±5,86), 2,02 (±0,87) para o medicamento referência. Intervalos de confiança (90%) para a razão das médias de AMO (teste/referência) para $ASC_{0\text{-}12h}$ e C_{max} foram: 0,961 – 1,149 e 0,914 – 1,142, respectivamente. Tanto para as concentrações plasmáticas quanto para as salivares não foram observadas diferenças estatisticamente significantes entre os dois medicamentos (Teste *t* pareado, $p>0.05$). As contagens de microrganismos provindos da saliva não mostraram diferenças estatísticas (ANOVA de medidas repetidas, $p>0.05$) entre as duas formulações durante cada tempo. A partir de 60 min, uma redução estatisticamente significante (ANOVA de medidas repetidas, $p<0.05$) foi observada

para todos os microrganismos. Os dois medicamentos foram considerados bioequivalentes baseado na razão e extensão de absorção e foram eficazes na redução dos microrganismos da microbiota oral avaliados até 12 horas.

ABSTRACT

The plasmatic and salivary concentration of amoxicillin (AMO) 875mg and its effects on oral microbiota in healthy volunteers were assessed in an open, randomized, two-period crossover design. A single 875 mg oral dose of AMO (Amoxicillin-EMS – test formulation and Amoxil® - reference formulation) was administered to all volunteers observing 1-week interval between doses. Blood and saliva samples were collected from pre-dose to 12 h. The concentrations of AMO, in plasma and saliva, were quantified by LC-ESI-MS and LC method, respectively. AUC was calculated by the trapezoidal rule extrapolation method. C_{max} and T_{max} were compiled from the plasmatic concentration-time data. Streptococci, anaerobe, and total microorganisms' counts were obtained in different culture conditions. The mean values (± SD) for AUC_{0-12h} ($\mu g.h.mL^{-1}$), AUC_{0-inf} ($\mu g.h.mL^{-1}$), C_{max} ($\mu g.mL^{-1}$) and T_{max} (h), were respectively: 55.42(±16.85), 55.42 (±16.85), 18.59 (±6.3), 2.04(±0.75) concerning the test formulation and 51.11 (±18.9), 51.29 (±19.12), 17.83 (±5.86), 2.02 (±0.87) concerning the reference formulation. Confidence intervals (90%) of amoxicillin means of AUC_{0-12h} and C_{max} ratios (test/reference) were: 0.961 – 1.149 and 0.914 – 1.142, respectively. No statistically significant differences were observed between the two formulations (*t* test, $p>0.05$) regarding AMO plasmatic and saliva concentrations. Saliva microorganisms counts did not show statistical differences (ANOVA repeated measures, $p>0.05$) between the two groups during each sampling time. Starting at 60 min, a statistically significant decrease (ANOVA repeated measures, $p<0.05$) was also observed for all microorganisms. Both formulations were bioequivalent based on both the rate and extent of absorption, and were effective in reduce microorganisms until 12 hours.

1. INTRODUÇÃO

A ineficácia clínica observada com o uso de alguns medicamentos, além de episódios de intoxicação, deram origem às investigações relacionadas a biodisponibilidade de produtos farmacêuticos (STORPIRTIS & CONSIGLIERI, 1995).

Diversos trabalhos foram realizados com diferentes medicamentos e mostraram que variações nas formulações promoviam diferentes biodisponibilidades. A partir destas constatações, a *Food and Drug Administration* (FDA/USA) promoveu estudos que resultaram na regulamentação de critérios relativos a biodisponibilidade e bioequivalência de medicamentos, tendo sido, a versão mais conhecida, publicada oficialmente em 1992 e que está atualmente revisada (FDA, 2004); ainda que, os medicamentos genéricos tenham surgido muito anteriormente nos EUA na década de 60, com um maior desenvolvimento destes a partir de 1984, com condições políticas e técnicas mais ideais.

No Brasil, estudos de biodisponibilidade e bioequivalência ganharam destaque somente a partir da Lei 9787/99 e resoluções complementares que propiciaram o desenvolvimento de medicamentos genéricos no Brasil (BRASIL, 1999, 2003); embora desde 1976 as indústrias tenham sido autorizadas a produzirem medicamentos similares e em 1991, com o projeto de Lei número 2002 que posteriormente deu origem ao Decreto 793/93, o qual não foi totalmente implantado, esboçavam os medicamentos genéricos no país. Estas resoluções atuais seguem parâmetros técnicos semelhantes aos dos Estados Unidos e Europa e permitem assegurar a qualidade, eficácia e segurança do medicamento genérico e garantir a sua intercambialidade com o produto de referência.

A biodisponibilidade relaciona-se com a quantidade de fármaco absorvida a partir de uma forma farmacêutica e a velocidade pela qual este processo ocorre (FDA, 2004).

Produtos bioequivalentes são equivalentes farmacêuticos cuja velocidade, avaliada pelo C_{max} (pico da concentração máxima do fármaco), e a extensão de absorção, avaliada pela ASC (área sob a curva da concentração sangüínea versus tempo), não mostram uma diferença significativa quando administrados na mesma dose molar, pela mesma via, em um mesmo indivíduo, sob condições experimentais semelhantes e em dose única ou múltiplas doses (BENET, 1999).

Duas formulações cuja velocidade e extensão de absorção diferem entre -20% até +25% são geralmente consideradas bioequivalentes, porém esta regra só é satisfeita e aceita utilizando o critério estatístico, o qual deve mostrar que o intervalo de confiança (IC) de 90% para as razões dos parâmetros farmacocinéticos deve estar entre 80 e 125%, critério este adotado pela maioria dos órgãos de regulamentação em medicamentos genéricos (BENET, 1999).

Medicamento Genérico é definido pela Agência Nacional de Vigilância Sanitária (ANVISA) como sendo o medicamento similar a um produto de referência ou inovador, que se pretende ser com este intercambiável, geralmente produzido após a expiração ou renúncia da proteção patentária ou de outros direitos de exclusividade, comprovada a sua eficácia, segurança e qualidade, designado pela DCB (denominação comum brasileira) ou, na sua ausência, pela DCI (denominação comum internacional). O medicamento de referência é classificado como sendo aquele produto inovador registrado no órgão federal responsável pela vigilância sanitária e comercializado no País, cuja eficácia, segurança e qualidade foram comprovadas cientificamente junto ao órgão federal competente, por ocasião do registro (BRASIL, 2003).

Dentre as diretrizes para os medicamentos genéricos, há a existência da determinação que somente centros autorizados pela Agência Nacional de Vigilância Sanitária podem realizar os testes de biodisponibilidade/bioequivalência (BRASIL, 2003). A Bioagri Laboratórios Ltda., a Faculdade de Odontologia de Piracicaba

(FOP-UNICAMP) e o Hospital dos Fornecedores de Cana de Piracicaba estão certificados pela ANVISA/MS para a realização das etapas clínica, analítica e estatística de estudos de biodisponibilidade/bioequivalência.

A farmacocinética em estudos de bioequivalência e biodisponibilidade é geralmente baseada na determinação das concentrações do fármaco no sangue. Entretanto, para alguns fármacos, como isoniazida (SURYAWATI & SANTOSO, 1986; HUTCHINGS *et al*., 1988), trimetoprima (WATSON & STEWART, 1986), metronidazol (MUSTOFA *et al*., 1991) e claritromicina (WUST & HARDEGGER, 1993), a concentração salivar tem sido mostrada como sendo proporcional aos níveis sanguíneos e foi então proposta para ser usada em estudos farmacocinéticos para estes fármacos.

Goddard *et al*. (1996) e Ortiz *et al*. (2002) não conseguiram quantificar a amoxicilina em saliva, entretanto Fricker *et al*. (1979) encontraram concentrações em 2 horas e Squinazi (1980) quantificou em 2, 6 e 8 horas após a administração de 500mg de amoxicilina.

A saliva tem sido apontada como um indicador da microbiota oral, sendo um reservatório de microrganismos normalmente derivado de biofilmes da placa dental, do periodonto, do dorso da língua e outras superfícies da mucosa oral (van der VELDEN *et al*., 1986, DAROUT *et al*., 2002).

Meylan *et al*. (1986) sugeriram que estudos *in vivo*, avaliando os efeitos de antimicrobianos que atuam na parede celular sobre a contagem de microrganismos viáveis, poderiam refletir melhor a eficácia clínica destes compostos do que os tradicionais métodos *in vitro*, como concentração bactericida mínima (CBM) ou a razão CBM/CIM (concentração inibitória mínima).

A amoxicilina é um antimicrobiano da classe das penicilinas, amplamente prescrito por décadas em diversos tipos de infecções. Diversos medicamentos contendo amoxicilina estão disponíveis no mercado farmacêutico brasileiro. São apresentações em cápsulas, suspensões orais, injetáveis e

comprimidos, incluindo concentrações de 125, 250 e 500mg, utilizadas normalmente a cada 8 horas e ainda 200, 400, 875 mg e 1g, cujas doses podem ser utilizadas a cada 12 horas (GRISETTI *et al.*, 1999).

