UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

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AVALIAÇÃO DA EFICÁCIA ANESTÉSICA E DA CONCENTRAÇÃO PLASMÁTICA DA ROPIVACAÍNA ENCAPSULADA EM LIPOSSOMAS, EM ANESTESIA ODONTOLÓGICA.

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.

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RESUMO

Uma nova forma farmacêutica de anestésico local, encapsulado em lipossomas, vem sendo estudada na Medicina e mais atualmente em Odontologia. Os objetivos deste trabalho foram avaliar a eficácia anestésica em anestesia tópica e infiltrativa e os parâmetros farmacocinéticos da ropivacaína encapsulada em lipossomas, em 4 estudos, cruzados, duplo-cegos e com ordem de aplicação aleatória, com intervalo de 1 semana entre as aplicações. **Capítulo 1**: foram comparadas a eficácia da anestesia tópica e a influência na resposta pulpar da ropivacaína 2% encapsulada em lipossomas (RL2), Benzocaína 20% (- B20), gel placebo lipossomal (PL) e gel placebo (P) aplicados em mucosa vestibular dos incisivos laterais superiores, em 40 voluntários. RL2 foi tão eficaz quanto B20 em reduzir dor à punção e na duração de anestesia em tecidos moles ($p>0,05$) e ambas foram superiores às formulações PL e P ($p<0,05$). Nenhuma das formulações exerceu influência na resposta pulpar. **Capítulo 2:** ropivacaína 2% encapsulada em lipossomas (RL2), ropivacaína 1% encapsulada em lipossomas (RL1), creme de lidocaína 2,5% e prilocaína 2,5% (EMLA) e gel placebo lipossomal (PL) foram avaliados quanto à eficácia em reduzir dor à punção e à injeção de anestésico local, quando aplicados topicamente na região palatina do canino superior esquerdo. O EMLA foi mais efetivo em diminuir a dor à punção ($p<0,05$), porém nenhuma das formulações testadas foi eficaz em diminuir a dor decorrente da injeção do anestésico local ($p>0,05$). Nenhuma das formulações lipossomais foi eficaz como anestésico tópico na mucosa palatina. **Capítulo 3**: foram injetados, no fundo de sulco vestibular do canino superior direito, 1,8mL de ropivacaína 0,5% encapsulada em lipossomas (RLipo), ropivacaína 0,5% com epinefrina 1:200.000 (Repi), ropivacaína a 0,5% (R) e lidocaína 2% com epinefrina 1:100.000 (Lepi), em 40 voluntários. Foram avaliadas latência e duração da anestesia pulpar por aplicação de estímulo elétrico e em tecidos moles por estímulo de pressão. Não houve diferença estatística entre os anestésicos com relação ao tempo de latência. Repi e Lepi apresentaram maior tempo de anestesia

pulpar quando comparados à RLipo e R ($p<0,05$). Repi promoveu anestesia mais prolongada em gengiva do que os outros anestésicos ($p<0,05$). A formulação lipossomal de ropivacaína não foi eficaz em anestesia infiltrativa na maxila. **Capítulo 4**: foram avaliados por cromatografia líquida de alta eficiência (CLAE) os níveis plasmáticos de ropivacaína, após infiltração de 1,8 mL, no fundo de sulco vestibular de canino superior direito, de ropivacaina 0,5 % associada à epinefrina 1:200.000 e ropivacaina 0,5% encapsulada em lipossomas em 14 voluntários. Não houve diferenças estatísticas ($p>0,05$) entre os parâmetros farmacocinéticos avaliados entre as duas soluções anestésicas. **Conclusão geral:** Não há vantagem no uso da ropivacaína 0,5% encapsulada em lipossomas em técnica infiltrativa ou 1 e 2% em anestesia tópica em mucosa palatina. Em mucosa vestibular, por apresentar eficácia semelhante à da benzocaína 20%, a ropivacaína 2% encapsulada em lipossomas pode ser uma opção a esse anestésico. A ropivacaína encapsulada em lipossomas apresenta perfil farmacocinético semelhante ao da ropivacaína com epinefrina.

Palavras-chave: Odontologia, Anestesia local, Farmacocinética, Portadores de fármacos.

ABSTRACT

A new pharmaceutical formulation of local anesthetic, liposome encapsulated, has been studied in medicine and recently in dentistry. The aims of the present study were to evaluate anesthetic efficacy in topical and infiltration anesthesia, and pharmacokinetic parameters of liposome-encapsulated ropivacaine in 4 random, crossed and double-blind studies, with a one week interval between sections. **Chapter 1**: liposome-encapsulated 2% ropivacaine (RL2), 20% Benzocaine (B20), liposomal placebo (PL) and placebo (P) were compared in relation to the efficacy of topical anesthesia and influence on pulpal response after topical application in the buccal fold of the upper lateral incisors, in 40 volunteers. RL2 was as efficacious as B20 in reducing pain during needle insertion and concerning soft tissue anesthesia ($p>0.05$) and both agents were better than PL e P formulations ($p<0.05$). None of the formulations influenced pulpal response. **Chapter 2:** liposome-encapsulated 2% ropivacaine (RL2), liposome-encapsulated 1% ropivacaine (RL1), 2.5% lidocaine and 2.5% prilocaine cream (EMLA) and liposomal placebo (PL) were evaluated concerning their efficacy in reducing pain during needle insertion and anesthetic injection after topical application at the palatal mucosa of the upper left canine. EMLA was the most effective in reducing pain during needle insertion ($p<0.05$), however none of the tested formulations was effective in reducing pain during anesthetic injection ($p>0.05$). None of the formulations was effective as a topical anesthetic in the palatine mucosa. **Chapter 3**: forty volunteers received 1.8mL of liposome-encapsulated 0.5% ropivacaine (RLipo), 0.5 % ropivacaine with 1:200,000 epinephrine (Repi), 0.5% ropivacaine (R) and 2% lidocaine with 1:100,000 epinephrine (Lepi), as an infiltration injection in the buccal fold of the right maxillary canine region. The onset and duration of pulpal anesthesia were evaluated through electric stimuli application and in soft tissue by pressure stimuli. No difference in onset of anesthesia was observed among anesthetic formulations ($p>0.05$). Repi and Lepi showed longer pulpal anesthesia when compared to RLipo and R