A posologia da amoxicilina não tem sido limitada pelos seus efeitos colaterais. As justificativas para o acréscimo nas concentrações das dosagens seriam para melhorar a adesão ao tratamento com a utilização de duas ao invés de três doses diárias (CRAIG & ANDES, 1996), e menos explicados, pelo aumento da tolerância de alguns microrganismos ao fármaco e o aparecimento de algumas cepas mais virulentas (DHAON, 2004).

Além dos parâmetros farmacodinâmicos do fármaco, para escolha de uma ótima posologia é essencial avaliar também os parâmetros farmacocinéticos, principalmente de fármacos como a amoxicilina que tem meia-vida relativamente curta de cerca de 1,7 h (CORTVRIENDT *et al.*, 1987, MOLINARO *et al.*, 1997).

Portanto, verificar a concentração sangüínea torna-se bastante importante para determinar novas doses de medicamentos e um critério exigido para determinação de bioequivalência entre medicamentos. Uma alternativa, a fim de não utilizar um método mais invasivo, como a colheita de sangue, poderia ser a determinação das concentrações do fármaco em outros fluídos biológicos, como por exemplo a saliva. Além da facilidade da colheita, para fármacos que possam ser utilizados para infecções da cavidade oral, como a amoxicilina, as concentrações salivares da amoxicilina poderiam estar diretamente relacionadas à sua atividade antimicrobiana, avaliada, dentre outros métodos, pela redução da microbiota oral.

2. PROPOSIÇÃO

1. Comparar a biodisponibilidade relativa de duas formulações de amoxicilina, amoxicilina 875mg EMS Sigma Pharma (medicamento teste) e de amoxicilina BD 875mg – Amoxil® GlaxoSmithKline (medicamento referência) administradas em regime de dose única em voluntários sadios.

2. Avaliar as concentrações plasmáticas e salivares destas formulações, correlacionando-as com o efeito sobre a microbiota oral.

3. CAPÍTULOS

Esta tese está baseada na Deliberação CCPG/001/98/Unicamp e na aprovação pela Congregação da Faculdade de Odontologia de Piracicaba em sua 105ª Reunião Ordinária em 17/12/2003, que regulamenta o formato alternativo para tese de Doutorado e permite a inserção de artigos científicos de autoria do candidato.

Assim sendo, esta tese é composta de dois capítulos contendo artigos que se encontram em fase de submissão para publicação em revistas científicas, conforme descrito a seguir:

Capítulo 1

Artigo "*Comparative bioavailability of 875mg amoxicillin tablets in healthy human volunteers*"

Este artigo foi submetido ao periódico: *International Journal of Clinical Pharmacology and Therapeutics* (vide Anexo 2).

Capítulo 2

Artigo "*Plasmatic and salivary amoxicillin concentrations: the effect against oral microbiota*"

Este artigo estará sendo submetido ao periódico: *Archives of Oral Biology*.

3.1 Capítulo 1

Comparative bioavailability of 875mg amoxicillin tablets in healthy human volunteers.

S. Baglie[1,2], P.L. Rosalen[1], L.M. Franco[3], A.P.D.B. Ruenis[1], R.C.C. Baglie[1], G.C.N. Franco[1], P. Silva[4] and F.C. Groppo[1]

[1]*Piracicaba Dental School, State University of Campinas (UNICAMP), SP, Brazil;*

[2]*Department of Pharmaceutical Sciences, Ponta Grossa State University, PR, Brazil;*

[3]*Faculty of Health Sciences, Methodist University of Piracicaba, SP, Brazil;*

[4]*Bioagri Laboratories Ltda, Piracicaba, SP, Brazil.*

Running Title: Comparative bioavailability of 875mg amoxicillin

Correspondence to:
Francisco Carlos Groppo
Faculdade de Odontologia de Piracicaba
Universidade Estadual de Campinas
Av. Limeira 901
Piracicaba, SP, Brazil
Zip-code 13414-903
fcgroppo@fop.unicamp.br

Abstract. Objective: To compare the bioavailability of amoxicillin 875mg tablets (EMS Sigma Pharma used as test formulation) and Amoxil® BD 875mg tablets (GlaxoSmithKline used as reference formulation) in 26 healthy volunteers. Material and methods: Twenty-six healthy volunteers (13 males and 13 females) received each formulation in an open, 2×2 crossover, randomized study with 7 days of washout period between doses. Plasma samples were obtained over a 12h interval after administration. Plasmatic amoxicillin concentrations were obtained by combined reversed-phase liquid chromatography and mass spectrometry with positive ion electrospray ionization using the select ion monitoring method. AUC was calculated by the trapezoidal rule extrapolation method. C_{max} and T_{max} were compiled from the plasmatic concentration-time data. Analysis of variance was carried out using logarithmically transformed $AUC_{0\text{-}inf}$, $AUC_{0\text{-}12h}$, C_{max} and untransformed T_{max}. Results: The mean values (± SD) for $AUC_{0\text{-}12h}$ ($\mu g.h.mL^{-1}$), $AUC_{0\text{-}inf}$ ($\mu g.h.mL^{-1}$), C_{max} ($\mu g.mL^{-1}$) and T_{max} (h), were, respectively: 55.42(±16.85), 55.42 (±16.85), 18.59 (±6.3), 2.04(±0.75) concerning the test formulation, and 51.11 (±18.9), 51.29 (±19.12), 17.83 (±5.86), 2.02 (±0.87) concerning the reference formulation. Confidence intervals (90%) of amoxicillin means of $AUC_{0\text{-}12h}$ and C_{max} ratios (test/reference) were: 0.961 – 1.149 and 0.914 – 1.142, respectively, agreeing whit the bioequivalence criteria established by the Brazilian National Health Surveillance Agency. Conclusion: Both formulations were bioequivalent based on both the rate and extent of absorption.

Key words

Amoxicillin - comparative bioavailability – bioequivalence – pharmacokinetics.

Introduction

Amoxicillin [[2S-[2α,5α,6β(S*)]]-6-[[Amino(4hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxilic acid] is an oral semi-syntheticpenicillin structurally related to ampicillin [Nathwani and Wood 1993]. A benzyl ring in the side chain of the molecule is responsible for the extended antibacterial activity against gram-negative bacteria [Tomas, 1979]. The amoxicillin mechanism of action has not been completely established, but it is usually accepted that penicillins may interfere with peptidoglycan bacterial cell-wall synthesis of the sensible organisms [Sutherland and Rolinson 1970, Neu 1974, Waxman and Strominger 1983].

Currently, numerous amoxicillin formulations are available in capsules, tablets or suspensions, offering a variety of doses such as 125, 200, 250, 375, 400, 500, 750, 875mg, and 1g [Brundusino et al. 1999].

The common oral dose of amoxicillin for adult ranges from 250mg to 500 mg tid [Langan et al. 1997, Georgopoulos et al. 2001], and the pediatric dose ranges from 25 to 50mg/kg/day [Garbutt et al. 2001, Agarwal et al. 2004]. The higher doses formulation has been widely prescribed twice a day, with 12 hours interval, which the same therapeutics efficacy for both adults and children [Lan et al. 2000, Bantar et al. 2000, Georgopoulos et al. 2001].

Bioavailability studies of amoxicillin have been previously reported. Oliveira et al. [2001] evaluated the bioavailability of two oral suspensions of 250mg/5mL and two 500mg capsules of commercial amoxicillin, in healthy subjects. A LC-MS-MS quantification method showed plasmatic amoxicillin levels of 4.94µg/mL (test medication)

and 5.31µg/mL (reference medication) and 7.11µg/mL (test) and 7.7µg/mL (reference), respectively for 250mg and 500mg doses. Similar results were observed by Pires de Abreu et al. [2003], which found plasmatic amoxicillin levels of 8.1µg/mL (test) and 8.15µg/mL (reference) after a single 500mg oral dose.

There is no data about bioequivalence of 875mg amoxicillin formulations in the indexed literature. The purpose of the present study was to compare the bioavailability of two formulations 875mg amoxicillin.

Subjects, material and methods

Test and reference medications

The test medication was Amoxicillin - 875mg tablets (EMS Sigma Pharma, lot n° AMO875LG02, Sao Paulo, Brazil), and the reference medication was Amoxil® BD - 875mg tablets (GlaxoSmithKline, lot n° DC0024, Rio de Janeiro, Brazil).

Clinical protocol

Twenty-six healthy volunteers (13 males and 13 females), ranging in age from 19 - 40 years (22.65 ± 4.08 years), in weight from 52.4 - 87.2kg (68.11 ± 10.44kg), and in height from 1.55 - 1.94m (1.72 ± 0.09m) completed the study. The volunteers were selected after passing a clinical screening procedure, and were free from significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal and hematological disease as well as psychiatric disorders, as determined by their medical history, physical examination, ECG

and routine laboratory tests (blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, γ-GT, total bilirubin, albumin, total protein, triglyceride, total cholesterol, hemoglobin, hematocrit, total and differential white cell counts, erythrocyte sedimentation rate, and routine urinalysis). All subjects were negative to HIV, HBV, HCV and addictive drugs. In female volunteers, βHCG was performed as pregnancy test and all were negative. Volunteers were excluded if they were possibly sensitive to penicillins, had a history of any illness of hepatic, renal, or cardiovascular systems, or had taken alcohol or other medications for a long period of time. This was done to ensure that the existing degree of variation would not be due to an influence of illness or other medications [Lerner et al. 2000].

This study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of Good Clinical Practice. The protocol of this study was approved by the Ethical Committee of Piracicaba Dental School of State University of Campinas (SP, Brazil). All participants signed a written informed consent after they had been informed of the nature and details of the study.

All subjects avoided using other drugs for at least 2 weeks prior to the study and until after its completion. They also refrained from alcoholic drinks and xanthine-containing foods and beverages; including tea, coffee, and cola, 48 hours prior to each dosing and until the collection of the last blood sample [Kim et al. 2001].

The study was conducted in an open randomized two-period crossover balanced design with a one week washout period between doses. During each period, the volunteers were hospitalized at night preceding the drug administration until last blood collection. After fasted 12 hours before dosing, the subjects received a single oral dose of either

amoxicillin medication with water (200 mL). Standard meal was provided 4, 8, 10 and 12 hours after drug administration. No other food was permitted during the hospitalization period. Water consumption was permitted *ad libitium* 2 hours after drug administration. Subjects were not allowed to remain in a supine position or to sleep during the whole blood collection period. Every 2 hours, body temperature, systolic and diastolic arterial pressure and heart rate were recorded. The volunteers were instructed to inform any adverse event during whole study.

Blood samples (8 mL) from antecubital vein, via heparinized cannula, were collected into sterile tubes with 100µL of 10% EDTA solution, at pre-dose (0), and 0h30min, 1h, 1h20min, 1h40min, 2h, 2h30min, 3h, 4h, 6h, 8h and 12 hours after the drug administration. The heparinized normal saline injection solution, 1 mL, was flushed into the cannula to prevent blood clotting, after each blood sampling. Right after each blood collection, the samples were centrifuged 2000 g during 15 min in a cooled centrifuge (4^{o}C) and immediately the plasma samples were frozen at -70°C until analysis.

Bioanalytical assay of amoxicillin in plasma

Amoxicillin and cefadroxil (internal standard) were extracted from plasma by solid-phase extraction using C-18 cartridges (Oasis, Waters Corp., USA). Samples were prepared by vortex-mixing (1min) 400µL of plasma, 1mL of sodium phosphate monobasic (pH 3.2), and 20µL of internal standard (360µg/mL). These samples were eluted in the preconditioned cartridges with 2mL of methanol and 1mL of phosphate buffer (pH 3.2).

Cartridges were washed by using 1mL of phosphate buffer and 1mL of water, drying the columns by vacuum (15min/5psi). The extraction were performed eluting 6mL of methanol:isopropanol (40:60) through the cartridges, which were dried under nitrogen flux at 40°C. Ammonium acetate buffer (40µL - pH 5.4) was added to eluate.

The samples were analyzed by a Hewlett Packard 1100 Series chromatography system (LC-ESI-MS), set in positive mode (ES+).

The chromatography was performed by using a reverse phase analytical column RP-18 (250mm x 2.1mm x 5µm) and reverse phase guard column RP-18 (4.0mm x 3.0mm x 5µm), with a flow rate of 0.3 mL/min at 40°C. The mobile phase involved a mixture of ammonium acetate buffer pH 4.0 (A) and acetonitrile (B), using a gradient of 95% A and 5% B (from t=0 to 3 min); 20% A and 80% B (from t=3 to 5 min); 20% A and 80% B (from t=5 to 7 min); and 95% A and 5% B (from t=7 to the end of the run time - 8 min). Amoxicillin's ions were monitored at 366m/z and cefadroxil at 364m/z.

Pharmacokinetic analysis

The maximum observed plasma concentration (C_{max}) and the time taken to achieve this concentration (T_{max}) were obtained directly from the curves. The areas under the amoxicillin plasma concentration vs. time curves from 0–12h (AUC_{0-12h}) were calculated by applying the linear trapezoid rule. Extrapolation of these areas to infinity (AUC_{inf}) was determined by using computer software calculation (PK Solutions, Non-compartmental Pharmacokinetics Data Analysis Excel Template, 2001, Summit Research Services, Montrose, CO, USA).

Statistical analysis of data

The statistical analysis was performed by using computer software (SAS 1999-2001, SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed on all variables (log transformed values), except for T_{max}. Confidence interval (CI) was obtained from ANOVA data, except for T_{max}. The treatment, sequence, study period and subject within sequence were considered in the ANOVA. The 90%CI of the geometric mean for the individual test/reference ratios for $AUC_{0\text{-}inf,}$ $AUC_{0\text{-}12h}$ and C_{max} were used to determine the bioequivalence between the formulations, observing the acceptance range of 80 to 125% (0.8-1.25), which is established for the Brazilian National Health Surveillance Agency.

Results and discussion

The method for amoxicillin quantification used in the present study was validated and provided high sensibility and specificity, as required by pharmacokinetic studies [Benet 1999]. Briefly, three amoxicillin solutions (0.90, 17 and 35µg/mL) were used as quality controls. The intra-day variabilities were 5.21, 9.38 and 7.80%, respectively. The inter-day variabilities were 9.65, 8.38, and 6.88%, respectively. The mean absolute recoveries of amoxicillin/cefadroxil were 75.67/94.45% at 0.90µg/mL, 58.15/85% at 17µg/mL, and 61.42/82.50% at 35µg/mL. Both the detection limit (0.2µg/mL) and the quantification limit (0.3µg/mL) were similar to other liquid chromatography methods, such as HPLC coupled to UV [Muth et al. 1996] or column switching ion-pair HPLC [Carlqvist

and Westerlund 1985]. However, the present method showed lower retention time for both amoxicillin and internal standard.

Twenty-six volunteers completed the study in good health conditions as confirmed by post-study clinical anamnesis and laboratorial tests. Both amoxicillin formulations were well tolerated after oral administration. Adverse effects related were headache 9.6% and nausea 1.9% of subjects considering both medications. These effects were not considered clinically significant since they were similar to the ones previously reported on medical literature [Grover and Tyagi 1993]. The fasting period could also contribute to the observed adverse effects. The sample size (n=26) assured reliable results for statistical analysis, providing 100% of power analysis (β=100%).

The mean of amoxicillin plasma concentrations regarding test and reference formulations are presented in the Figure 1. The T_{max} observed in present study result was similar to results reported by Pires de Abreu et al. [2003] and Oliveira et al. [2001] both using capsules of 500mg amoxicillin. The authors observed a T_{max} between 1.7h and 2.0h.

It is well established that the dose affects the C_{max} and the area under the curve. The dose of amoxicillin in our study (875mg) provided, as expected, higher C_{max} than the ones observed in studies using 500mg amoxicillin [Pires de Abreu et al. 2003; Oliveira et al. 2001].

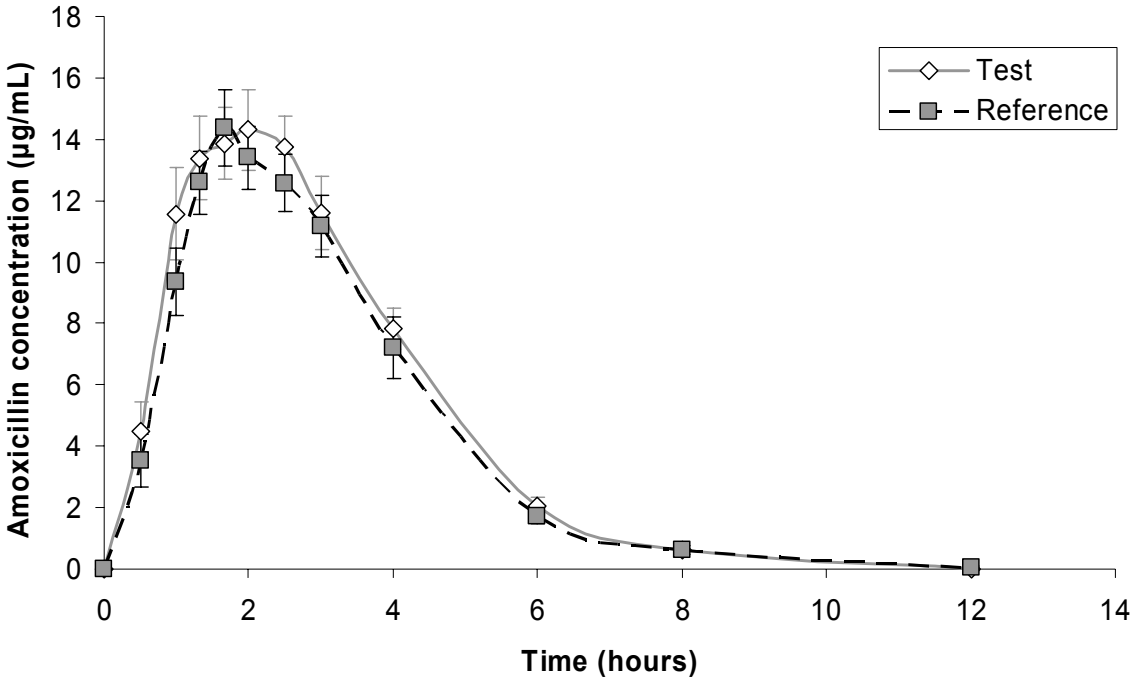

Figure 1. Mean (± SEM) plasma concentration-time profiles of amoxicillin after single oral administration of 2 amoxicillin 875mg tablets (amoxicillin EMS as test and Amoxil as reference).