($p<0.05$). Repi provided longer gingival anesthesia than the other formulations ($p<0.05$). Liposome-encapsulated ropivacaine was not effective in maxillary infiltration anesthesia. **Chapter 4**: plasma levels of ropivacaine were analyzed by high performance liquid chromatography (HPLC) after infiltration of 1.8mL of 0.5% ropivacaine with 1:200,000 epinephrine and liposome-encapsulated 0.5% ropivacaine in the buccal fold of the maxillary right canine region in 14 volunteers. There were no statistically differences ($p>0.05$) among pharmacokinetics parameters between the two anesthetic formulations. **Final conclusion:** There is no advantage in the use of liposome-encapsulated 0.5% ropivacaine in infiltration anesthesia or liposome-encapsulated 1 and 2% ropivacaine in topical anesthesia in palatal mucosa. In the buccal mucosa, as it showed similar efficacy of 20% benzocaine, liposome-encapsulated 2% ropivacaine can be an option to this anesthetic. Liposome-encapsulated ropivacaine and ropivacaine with epinephrine showed similar pharmacokinetic.

Key Words: Dentistry, Local anesthesia, Pharmacokinetics, Drug carriers.

SUMÁRIO

INTRODUÇÃO

A ansiedade gerada pelo medo de sentir dor ainda é uma barreira para o atendimento odontológico (Nuttall, 2001). A anestesia local elimina a dor durante o atendimento odontológico; no entanto, este procedimento é um dos mais poderosos agentes indutores de estresse e ansiedade (Meechan, 2002). Assim, a obtenção de anestesia pulpar clinicamente útil, sem a necessidade do uso de agulha seria um enorme avanço no controle da dor em Odontologia.

Alguns autores relataram atingir anestesia pulpar por meio da aplicação tópica do creme para uso dermatológico formado pela mistura eutética de prilocaína e lidocaína, ambos a 2,5% (EMLA® AstraZeneca, Cotia, Brazil) no fundo de sulco vestibular da maxila com tempos de aplicação que variaram de 15 a 30 minutos (Vickers & Punnia-Moorthy, 1993; Vickers *et al.*, 1997; Munshi *et al.*, 2001), permitindo a execução de procedimentos como instrumentação periodontal (Svensson *et al.*, 1994), dentística (Vickers & Punnia-Moorthy, 1993; Vickers *et al.*, 1997), exodontias e terapias pulpares em odontopediatria (Munshi *et al.*, 2001) e biópsia na região de mucosa palatina (Meechan, 2001). O EMLA também reduziu o desconforto da colocação de grampos em isolamento absoluto usado para realização de procedimentos restauradores e endodônticos (Lim & Julliard, 2004) e injeções intraligamentares (Meechan & Thomason, 1999).

Também tem sido relatada maior eficácia do EMLA® em comparação à benzocaína e à lidocaína em reduzir dor à punção e à injeção tanto em mucosa vestibular como em mucosa palatina (Roghani *et al.,* 1999; McMillan *et al.,* 2000; Abu Al-Melh et al., 2005; Nayak & Sudha, 2006; Al-Melh & Andersson, 2007).

No entanto, a superioridade do EMLA foi questionada em relação aos outros anestésicos disponíveis, pois foi demonstrada por Primosch & Rolland-Asensi (2001) equivalência entre benzocaína a 20% e EMLA em reduzir a dor associada à anestesia em mucosa palatina em crianças. Além disso, estes autores também relatam que a benzocaína tem vantagens sobre o EMLA, como maior preferência pelos voluntários e gosto mais aceitável. As desvantagens

relacionadas ao EMLA incluem: gosto amargo, alto custo e pouca viscosidade, resultando em dificuldade de manter o creme no local desejado.

Em um estudo piloto com o objetivo de reproduzir resultados anteriores (Vickers & Punnia-Moorthy, 1993; Vickers *et al*., 1997; Munshi *et al*., 2001) a aplicação do EMLA por 30 minutos na região de incisivo lateral superior direito promoveu uma lesão ulcerativa no local de aplicação em 4 voluntárias sem induzir anestesia pulpar (Franz-Montan *et al*., 2008). Desta forma, anestesia pulpar por meio da aplicação tópica de um anestésico indicado para uso em mucosa bucal ainda não está disponível na rotina do cirurgião-dentista.