Table 1 shows the means of each pharmacokinetic parameter ($AUC_{0\text{-}12h}$, $AUC_{0\text{-}inf}$, C_{max} and T_{max}) of 875mg amoxicillin tablets. Pires de Abreu et al. [2003] observed mean values of $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}inf}$ of 21.7 (range 14.1 - 33.8) and 24.3 (range 15.6 - 38.8)µg/mL.h, considering 500mg amoxicillin capsules. In this study, higher values (more than two times higher) were observed (mean $AUC_{0\text{-}12h}$ = 53.3 µg/mL.h and mean $AUC_{0\text{-}inf}$ =53.4µg/mL.h) certainly due to the use of 875mg amoxicillin. Similar comparison is valid considering the results observed by Oliveira et al. [2001].

Table 1. Mean (± SD) pharmacokinetic parameters after oral administration of amoxicillin 875mg tablets (test and reference) to 26 healthy volunteers.

Pharmacokinetic parameters	**Amoxicillin EMS (test)**	**Amoxil (reference)**
$AUC_{0\text{-}12h}$ ($\mu g.h.mL^{-1}$)	55.42(±16.85)	51.11 (±18.9)
$AUC_{0\text{-}inf}$ ($\mu g.h.mL^{-1}$)	55.42 (±16.85)	51.29 (±19.12)
C_{max} ($\mu g.mL^{-1}$)	18.59 (±6.3)	17.83 (±5.86)
T_{max} (h)	2.04(±0.75)	2.02 (±0.87)

Confidence interval (90%) ratio considering test and reference formulations was 0.914 – 1.142 for C_{max} and 0.961 – 1.149 for $AUC_{0\text{-}12h}$.

Amoxicillin/Amoxil 875mg tablets showed the 90% confidence interval similar to described by Oliveira et al. [2001] using Amoxicillin/Amoxil 500mg capsule. These authors observed an IC 90% range of 0.850 - 1.023 for $AUC_{0\text{-}8h}$ and 0.801 - 1.027 for $C_{max.}$ The results demonstrated are within the 80 to 125% confidence, which is the interval recommended the by regulatory agencies [ANVISA/Brazil 2004, FDA/USA 2004] for bioequivalent formulations.

Conclusion

Both formulation - amoxicillin 875 mg tablets (amoxicillin 875 mg tablets - EMS Sigma Pharma and Amoxil® BD – GlaxoSmithKline 875 mg tablets) are bioequivalent, based on rate and extent of absorption, and thus may be prescribed interchangeably.

Acknowledgments

This study was supported by the contract between EMS Sigma Pharma and Bioagri Laboratorios Ltda – Bioequivalence division, Sao Paulo, Brazil. The author Baglie S. was supported by a scholarship provided by CAPES.

References

Agarwal G, Awasthi S, Kabra SK, Kaul A, Singhi S, Walter SD; Iscap Study Group (2004) Three day versus five day treatment with amoxicillin for non-severe pneumonia in young children: a multicentre randomised controlled trial BMJ 328: 791

Anvisa - National Health Surveillance Agency - Brazil (2003) Resolution - RE nº 397 of Nov 12nd 2004. Available in: <http://www.anvisa.gov.br/eng/legis/resol/397__12_11_04_re_e.htm > Access in: 2004 Dec 14th

Bantar, C, Nicola F, Fernandez Canigia L, Arenoso HJ, Soutric J, Montoto M, Blanco M, Smayevsky J, Jasovich A (2000) A pharmacodynamic model to support a 12-hour dosing interval for amoxicillin/sulbactan, a novel oral combination, in the treatment of community-acquired lower respiratory tract infections J Chemother 12: 223-277

Benet, LZ (1999) Understanding bioequivalence testing Transplant Proc 31: 7S-9S

Brundusino A, Vasconcellos LH, Pinto Coelho DSB, Oliani C (1999) Randomized cross-over pharmacokinetic study with two oral amoxicillin pharmaceutical forms (tablets and capsules) at three different dosages European Bulletin of Drug Research 7: 32-53

Carlqvist J, Westerlund D (1985) Automated determination of amoxycillin in biological fluids by column switching in ion-pair reversed-phase liquid chromatographic systems with post-column derivatization J Chromatogr 344:285-96

Food and Drug Administration (FDA/USA) (2004) Title 21, Part 320 Bioavailability and Bioequivalence Requirements Available in: <http://www.fda.gov> Access in: 2004 nov. 04th

Garbutt JM, Goldstein M, Gellman E, Shannon W, Littenberg B (2001) A randomized, placebo-controlled trial of antimicrobial treatment for children with clinically diagnosed acute sinusitis Pediatrics 107: 619-625

Georgopoulos A, Borek M, Ridl W (2001) Amoxycillin Bronchitis Study Group Randomized, double-blind, double-dummy study comparing the efficacy and safety of amoxycillin 1 g bd with amoxycillin 500 mg tds in the treatment of acute exacerbations of chronic bronchitis J Antimicrob Chemother 47:67-76

Grover JK, Tyagi D (1993) Adverse reactions associated with aminopenicillins in Indian population Indian J Physiol Pharmacol 37:247-248

Kim YG, Kim HJ, Kwon JW, Kim, WB, Lee MG (2001) Bioequivalence of clarithromycin tablet formulations assessed in Korean males Int J Pharmacol Ther 39:356-361

Lan AJ, Colford, JM Colford-Jr JM (2000) The impact of dosing frequency on the efficacy of 10-day penicillin or amoxicillin therapy for streptococcal tonsillopharyngitis: A meta-analysis Pediatrics 105:E19

Langan CE, Cranfield R, Breisch S, Pettit R (1997) Randomized, double-blind study of grepafloxacin versus amoxycillin in patients with acute bacterial exacerbations of chronic bronchitis J Antimicrob Chemother 40 Suppl A:63-72

Muth P, Metz R, Beck H, Bolten WW, Vergin H (1996) Improved high-performance liquid chromatographic determination of amoxicillin in human plasma by means of column switching J Chromatogr A. 729:259-66

Lerner FE, Caliendo G, Santagada V, Santana GSM, Moraes MEA, De Nucci G (2000) Clarithromycin bioequivalence study of two oral formulations in healthy human volunteers Int J Clin Pharmacol Ther 38:345-354

Nathwani D, Wood MJ (1993) Penicillins. A current review of their clinical pharmacology and therapeutic use Drugs 45: 866-894

Neu HC (1974) Antimicrobial activity and human pharmacology of amoxicillin J Infect Dis 129: S123-131

Oliveira CH, Abib E, Vannuchi YB, Sucupira M, Ilha J, De Nucci G (2001) Comparative bioavailability of 4 amoxicillin formulations in healthy human volunteers after a single dose administration Int J Clin Pharmacol Ther 39:167-172

Pires de Abreu LR, Ortiz RM, De Castro SC, Pedrazzoli J Jr (2003) HPLC determination of amoxicillin comparative bioavailability in healthy volunteers after a single dose administration J Pharm Pharm Sci 6: 223-230

Sutherland R, Rolinson GN (1970) Alfa-amino-p-hydroxybenzylpenicillin (BRL 2333), a new semisynthetic penicillin: in vitro evaluation Antimicrobial Agents Chemother 10: 411-415

Tomas A (1979) From penicillin-binding proteins to the lysis and death of bacteria: a 1979 review Rev Infect Dis 1: 434-467

Waxman DJ, Strominger JL (1983) Penicillin-binding proteins and the mechanism of action of beta-lactam antibiotics Annu Rev Biochem 52: 825-869

3.2 Capítulo 2

Plasmatic and salivary amoxicillin concentrations: the effect against oral microbiota.

Sinvaldo Baglie, PharmD, MS[1,2]
Ana Paula Del Bortolo Ruenis, PharmD, MS, CCRP[2]
Gilson Cesar Nobre Franco DDs[2]
Luiz Madaleno Franco, PharmD, MS[3]
Roberta Cristiane Catelli Baglie, PharmD, MS[2]
Rogério Heládio Lopes Motta DDs, MS[2]
Pedro Luiz Rosalen, PharmD, PhD[2]
[*]Francisco Carlos Groppo, DDs, PhD[2]

[1] Department of Pharmaceutical Sciences – Ponta Grossa State University

[2] Piracicaba Dental School – The State University of Campinas - Area of Pharmacology, Anesthesiology and Therapeutics - FOP/UNICAMP

[3] Faculty of Health Sciences – The Methodist University of Piracicaba - UNIMEP

Correspondence author:
[*]Francisco Carlos Groppo
Faculdade de Odontologia de Piracicaba
Universidade Estadual de Campinas – UNICAMP
Av. Limeira, 901; 13414-903
Piracicaba, SP, Brazil
E-mail: fcgroppo@fop.unicamp.br

Abstract

Objective: To quantify both plasmatic and salivary concentrations after a single 875mg oral dose of two amoxicillin (AMO) formulations. In addition, to assess the effect of amoxicillin against oral microbiota. Design: Twenty healthy volunteers were evaluated in an open, randomized, two-period crossover design. Tablets of AMO (Group 1 – Amoxicillin-EMS and Group 2 - Amoxil®) was administered to all volunteers observing 1-week interval between each administration. Saliva and blood samples were collected previously and after 0.30, 1, 2, 4, 8 and 12 hours of drug administration. Blood and saliva samples were quantified by liquid chromatography methods (LC-ESI-MS and LC, respectively). Streptococci, anaerobe, and total microorganism counts were obtained on different culture conditions. Results: No statistically significant differences were observed between the two formulations (*t* test, $p>0.05$) regarding AMO plasmatic and saliva concentrations. Salivary microorganism counts did not show statistical differences (Repeated Measures ANOVA, $p>0.05$) between the two formulation during each sampling time. Starting at 60 min, a statistically significant decrease (Repeated Measures ANOVA, $p<0.05$) was also observed considering all microorganisms. Conclusion: Both formulation of amoxicillin in a single 875mg dose were effective in reduce oral microorganisms until 12 hours.

Key words: amoxicillin, saliva, microorganism, plasmatic concentration

Introduction

Amoxicillin is widely prescribed penicillin, which is mainly used due to its extended spectrum, a rapid and extensive oral absorption, unhindered by food and good tolerance by patients[1].

Penicillin kills susceptible bacteria by specifically inhibiting the transpeptidase catalyzing the final step in cell-wall biosynthesis, the cross-linking of peptidoglycan[2]. Previus studies using time kill assay have shown that the bactericidal activity of amoxicillin is more effective than other betalactam antibiotics.[3,4,5]

The oral daily recommended dosage to adult patients is usually 250 to 500mg tid.[6,7] For pediatric patients depending on the infection severity and the etiological agent, the dosage is usually 25 to 50mg/kg tid.[8,9] Higher doses has been widely prescribed twice a day, with 12 hours interval, which the same therapeutics efficacy for both adults and children.[7,10,11]

Amoxicillin has been used as the first choice in several odontogenic infections and bacterial endocarditis prophylaxis.[12] *Viridans* streptococci (alpha-hemolytic streptococci) and *S. aureus* are the most common cause of endocarditis following dental or oral procedures, upper respiratory tract procedures, bronchoscopy with a rigid bronchoscope, surgical procedures involving the respiratory mucosa, and esophageal procedures.[13,14,15] The standard bacterial endocarditis prophylaxis regimen includes 2g single dose of amoxicillin, one hour

before the procedure.[12]

Measurement of amoxicillin in body fluids can be performed using high performance liquid chromatography (HPLC) techniques previously described[16,17]. Also, reversed phase liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS) was used to quantify the compound.[18]

The aim of this study was to compare both plasmatic and salivary concentration of two amoxicillin formulations and their effect against oral microbiota of healthy volunteers.

Materials and methods

This study enrolled 20 healthy volunteers (50% of males), ranging in age from 19 to 40 years (22.4 ±4.6), in weight from 52 to 87 kg (69.38 ± 11.36 kg) and in body mass index from 21 to 25 (22.8±1.98). The clinical trial was conducted according to the I.C.H Harmonized Tripartite Guideline for Good Clinical Practice (1996) and the Declaration of Helsinki (1965).

The subjects were informed of the purpose, protocol and risks of the study. Individuals using any medications 14 days prior to or throughout the study, including over-the-counter products, and showing hypersensitivity to amoxicillin were excluded. The FOP-UNICAMP Ethics Committee approved the protocol and all subjects provided written informed consent.

The volunteers were hospitalized and fasted 12 hours before dosing. The subjects received a single oral dose of either Amoxicillin EMS (875mg tablets, EMS Sigma Pharma, Sao Paulo, Brazil), and the Amoxicillin - Amoxil® BD (875mg tablets, GlaxoSmithKline, Rio de Janeiro, Brazil) in an open, randomized, two-period crossover design with 1-week wash-out period between doses.

Standardized meals were given 4, 8, 10 and 12 hours after drug administration. No other food was permitted during the sampling period. Except xanthine-containing drinks including tea, coffee, and cola derivatives, liquid consumption was permitted *ad libitium* 2 hours after drug administration. All the volunteers were instructed not to perform tooth brushing and dental flossing from the beginning of the fasting period to the final saliva sampling (12 h).

Non-stimulated saliva samples and blood samples were collected at the following time periods: pre-dose, 30 minutes, 1, 2, 4, 6 and 12 hours after drug administration in order to assess pharmacokinetics parameters.

The blood samples were centrifuged at 2000 g during 15 minutes (4^{o}C) and plasma was stored at -70^{o}C for further LC-ESI-MS analysis. Aliquots of saliva samples were stored at -70^{o}C for further LC analysis.

Amoxicillin and cefadroxil (internal standard) were extracted from plasma by solid-phase extraction using C-18 cartridges (Oasis, Waters Corp., USA). Samples were prepared by vortex-mixing (1min) 400µL of plasma, 1mL of sodium phosphate monobasic (pH 3.2), and 20µL of internal standard (360µg/mL). The

samples were analyzed by a Hewlett Packard 1100 Series chromatography system (LC-ESI-MS), set in positive mode (ES+).

The chromatography was performed by using a reverse phase analytical column RP-18 (250x2.1mmx5µm) and reverse phase guard column RP-18 (4.0x3.0mmx5µm), with a flow rate of 0.3 mL/min at 40°C. The mobile phase involved a mixture of ammonium acetate buffer pH 4.0 and acetonitrile (run time - 8 min). Amoxicillin's ions were monitored at 366m/z and cefadroxil (internal standard) at 364m/z.

Saliva samples added of cefadroxil (30µL of 200µg/mL) as internal standard were submitted to liquid-liquid extraction by using perchloric acid.[19] Aliquots of 50µL were injected in the liquid-chromatography device (9012 HPLC pump, UV-VIS detector 9050 and Star 9050 integrator software - Varian Inc. Corporate, Palo Alto, USA) using a 5-µm particle size Lichrocart column 100 RP18 (250×4 mm I.D., Merck, Darnstadt, Germany). Samples were eluted with a mobile phase consisting of 0.01 M aqueous solution of sodium monophosphate (pH=4.8) and acetonitrile (99:1). The flow-rate was 2.0 mL/min and the wavelength was set at 229 nm. Total analysis time was 6 min. All analyses were performed at room temperature.

Aliquots of saliva were diluted right after collection and 50 µL of each sample were logarithmically plated by an automatic spiral plater (Whitley

Automatic Spiral Plater, Don Whitley Scientific Limited, Shipley, UK) on three different culture conditions, in duplicate.

Total microorganisms were recovered from 5% Blood Sheep Agar (BSA) after 24 hours of incubation at 37°C, 10% CO_2, and afterwards at 37°C in aerobic conditions. Streptococci were recovered from Mitis Salivarius Agar (MSA) after 48 hours of incubation at 37°C, 10% CO_2. Anaerobes were recovered from 5% Blood Sheep Agar (BSA), after 7 days of incubation at 37°C in anaerobic environment (80% N_2, 10% CO_2 and 10% H_2).

Paired t test (alpha=0.05) was performed to verify differences between the two formulations considering plasmatic and salivary concentration.

The number of colony forming units (cfu/mL) was obtained by manual counting device, considering each time of saliva collection and each amoxicillin formulation. ANOVA repeated measures test (alpha=0.05) and Tukey's Studentized Range (HSD) was used to analyze the microorganisms counts comparing the time periods for each formulation and comparing both formulations considering each time period.

Results

No statistically significant differences were observed between the two formulations (p>0.05) considering the amoxicillin plasmatic and salivary

concentrations. The concentration (mean ± SEM) observed considering both formulations at each sampling time points are illustrated in Figure 1.