Também não há, até o momento, comprovação da eficácia de um anestésico tópico indicado para uso oral que elimine completamente a dor da anestesia local odontológica, especialmente na mucosa palatina. Esta, por apresentar tecido conjuntivo fibroso, estar firmemente aderida ao osso palatino adjacente, e ser ricamente inervada, é extremante sensível em comparação a outras regiões da cavidade bucal (McArdle, 1997; Meechan, 2002; Primosch & Rolland-Asensi, 2001; Meechan *et al*., 2005).

Desta forma o modelo de avaliação de anestesia tópica na mucosa palatina é o maior desafio a que um anestésico tópico pode ser submetido por esta ser uma das regiões mais dolorosas da cavidade bucal (Svensson & Petersen,1992; Meechan *et al*., 2005). Assim um anestésico tópico capaz de eliminar a dor durante a punção e a injeção de uma solução anestésica nesta região, seria um benefício à Odontologia.

A ropivacaína, um anestésico de longa duração, quimicamente homóloga à bupivacaína e à mepivacaína, disponível comercialmente apenas para uso médico, tem sido relatada como potencialmente menos tóxica do que a bupivacaína para os sistemas nervoso central e cardiovascular (Scott *et al*., 1989; Knudsen *et al*., 1997, Leone *et al*., 2008; Zink & Graf, 2008).

Em odontologia, a eficácia anestésica da ropivacaina foi comprovada tanto em anestesia infiltrativa na maxila, como em bloqueio do nervo alveolar inferior (Kennedy *et al*., 2001; Ernberg & Kopp, 2002; Axelsson & Isacsson, 2004;

Palma, 2004; El-Sharrawy & Yagiela, 2006). Segundo Buric (2006) este anestésico local foi eficaz no controle de dor transoperatória de cirurgias orais como cistectomia, apicectomia e extrações de terceiros molares inclusos.

Recentemente a ropivacaína foi avaliada na forma de gel para uso tópico em mucosa bucal e foram observadas boa eficácia e segurança quando comparada a outros anestésicos tópicos como benzocaína e EMLA® em reduzir a dor à punção na mucosa vestibular (Franz-Montan *et al.*, 2007a).

Nos últimos anos tem crescido o interesse por formas de liberação controlada de medicamentos que permitam o aumento da duração do efeito e diminuição da toxicidade. Dentre estas, a encapsulação em lipossomas tem sido bastante estudada (Gesztes & Mezei, 1988; Singh & Vyas 1996; Zed et al., 1996; Hung *et al.*, 1997; Bucalo *et al.*, 1998; Fisher *et al.*, 1998; Friedman *et al.*, 1999; Franz-Montan et al., 2007b).

Os lipossomas consistem de esferas microscópicas formadas por uma ou mais bicamadas lipídicas. Anestésicos locais encapsulados em lipossomas demonstraram promover maior duração da anestesia devido à liberação lenta da droga, bem como redução da toxicidade para os sistemas cardiovascular e nervoso central (Boogaerts *et al.*, 1993; Boogaerts *et al.*, 1994; Grant *et al.*, 1994; Mowat *et al.*, 1996; Yu *et al.*, 2002; Grant *et al.*, 2001; Cereda *et al.*, 2004).

Além disso, os lipossomas são biocompatíveis, biodegradáveis, com reduzido risco de toxicidade, imunogenicidade, antigenicidade e lesões histológicas, principalmente devido à semelhança dos monômeros constituintes dos lipossomas (fosfatildilcolina e colesterol) com os das membranas biológicas (Malinovsky *et al.*, 1997; Grant, 2002).

A eficácia de anestésicos encapsulados em lipossomas, como a lidocaína e a tetracaína já foram demonstradas na aplicação tópica em pele humana (Gesztes & Mezei, 1988; Singh & Vyas 1996; Hung *et al.*, 1997; Bucalo *et al.*, 1998; Fisher *et al.*, 1998; Friedman *et al.*, 1999).

Em mucosa bucal dois estudos avaliaram a eficácia de anestésicos em formulação lipossomal. Zed et al (1996) observaram maior redução na dor à

punção e infiltração de anestésico local após aplicação de tetracaína encapsulada em lipossomas do que com a benzocaína 20%.

Franz-Montan *et al.* (2007b) verificaram que o gel de ropivacaína 1% encapsulada em lipossomas apresentou eficácia superior em reduzir dor à punção durante uma simulação de anestesia local na técnica infiltrativa na região anterior de maxila, em comparação ao gel de benzocaína 20%. Não houve, porém, alteração da resposta pulpar após aplicação tópica por 2 minutos, o que talvez pudesse ocorrer com o aumento da concentração do sal anestésico e do tempo de aplicação da formulação.

Em técnica infiltrativa foi observado aumento da duração de ação do anestésico local encapsulado em lipossomas. Tofoli *et al.*, (2008) observaram que a mepivacaina 2% encapsulada em lipossomas foi capaz de promover anestesia pulpar com tempo de duração semelhante ao obtido com a formulação comercial de mepivacaína 3%, permitindo assim uso de menor concentração do sal anestésico com a mesma eficácia.

Por apresentar estrutura química semelhante à da mepivacaína, a ropivacaína também poderia ser beneficiada com a encapsulação em lipossomas para uso em técnica infiltrativa.

Esses resultados demonstram que o uso destas formulações poderia representar uma nova alternativa aos anestésicos locais para uso em odontologia, com prolongada duração de ação e elevada segurança, o que levou à realização dos quatro estudos que compõem esta tese.

Esta tese está de acordo com a deliberação da Comissão Central de Pós-Graduação (CCPG) da Universidade Estadual de Campinas (UNICAMP) nº 001/98, que regulamenta o formato alternativo para dissertação e tese, permitindo a inserção de artigos científicos de autoria ou co-autoria do candidato, sendo composta de quatro capítulos contendo artigos que se encontram em fase de submissão para publicação em revista científica, conforme descrito a seguir:

CAPÍTULO 1

Artigo: *"Efficacy of liposome encapsulated 2% ropivacaine as topical anesthetic and its influence in pulpal anesthesia. "*

Este Artigo será submetido ao periódico: Journal of the American Dental Association.

CAPÍTULO 2

Artigo: *"Efficacy of two concentrations of liposome-encapsulated ropivacaine for topical anesthesia in the palatal mucosa."*

Este artigo será submetido ao periódico: Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology.

CAPÍTULO 3

Artigo: *"Efficacy of liposome-encapsulated 0.5% ropivacaine in maxillary dental anesthesia."*

Este Artigo foi submetido ao periódico: Anesthesia & Analgesia em 23 de janeiro de 2009. (Anexo 3).

CAPÍTULO 4

Artigo: *"Pharmacokinetics of ropivacaine with epinephrine or encapsulated in liposome after dental anesthesia."*

Este Artigo será submetido ao periódico: Journal of Controlled Release

CAPÍTULO 1: Efficacy of liposome encapsulated 2% ropivacaine as a topical anesthetic and its influence in pulpal anesthesia.

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Abstract

Aim. The aim of the present study was to evaluate the efficacy of liposome encapsulated 2% ropivacaine as a topical anesthetic in dentistry and to verify its influence in pulpal response. *Material and methods.* In this crossover, double blind, placebo-controlled and two period design study, 40 volunteers randomly received equal amounts (0.3g) of the following topical formulations: liposome encapsulated 2% ropivacaine gel, liposomal placebo gel, placebo gel and 20% benzocaine gel at maxillary lateral incisor buccal fold (right and left sides) for 30 minutes. Teeth 12 and 22 were tested with a pulp tester every ten minutes. At the end of topical anesthesia application, a 30G needle was inserted until contacting the periosteum. Pain associated with needle insertions were measured using a visual analogue pain scale (VAS). Duration of soft tissue anesthesia was accessed by pinprick test. *Results*. Liposome encapsulated 2% ropivacaine and 20% benzocaine showed lower VAS mean values and longer soft tissue anesthesia when compared to placebo and liposomal placebo ($P=0.0003$ and $P<0.0001$, respectively), however liposome encapsulated 2% ropivacaine was not different from 20% benzocaine ($p>0.05$) concerning VAS and duration. Neither liposome encapsulated 2% ropivacaine nor 20% benzocaine were able to induce pulpal anesthesia. *Conclusion.* Liposome encapsulated 2% ropivacaine performed a similar efficacy in reducing pain during needle insertion and in duration of soft tissue when compared to 20% benzocaine however, neither one were able to induce pulpal anesthesia after a 30-min application.

Clinical implication: The liposome formulation of ropivacaine could be an alternative topical anesthetic in dentistry since it performed similar efficacy to the commercially available 20% Benzocaine

***Key words*:** Local Anesthesia, Topical Anesthesia, Ropivacaine, Liposomes, Benzocaine.

Introduction

Pulpal anesthesia achieved simply by topical application of a local anesthetic is still not achieved in routinely dental practice.

While few studies demonstrated pulpal anesthesia after a topically applied local anesthetic (Vickers and Punnia-Moorthy, 1993; Vickers et al., 1997; Munshi et al., 2001), others failed to achieve the same results (Meechan and Donaldson, 1994; Franz-Montan et al., 2007).

In a pioneer study, Vickers and Punnia-Moorthy (1993) showed pulpal anesthesia (evaluated by electric pulp tester) in 92% of the subjects after a 15 to 30 minute topical application of EMLA in the oral mucosa. A successful rate of 75% in a clinical evaluation of topically applied EMLA before restorative procedures including high- and low-speed drilling was also reported (Vickers et al., 1997) and Munshi et al. (2001) concluded that EMLA could eliminate the use of conventional anesthetic injection in pediatric dentistry.

However, the use of EMLA in oral mucosa is not recommended by its manufacturer (Primosch & Rolland-Asensi, 2001; Meechan, 2002). In addition it was reported in the literature that oral use of EMLA promoted painful ulceration and desquamation of gingival mucosa in a 30-minute application (Franz-Montan et al., 2008).

Liposomes are lipid vesicles considered safe and effective drug carrier systems (Grant et al., 1994; Boogaerts et al., 1993; Boogaerts et al., 1995; Mowat et al., 1996; Yu et al., 2002; Grant et al., 2004). Liposomal formulations of local anesthetics, such as tetracaine and lidocaine, were demonstrated to be effective for topical anesthesia of intact skin (Gesztes & Mezei, 1988; Hung et al., 1997; Fisher et al., 1998; Friedman et al., 1999).

Franz-Montan et al. (2007) demonstrated *in vivo* that liposome-encapsulated ropivacaine gel was equivalent to EMLA as an oral topical anesthetic in reducing pain during needle insertion after a 2-min application in the buccal fold. This application time however, was not sufficient to achieve pulpal anesthesia. Therefore, this study was conducted to evaluate the efficacy of liposome-

encapsulated ropivacaine gel in higher concentration and longer application time to provide pulpal and soft tissue anesthesia.