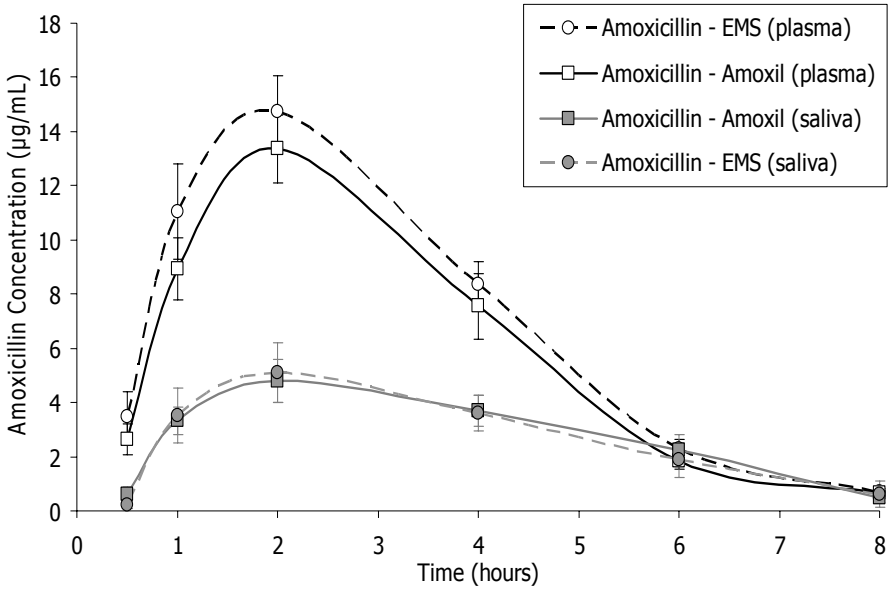

Figure 1. Concentration versus time curves. Mean plasmatic concentrations (µg/mL ± SEM) and mean salivary concentration (µg/mL ± SEM) of the two amoxicillin formulations obtained from 20 volunteers.

There were no statistically significant differences (p>0.05) between the formulations regarding each microorganism (streptococci, total microorganisms or anaerobe) counts considering each sampling time. However, a comparison among the time points regarding each formulation showed statistically significant differences from 1hour to 12 hours (p<0.05) for all studied microorganisms.

The Figures 2, 3 and 4 present, respectively, the log of mean (±SEM) of streptococci, total and anaerobic microorganism counts from saliva sampling after the administration of both amoxicillin formulations tested.

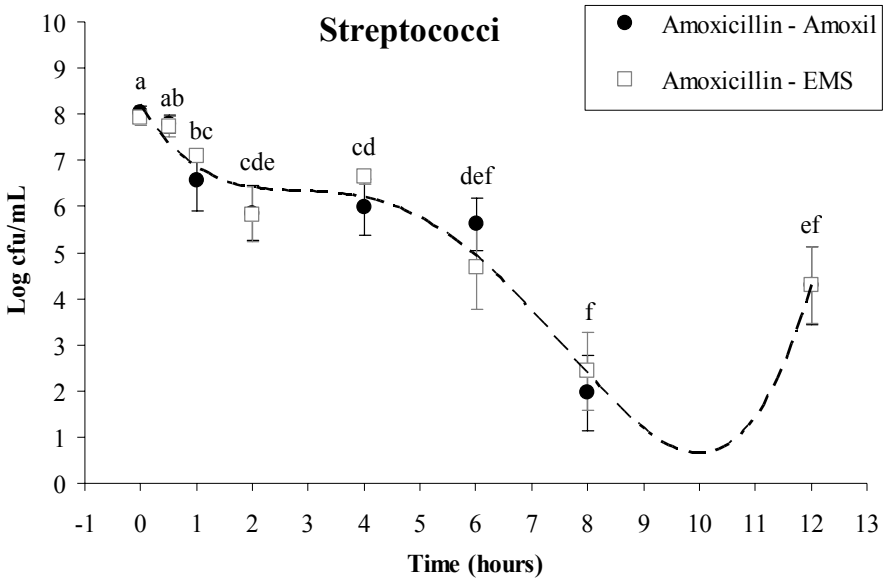

Figure 2. Mean values (±SEM) of streptococci counts (log cfu/mL) from saliva sampling (black-dashed line represents the regression curve). Different letters means statistically significant differences ($p<0.05$).

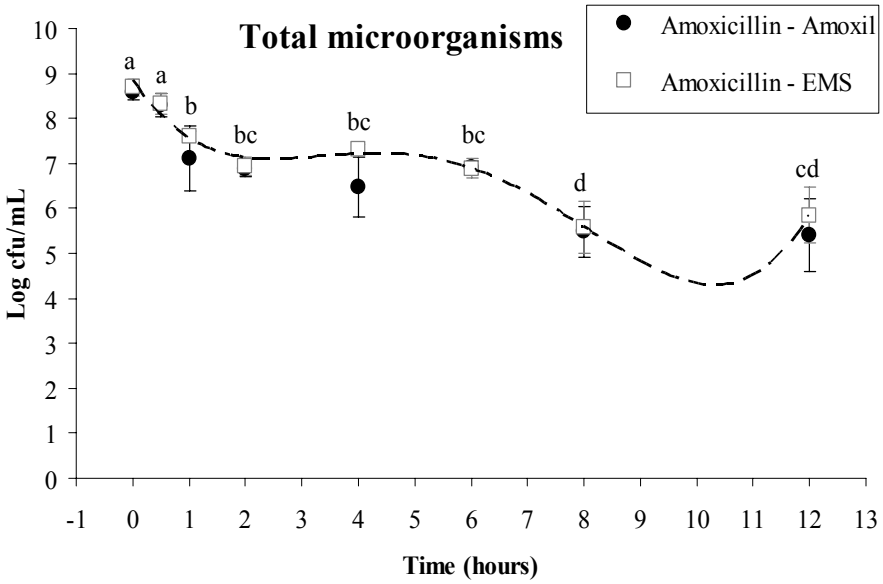

Figure 3. Mean values (±SEM) of total microorganism counts (log cfu/mL) from saliva sampling (black-dashed line represents the regression curve). Different letters means statistically significant differences ($p<0.05$).

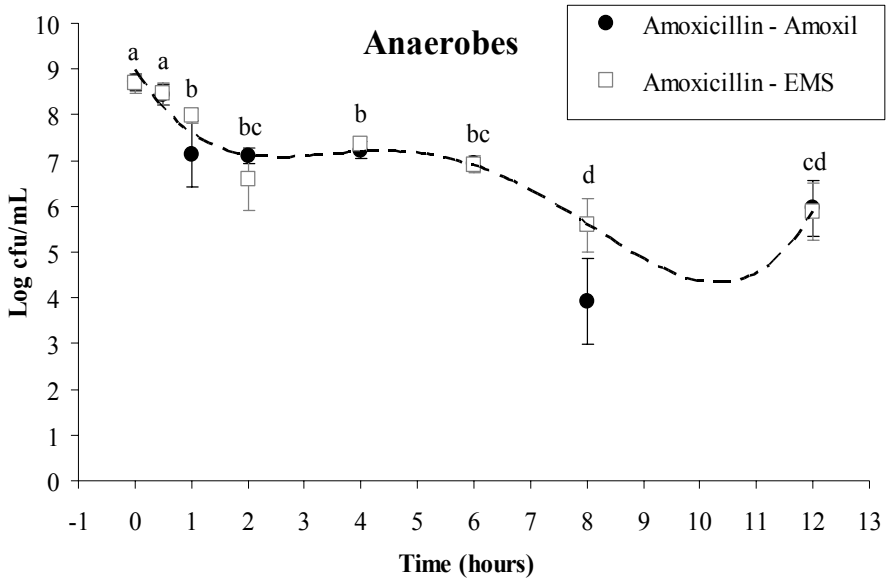

Figure 4. Mean values (± SEM) of anaerobic microorganism counts (log cfu/mL) from saliva sampling (black-dashed line represents the regression curve). Different letters means statistically significant differences ($p<0.05$).

Discussion

Several methods have been described to assay amoxicillin in body fluids. High performance liquid chromatography (HPLC) techniques were previously described.[16,17] Also, reversed phase liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS) was used to quantify the antibiotic.[18] In the present study, we validated a LC-ESI-MS assay to quantify amoxicillin plasmatic concentrations which showed linearity, specificity, precision, accuracy (data not shown) after a solid extraction procedure. Quantification in saliva samples were performed by a slightly modified HPLC method[17] resulting in a good chromatographic separation and quantification.

There is no similar data regarding both plasmatic and salivary concentrations of 875mg amoxicillin in the literature. As expected, the results obtained in the present study for plasmatic concentration were higher than those reported in previous studies using healthy volunteers submitted to a single 500mg oral dose of amoxicillin[17,18] and lower than those using 1000mg of amoxicillin[16]. However, the T_{max} parameter was similar to the previous reported studies.[16,17,18] In addition, both formulations tested showed the same profile either considering salivary or plasmatic concentration, i.e., both could be considered therapeutically equivalent.