Material and Methods

The Ethical Committee of Piracicaba Dental School, University of Campinas, SP, Brazil (#093/2006) approved this research. After informed consent was obtained, 40 healthy volunteers (20 females and 20 males), 18 to 43 years-old (21.3 ± 4.6) were included in this research. All volunteers were in good health, had no history of allergy to any of the local anesthetics used, and were not taking any medication that would alter pain perception, as determined by oral questioning and written health history. The teeth undergoing testing were vital and free of caries, large restorations, periodontal disease, past endodontic treatment and history of trauma or sensitivity.

A crossover, double blind, placebo-controlled and two period design was used. In a randomized manner, equal amounts (0.3g) of two of the topical anesthetics: liposome encapsulated 2% ropivacaine gel, liposomal placebo gel, placebo gel and 20% benzocaine gel (Benzotop®, DFL Ind Com Ltda, Rio de Janeiro, Brazil) were applied at the right and left sides of maxillary buccal fold of the lateral incisor region according to a latin square design.

The 20% benzocaine gel was selected for being the most commonly used in dentistry (Rosa et al., 1999; Primosch RE, Rolland-Asensi, 2001; Alqareer et al., 2006).

Liposome formulations were prepared at the Department of Biochemistry, Institute of Biology, University of Campinas. The liposomes consisted of large unilamellar vesicles of homogenised sizes (400nm), prepared as described previously (de Araújo et al., 2008). All the formulations not commercially available were prepared by the same operator (not involved in application or anesthetic efficacy evaluation) with identical colour, taste, smell and fluidity to resemble that of the commercial benzocaine. The gel formulations were placed into coded flasks to ensure blindness of the volunteers and the investigator involved in application and

evaluation of anesthetic efficacy.

At the beginning of each session, before topical application, a cheek and lip retractor was positioned and cotton rolls were applied in the buccal fold of teeth 13 and 23 to allow proper isolation of the region to be tested. After this procedure, teeth 12 and 22 and their respective buccal mucosa were dryed with sterile gauze followed by pulp testing of these teeth with an eletric pulp tester (Vitality Scanner 2006, Analytic Technology, Redmond, WA) three times to record baseline vitality. The pulp tester emits 0 to 300 V (0-80 units in the digital scale) at 0.08 mA (10 pulses at each 6 milliseconds). The probe tip of the pulp tester was placed in the center of the teeth on the buccal side and a fluoride gel was used as the conductive agent (Branco et al., 2006).

The topical anesthetics (previously weighed) were applied by using a cotton swab and kept in place for 30 minutes.

With the topical anesthetic in place at the mucosal surface, teeth 12 and 22 were tested three times every ten minutes (at the 10^{th}, 20^{th} and the 30^{th} minutes of application) with the pulp tester to evaluate any change in the pulpal response. Pulpal anesthesia was defined as the absence of the subject's response to the maximal output (300 V, 0.08 mA) of the pulp tester, indicated as the "80" reading (McLean et al., 1993).

At the end of topical application, the mucosa was wiped gently with sterile gauze followed by a water rinse. After this procedure thirty-gauge needles attached to aspirating syringes were inserted until periosteum contact, at both sides, simulating a local anesthetic injection. Pain associated to needle insertion was measured using a visual analogue pain scale (VAS), which consists of a 10-cm line where 0 indicates "no pain" and 10 "unbearable pain." Subjects were asked to make a mark on the line according to their level of perceived pain, and then a ruler was used to measure the distance from the end-point marked "no pain" to the mark made by the volunteer on the VAS.

After pain intensity was measured, all volunteers were asked to verify the duration of oral mucosa anesthesia, using a pinprick test (Franz-Montan et al.,

2007), every one minute, up to cessation of numbness.

VAS scores were analyzed by ANOVA and Tukey test (Bioestat 4.0, Mamirauá Institute, Belém, PA, Brazil); duration of soft tissue anesthesia and pulpal response values were compared by Friedman test; comparisons were considered significant at $P < 0.05$.

Results

Liposome-encapsulated 2% ropivacaine and 20% benzocaine were significantly better then placebo and liposomal placebo in reducing pain during needle insertion (P=0.0003). However, there was no difference between liposome-encapsulated 2% ropivacaine and 20% benzocaine (P>0.05). Figure 1 shows means of VAS for all groups concerning pain during needle insertion.

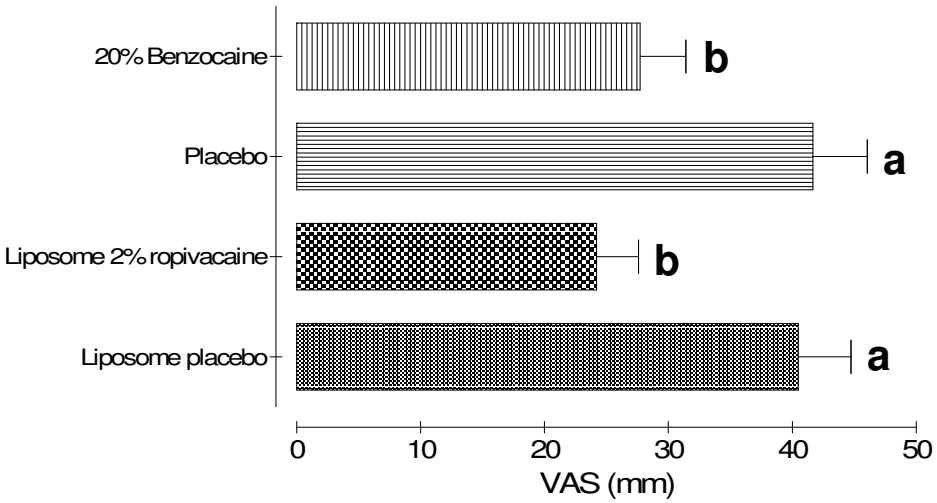

Figure 1. VAS scores (mean ± S.E.M.) rated by volunteers after needle insertion (different letters represent statistically significant differences - p<0.05).