Previous studies have showed undetectable concentrations in saliva after an intravenously single-dose of 500mg amoxicillin.[20,21] However, in the present study it was possible to quantify the antimicrobial agent until 8 hours after administration, probably due to the higher dose of amoxicillin used. The salivary concentration certainly had influenced the decrease of all microorganisms in the saliva samples.

Although whole saliva has no distinctive microbiota of its own[22], it harbors as much as 10^8 bacteria/mL[23] and is a reservoir of microorganisms regularly derived from dental plaque biofilms adhering to gingival crevices, periodontal pockets, the dorsum of the tongue, and other oral mucosal surfaces[24], reflecting the oral microbiota.

The study of the *in vivo* effect of amoxicillin over viable microorganism counts is more accurate to reflect clinical efficacy than the traditional *in vitro* methods, such as MBC or the MBC/MIC ratio.[25] Both amoxicillin formulations demonstrated effectiveness against the microorganisms, since a decrease in bacterial counts were observed starting from 60 min after drug administration, which was maintained over the sampling period for all microorganisms evaluated. Streptococci showed the largest decrease among the microorganisms.

A previous study using a single dose of three grams of amoxicillin observed a marked drop in the concentration of streptococci after four hours but almost all subjects substantially recovered the microorganisms after 48h of drug administration. All subjects recovered the initial number at seven days.[26] The present study showed that the number of streptococci was keep lower than the initial number after 12 hours showing a tendency to increase the number after this period.

It is currently accepted that oral streptococci, especially *viridans* group isolated from dental infections, are still susceptible to many antimicrobial agents. A previous study showed a range between 0.016 to 3µg/mL considering the MIC_{50} for *S. mitis*, *S. oralis* and *S. sanguis* isolated from aspirates of pus of dento-alveolar infections.[27,28] In the present study, we showed that amoxicillin remained very closed to these MIC values until 6 hours, which could explain the reduced levels of streptococci until 12 hours after drug administration.

Beta-lactam antibiotics are still considered to be the antimicrobial of choice for orofacial endodontic infections, which are caused mainly by anaerobe microbiota.[29,30] Despite some penicillin-resistant anaerobe species have been reported (especially Gram negative anaerobes), the prevalence of these bacteria is still considered low.[29] This low resistance could be also responsible for the significative reduction of anaerobes strains verified after 8 hours of both drug administration in the present study.

In conclusion, both formulations of amoxicillin showed similar pharmacokinetics in plasma and in saliva, and were effective in reduce the oral microbiota.

Acknowledgment

The author Baglie S. was supported by a scholarship provided by CAPES.

References

1. Dhaon NA. Amoxicillin tablets for oral suspension in the treatment of acute otitis media: a new formulation with improved convenience. *Adv Ther* 2004; **21**: 87-95.
2. Yocum RR, Rasmussen JR, Strominger JL. The mechanism of action of penicillin. Penicillin acylates the active site of Bacillus stearothermophilus D-alanine carboxypeptidase. *J Biol Chem* 1980; **255**: 3977-3986.
3. Spangler SK, Lin G, Jacobs MR, Appelbaum PC. Postantibiotic effect of sanfetrinem compared with those of six other agents against 12 penicillin-susceptible and -resistant pneumococci. *Antimicrob Agents Chemother* 1997; **41**: 2173-2176.
4. Perez-Trallero E, Alkorta M, Garcia-Arenzana JM, Iturzaeta A, Gomariz M. In-vitro, in-vivo and ex-vivo studies with oral beta-lactams against Streptococcus pneumoniae. *J Antimicrob Chemother* 1998; **41**: 629-634.
5. Thorburn CE, Knott SJ, Edwards DI. In vitro activities of oral beta-lactams at concentrations achieved in humans against penicillin-susceptible and -resistant pneumococci and potential to select resistance. *Antimicrob Agents Chemother* 1998; **42**:1973-1979.

6. Langan CE, Cranfield R, Breisch S, Pettit R. Randomized, double-blind study of grepafloxacin versus amoxycillin in patients with acute bacterial exacerbations of chronic bronchitis. *J Antimicrob Chemother* 1997; **40** Suppl A: 63-72.
7. Georgopoulos A, Borek M, Ridl W; Amoxycillin Bronchitis Study Group. Randomized, double-blind, double-dummy study comparing the efficacy and safety of amoxycillin 1 g bd with amoxycillin 500 mg tds in the treatment of acute exacerbations of chronic bronchitis. *J Antimicrob Chemother* 2001; **47**: 67-76.
8. Garbutt JM, Goldstein M, Gellman E, Shannon W, Littenberg B. A randomized, placebo-controlled trial of antimicrobial treatment for children with clinically diagnosed acute sinusitis. *Pediatrics* 2001; **107**: 619-625.
9. Agarwal G, Awasthi S, Kabra SK, Kaul A, Singhi S, Walter SD; ISCAP Study Group. Three day versus five day treatment with amoxicillin for non-severe pneumonia in young children: a multicentre randomised controlled trial. *BMJ* 2004; **328**: 791.
10. Lan AJ, Colford JM, Colford JM Jr. The impact of dosing frequency on the efficacy of 10-day penicillin or amoxicillin therapy for streptococcal tonsillopharyngitis: A meta-analysis. *Pediatrics* 2000; **105**: E19.

11. Bantar C, Nicola F, Fernandez Canigia L, Arenoso HJ, Soutric J, Montoto M, et al. Rationale for treating community-acquired lower respiratory tract infections with amoxicillin/sulbactam combination through pharmacodynamic analysis in the setting of aminopenicillin-resistant organisms. *J Chemother* 2001; **13**: 402-406.
12. Dajani AS, Taubert KA, Wilson W, Bolger AF, Bayer A, Ferrieri P, et al. Prevention of bacterial endocarditis: recommendations by the American Heart Association. *Clin Infect Dis* 1997; **25**: 1448-1458.
13. Cowper TR. Pharmacologic management of the patient with disorders of the cardiovascular system. Infective endocarditis. *Dent Clin North Am* 1996; **40**: 611-647.
14. Dudkiewicz B, Mikucki J, Ostrowski S. Infective endocarditis--changes in the microbiological profile. *Mater Med Pol* 1996; **28**: 49-56.
15. Benn M, Hagelskjaer LH, Tvede M. Infective endocarditis, 1984 through 1993: a clinical and microbiological survey. *J Intern Med* 1997; **242**: 15-22.
16. Brundusino A, Lopes HV, Coelho DSB, Oliani, C. Randomized crossover pharmacokinetic study with two oral amoxicillin pharmaceutical forms (tablets and capsules) at three different dosages. *Eur Bull Drug Res* 1999; **7**: 5-10.

17. Pires de Abreu LR, Ortiz RM, de Castro SC, Pedrazzoli J Jr. HPLC determination of amoxicillin comparative bioavailability in healthy volunteers after a single dose administration. *J Pharm Pharm Sci* 2003; **6**: 223-230.
18. Oliveira CH, Abib E, Vannuchi YB, Sucupira M, Ilha J, De Nucci G. Comparative bioavailability of 4 amoxicillin formulations in healthy human volunteers after a single dose administration. *Int J Clin Pharmacol Ther* 2001; **39**: 167-172.
19. Carlqvist J, Westerlund D. Automated determination of amoxycillin in biological fluids by column switching in ion-pair reversed-phase liquid chromatographic systems with post-column derivatization. *J Chromatogr* 1985; **344**: 285-296.
20. Ortiz RA, Calafatti SA, Corazzi A, Souza JM, Deguer M, De Souza CA, et al. Amoxicillin and ampicillin are not transferred to gastric juice irrespective of Helicobacter pylori status or acid blockade by omeprazole. *Aliment Pharmacol Ther* 2002; 16: 1163-1170.
21. Goddard AF, Jessa MJ, Barrett DA, Shaw PN, Idstrom JP, Cederberg C, et al. Effect of omeprazole on the distribution of metronidazole, amoxicillin, and clarithromycin in human gastric juice. *Gastroenterology* 1996; **111**: 358-367.

22. Beighton D, Hellyer PH, Lynch EJ, Heath MR. Salivary levels of mutans streptococci, lactobacilli, yeasts, and root caries prevalence in non-institutionalized elderly dental patients. *Community Dent Oral Epidemiol* 1991; **19**: 302-307.

23. Marsh PD. Microbiologic aspects of dental plaque and dental caries. *Dent Clin North Am* 1999; **43**: 599-614.

24. Van der Velden U, Van Winkelhoff AJ, Abbas F, De Graaff J. The habitat of periodontopathic micro-organisms. *J Clin Periodontol* 1986; **13**: 243-248.

25. Meylan PR, Francioli P, Glauser MP. Discrepancies between MBC and actual killing of viridans group streptococci by cell-wall-active antibiotics. *Antimicrob Agents Chemother* 1986; **29**: 418-423.

26. MacGregor AJ, Hart P. The effect of a single large dose of amoxycillin on oral streptococci. *J Antimicrob Chemother* 1986; **18**: 113-117.