Liposome-encapsulated 2% ropivacaine and 20% benzocaine showed longer soft tissue anesthesia when compared to the placebo formulations (P<0.0001). No significant differences were found between liposome-encapsulated

2% ropivacaine and 20% benzocaine and between placebo and liposome placebo (P > 0.05). Figure 2 shows the means of soft tissue anesthesia, in minutes.

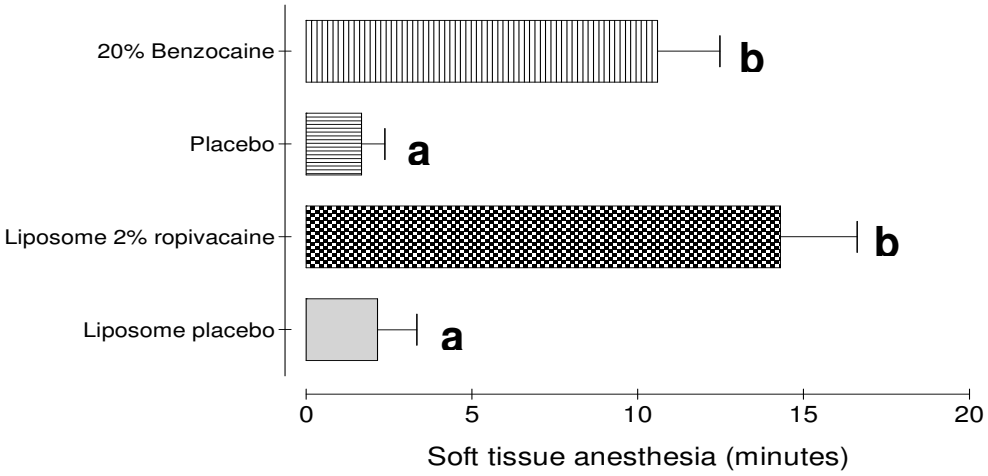

Figure 2. Duration of soft tissue anesthesia (mean ± S.E.M.), in minutes (different letters represent statistically significant differences - p<0.05) after 30 min application.

There were no statistically significant differences concerning pulpal response (teeth 12 and 22) among the different periods or formulations tested (p>0.05). The maximum setting of the pulp tester (300V, 80 reading) was not achieved by any volunteer. Figure 3 shows medians of pulpal response evaluated every ten minutes during topical application.

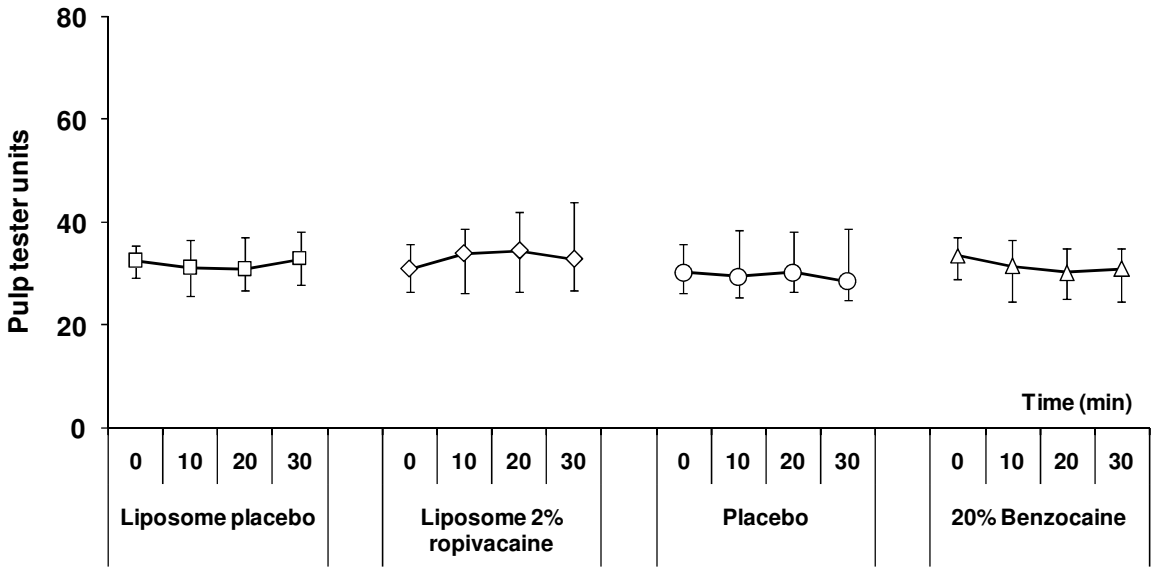

Figure 3. Pulpal response (median ± interquartile range, in electric pulp tester units) measured with the electric pulp tester every ten minutes during the 30-min application of topical anesthesia.

Discussion

Pulpal anesthesia achieved by the topical application of a local anesthetic would be a significant advance in dental care, since the fear of feeling pain during local anesthetic injections is a great source of anxiety for many patients (Hutchins et al., 1997; Meechan, 2005; Alqareer et al., 2006).

Studies in which pulpal anesthetic success was obtained solely with topical anesthetic use high amounts of EMLA (0.5 to 1g) was applied for longer periods of time than normally used in dental treatment (15 to 37 minutes) (Vickers & Punnia-Moorthy, 1993; Vickers et al., 1997; Munshi et al., 2001).

Franz-Montan et al. (2007) tried to reproduce those results by topically applying EMLA and liposome-encapsulated 1% ropivacaine, however, the application time (2min) and the amount of topical anesthetic (60mg) was not enough to induce pulpal anesthesia. Therefore, it was suggested that a higher amount of topical anesthetic and a longer application time should be necessary.

Nevertheless in a pilot study, Franz-Montan et al. (2008) aiming to achieve pulpal anesthesia with topical anesthetics, reported painful ulceration and desquamation of gingival mucosa after a 30-minute application of EMLA (0.3g) in adult volunteers. Therefore this anesthetic was not tested in the present study.

In the present study, even in higher amount (0.3g) and concentration (2%) with a longer application period (30min), liposome-encapsulated ropivacaine was not able to induce pulpal anesthesia, as assessed by electric pulp tester. The efficacy in promoting soft tissue anesthesia, otherwise was comparable to that obtained with 20% benzocaine. Interestingly the increase in amount, concentration and time of application did not reduce the VAS scores as compared to the previous study (Franz-Montan et al., 2007), but increased the duration of soft tissue anesthesia (7 and 12 minutes in the previous study and 11 and 14 minutes, respectively for benzocaine and liposome ropivacaine).

The enhanced skin deposition of several formulations based on conventional liposomes were demonstrated in many *in vivo* and *in vitro* transport studies reporting a reduction (or no effect) in percutaneous permeation or systemic absorption of a number of drugs (Wohlrab & Lasch, 1989; Foldvari et al., 1990, Fresta & Puglisi, 1997, Ferreira et al., 2004, Puglia et al., 2004, Kitagawa & Kasamaki, 2006).

The hypothesis of an enhanced penetration of liposome-encapsulated local anesthetics in oral mucosa as observed in the skin (Gesztes & Mezei, 1988; Hung et al., 1997; Fisher et al., 1998; Friedman et al., 1999) was not demonstrated in the present study.

The skin penetration of liposomes can be influenced by their physico-chemical characteristics such as size, charge and lamellarity (Katahira et al., 1999; Ogiso et al., 2001; Manosroi et al., 2004; Choi & Maibach, 2005; Sinico et al., 2005).

According to Elsayed et al. (2007) concerning dermal application, in most cases the conventional liposomes do not deeply penetrate skin and stay limited to upper layers of the stratum corneum. This hypothesis could somehow explain why

the topical formulation of liposomal ropivacaine was not able to deeply penetrate and achieve pulpal tissue. One possible explanation for this inefficacy might be the size of liposome used in the present study. Although the buccal oral mucosa is not as keratinised as the skin, the large size of the liposomes (400nm, unilamelar vesicles) used here could possibly unable them to cross the periosteum and the bone to reach the nerve fibber endings that innervate dental pulp.

The use of different liposome preparations, such as small size vesicles or the recently introduced ultraflexible vesicles, which have been shown to penetrate the skin with superior efficiency compared to the conventional liposomes (Elsayed et al., 2007) could improve diffusion allowing penetration of the local anesthetic till the tooth apex. Further studies will be necessary to test this hypothesis.

In conclusion, the liposome-encapsulated 2% ropivacaine presented similar efficacy in reducing pain during needle insertion and in duration of soft tissue anesthesia when compared to 20% benzocaine, however, this liposomal formulation was not able to induce pulpal anesthesia after a 30-min application.

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CAPÍTULO 2: Efficacy of two concentrations of liposome-encapsulated ropivacaine for topical anesthesia in the palatal mucosa.

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Abstract

Objective: The aim of this study was to evaluate the efficacy of liposome-encapsulated ropivacaine in different concentrations for topical anesthesia in the palatal mucosa.

Study Design: In this single-blinded, placebo controlled and crossover study 40 (20 male) healthy volunteers randomly received: liposome-encapsulated 2% ropivacaine, liposome-encapsulated 1% ropivacaine, euthetic mixture of 2.5% lidocaine and 2.5% prilocaine (EMLA), and liposomal placebo gel, topically in the palatal mucosa of the right- canine region during 5 minutes, in four different sessions. Pain associated with the 30G-needle insertion and local anesthetic injection was rated on a visual analogue scale (VAS).

Results: EMLA elicited lower VAS scores ($P<0.05$) during needle penetration than the other agents in female and male volunteers, with no difference among the others ($P>0.05$). VAS scores in females with EMLA were lower than in males ($P<0.05$). In both female and male volunteers the topical agents were not statistically different concerning local anesthetic injection ($P>0.05$).

Conclusion: EMLA was superior in reducing pain during needle insertion, but none of the topical agents were effective in reducing pain during local anesthetic injection.

***Key words*:** Local Anesthesia, Ropivacaine, Liposomes, palatal mucosa, EMLA.

Introduction

Local anesthesia in the palatal mucosa is important to allow palatal soft tissue manipulation without pain in different dental procedures (Meechan et al., 2000).