27. Smith AJ, Jackson MS. Susceptibility of viridans group streptococci isolated from dento-alveolar infections to eight antimicrobial agents. *J Antimicrob Chemother* 2003; **52**: 1045-1046.

28. Tuohy M, Washington JA. Antimicrobial susceptibility of viridans group streptococci. *Diagn Microbiol Infect Dis* 1997; **29**: 277-280.

29. Khemaleelakul S, Baumgartner JC, Pruksakorn S. Identification of bacteria in acute endodontic infections and their antimicrobial susceptibility. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **94**: 746-755.

30. Baumgartner JC, Xia T. Antibiotic susceptibility of bacteria associated with endodontic abscesses. *J Endod* 2003; **29**: 44-47.

4. CONCLUSÃO

1. Os medicamentos teste e referência de amoxicilina 875mg em comprimidos foram considerados bioequivalentes, podendo ser intercambiáveis.
2. Os medicamentos não mostraram diferenças entre as concentrações plasmáticas e nem entre as concentrações salivares.
3. Os medicamentos mostraram eficácia antimicrobiana similares, pois ambos foram capazes de causar redução significativa da microbiota salivar.

REFERÊNCIAS*

1. Benet LZ. Understanding bioequivalence testing. ***Transplant Proc***. 1999 31(Suppl 3A): 7S-9S.
2. Brasil. Lei nº 9.787, de 10 de fevereiro de 1999. ***Diário Oficial da República Federativa do Brasil*** , Brasília, 10 de fevereiro de 1999.
3. Brasil. Resolução – RDC nº 135, de 29 de maio de 2003. ***Diário Oficial da República Federativa do Brasil*** , Brasília, 29 de maio de 2003.
4. Cortvriendt WR, Verschoor JS, Hespe W. Bioavailability study of a new amoxicillin tablet designed for several modes of oral administration. ***Arzneimittelforschung***. 1987; 37(8): 977-979.
5. Craig WA, Andes D. Pharmacokinetics and pharmacodynamics of antibiotics in otitis media. ***Pediatr Infect Dis J***. 1996; 15(3): 255-259.
6. Darout IA, Albandar JM, Skaug N, Ali RW. Salivary microbiota levels in relation to periodontal status, experience of caries and miswak use in Sudanese adults. ***J Clin Periodontol***. 2002; 29(5): 411-420.
7. Dhaon NA. Amoxicillin tablets for oral suspension in the treatment of acute otitis media: a new formulation with improved convenience. ***Adv Ther***. 2004; 21(2): 87-95.

* De acordo com a norma da FOP/UNICAMP, baseada no modelo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline

8. Food and Drug Administration (FDA/USA) (2004) Title 21, Part 320 Bioavailability and bioequivalence requirements. Disponível em: URL: http://www.fda.gov [2004 Nov 4].

9. Fricker U, Osborn JF, Wust J. Determination of serum and saliva concentrations of clindamycin (Dalacin C) and amoxicillin (Clamoxyl). ***SSO Schweiz Monatsschr Zahnheilkd***. 1979; 89(8): 738-747.

10. Goddard AF, Jessa MJ, Barrett DA, Shaw PN, Idstrom JP, Cederberg C, et al. Effect of omeprazole on the distribution of metronidazole, amoxicillin, and clarithromycin in human gastric juice. ***Gastroenterology*** 1996; 111(2): 358-367.

11. Gristetti M, Brundosino A, Lopes VH, Lai HJ, Oliani C. Pharmacokinetic profile of three different daily dosages of amoxicillin. ***Eur Bull Drug Res***. 1999; 7(1): 15-20.

12. Hutchings AD, Monie RD, Spragg BP, Routledge PA. Saliva and plasma concentrations of isoniazid and acetylisoniazid in man. ***Br J Clin Pharmacol***. 1988; 25(5): 585-589.

13. Meylan PR, Francioli P, Glauser MP. Discrepancies between MBC and actual killing of viridans group streptococci by cell-wall-active antibiotics. ***Antimicrob Agents Chemother***. 1986; 29(3): 418-423.

14. Molinaro M, Corona G, Fiorito V, Spreafico S, Bartoli AN, Zoia C. Bioavailability of two different oral formulations of amoxicillin in healthy subjects. ***Arzneimittelforschung***. 1997; 47(12): 1406-1410.

15. Mustofa, Suryawati S, Santoso B. Pharmacokinetics of metronidazole in saliva. ***Int J Clin Pharmacol Ther Toxicol***. 1991; 29(12): 474-478.

16. Ortiz RA, Calafatti SA, Corazzi A, Souza JM, Deguer M, De Souza CA, et al. Amoxicillin and ampicillin are not transferred to gastric juice irrespective of Helicobacter pylori status or acid blockade by omeprazole. ***Aliment Pharmacol Ther***. 2002; 16(6): 1163-1170.

17. Squinazi F. Salivary diffusion of oral amoxicillin. ***Rev Odontostomatol***. 1980; 9(3): 55-57.

18. Storpirtis S, Consiglieri VO. Biodisponibilidade e bioequivalência de medicamentos: aspectos fundamentais para o planejamento e execução de estudos. ***Rev Farm Bioquim Univ Sao Paulo***. 1995; 31(2): 63-70.

19. Suryawati S, Santoso B. Determination of isoniazid half-life from salivary samples. ***Int J Clin Pharmacol Ther Toxicol***. 1986; 24(1): 18-22.

20. Van der Velden U, Van Winkelhoff AJ, Abbas F, De Graaff J. The habitat of periodontopathic micro-organisms. ***J Clin Periodontol***. 1986; 13(3): 243-248.

21. Watson ID, Stewart MJ. Trimethoprim: prediction of serum concentrations from saliva measurements. ***Eur J Clin Pharmacol***. 1986; 30(4): 457-461.

22. Wust J, Hardegger U. Penetration of clarithromycin into human saliva. ***Chemotherapy***. 1993; 39(5): 293-6.

ANEXO 1 – CERTIFICADO DO COMITÊ DE ÉTICA EM PESQUISA

UNICAMP

COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "Estudo de biodisponibilidade comparativa entre comprimidos de 875mg de amoxicilina bd do grupo ems-sigma pharma® e comprimidos de 875mg de amoxicilina bd da glaxosmithkline®", sob o protocolo nº **138/2002**, do Pesquisador ***SINVALDO BAGLIE***, sob a responsabilidade do(a) Prof(a). Dr(a). **PEDRO LUIZ ROSALEN**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP.

Piracicaba, 06/11/2002

We certify that the research project with title "Comparative bioavalibility study between two tablets of 875 mg amoxicilin bd from ems-sigma pharma® and glaxosmithkline®", protocol nº **138/2002**, by Researcher ***SINVALDO BAGLIE***, responsibility by Prof. Dr. **PEDRO LUIZ ROSALEN**, is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Research at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, November 06 2002

Prof. Dr. Thales Rocha de Mattos Filho
Diretor
FOP/UNICAMP

Prof. Dr. Antonio Bento Alves de Moraes
Coordenador
CEP/FOP/UNICAMP

ANEXO 2 – COMPROVANTE DE SUBMISSÃO À PUBLICAÇÃO DO CAPÍTULO 1

De: Assoc. Prof. Barry G. Woodcock [mailto:Woodcock@t-online.de]
Enviada em: sexta-feira, 14 de janeiro de 2005 14:41
Para: fcgroppo
Assunto: Ms 05-498/submission/Baglie

Ms CPH05-498: "Comparative bioavailability of 875mg amoxicillin tablets in healthy human volunteers" by Baglie et al.
Dear Professor Groppo,
The above manuscript has been received by this office (date received: 14.01.2005). It has been given the submission number CPH05-498 and will be peer-reviewed. You will be informed of the results without delay.
Sincerely Yours,
Office of the Editor-in-Chief,
Prof. Barry G. Woodcock
Associate Professor of Clinical Pharmacology
International Journal of Clinical Pharmacology and Therapeutics
Address for correspondence:
P.O. Box 20 02 32, D-63308 Roedermark, Germany
and
Johann Strauss Strasse 9, 63322 Roedermark, Germany
Tel: +49 6074 96183
Fax: +49 6074 6961154
Email: woodcock@em.uni-frankfurt.de

"fcgroppo" <fcgroppo@fop.unicamp.br> schrieb:
> Dear Dr. Woodcock,
> Assoc. Professor of Clinical Pharmacology and Editor-in-Chief of
> International Journal of Clinical Pharmacology and Therapeutics
> We would be grateful if the attached manuscript entitled "Comparative
> bioavailability of 875mg amoxicillin tablets in healthy human volunteers"could
be considered for publication in the International Journal of Clinical>
Pharmacology and Therapeutics.
> Best regards,
> Francisco
> Prof Dr Francisco Carlos Groppo
> Associate Professor Pharmacology, Anesthesiology and Terapeutics
> Piracicaba Dental School - UNICAMP
> Phone 55 19 3412 5310
> Fax 55 19 3412 5218