However it is known that this region has a thick keratinized layer that is more resistant to the effects of topical anesthetics than other intraoral sites, specially the anterior portion (Meechan, 2002; Meechan et al., 2005). Infiltration anesthesia in palatal mucosa can be extremely painful because this mucosa is firmly attached to underlying periosteum and has numerous accessory nerves (McArdle, 1997). According to Harker (1997) the pain during palatal injections is more associated with the dislocation of the muco-periosteum than the needle punction.

Because palatal mucosa is one of the most painful sites to perform local anesthesia in the mouth, it is the strictest test that a topical anesthetic can be submitted to for assessing its efficacy (Svensson & Petersen, 1992; Meechan et al., 2005).

An effective topical agent to reduce pain during local anesthesia in the palate is been pursued since 1979 (Gill & Orr, 1979). Several studies demonstrated that the most used topical agent, 20% benzocaine, failed to reduce pain from needle insertion and from local anesthetic injection in this region (Gill & Orr, 1979; Keller et al., 1985; Hutchins et al., 1997; Fukayama et al., 2002).

In the 20 century the first studies with EMLA, in that time called a new euthetic mixture of local anesthetics (2.5% lidocaine and 2.5% prilocaine) for dermal use, were performed at the oral mucosa showing promising results. In most of the studies this topical cream was effective in the palatal mucosa in alleviating pain from needle insertion (Holst & Evers,1985; Svensson & Petersen, 1992; Al-Melh & Andersson, 2007; Al-Melh & Andersson, 2008), local anesthetic injection (Hutchins et al., 1997; Meechan & Winter, 1996) and removal of a leaf fibroma (Meechan, 2001). According to Meechan (2002) this was the unique effective topical anesthetic in reducing pain during palatal injection.

The liposome encapsulation of local anesthetics has been widely studied for dermal topical application. Liposomes are phospholipid vesicles used as drug carriers that were demonstrated to enhance cutaneous and percutaneous penetration providing slow release of the local anesthetic and better superficial anesthesia (Gesztes & Mezei, 1988; Foldvari, 1994; Bucalo et al., 1998; Fisher et al., 1998; Friedman et al., 1999).

In dentistry the liposome encapsulated ropivacaine was observed to perform similar efficacy to EMLA as an oral topical anesthetic in reducing pain during needle insertion in the maxillary buccal fold after a 2-minute application (Franz-Montan, et al., 2007).

The aim of the present study was to evaluate the efficacy of liposome-encapsulated ropivacaine in different concentrations in reducing pain during needle insertion and local anesthetic injection in the palatal mucosa.

Material and Methods

Forty health volunteers (20 female and 20 male) from 19 to 29 years-old (21.9 ± 2.7) were selected for this single-blind, randomized, crossover and four-period study. All the volunteers were undergraduate or graduate students at Piracicaba Dental School. The study was approved by the Ethics Committee of Piracicaba Dental School, University of Campinas, SP, Brazil (#059/2008).

All subjects were in good health, had no history of allergy to any of the local anesthetics used, and were not taking anti-inflammatory or analgesic drugs that would alter pain perception. After being verbally informed about the study, the volunteers that accepted to participate were asked to read and sign the informed consent.

The volunteers received 100mg (previously weighted) of the following topical anesthetics: liposome-encapsulated 2% ropivacaine; liposome-encapsulated 1% ropivacaine; liposome-placebo gel; and EMLA® (2.5% lidocaine and 2.5% prilocaine) in four different appointments spaced at least one week apart. EMLA® was used as a positive control due to its efficacy in reducing pain in the palatal

mucosa related to needle insertion (Holst & Evers,1985; Svensson & Petersen, 1992; Al-Melh & Andersson, 2007; Al-Melh & Andersson, 2008), and local anesthetic injection (Hutchins et al., 1997; Meechan & Winter, 1996).

Liposomal formulations were prepared at the Department of Biochemistry, Institute of Biology, University of Campinas. The liposomes consisted of large unilamellar vesicles of homogenised sizes (400nm) prepared following a previous described methodology (de Araújo et al., 2008).

Before topical anesthesia, the palatal mucosa at the right canine region was dried using sterile gauze and then the topical anesthetic was applied by using a cotton swab for 5 minutes. After removal of the topical anesthetic, a 30-gauge needle with an aspirating syringe was inserted at the same place of topical application (approximately 0.5 to 1.0 cm away from the gingival margin) until bone contact and 0.3mL of 2% lidocaine with 1:100.000 epinephrine (Alphacaine® – DFL Ind. Com. Ltda) was injected.

Following this procedure, the volunteers were asked to rate pain during needle penetration, and during anesthetic injection in two different visual analogue pain scales (VAS). VAS consists of a 100mm nongraded line where the left end (0) indicates “no pain” and the right end (10) indicates “unbearable pain”.

Statistical analyses

Data were analyzed with BioEstat, version 5.0 (Mamiraua Institute, Belem, PS, Brazil). Data were submitted to Kruskal-Wallis and Student Newman Keuls test considering gender and treatment group. Comparisons were considered significant at $P < .05$.

Results

Figure 1 shows medians of VAS pain scores during needle insertion. There was a gender-related effect in VAS scores during needle penetration ($P < .05$). EMLA was more effective in reducing pain during needle insertion ($P < .05$) than liposome-encapsulated ropivacaine at 1 or 2%, and also than liposomal placebo for

male and female volunteers. Liposome ropivacaine at 1% and 2% was not different from placebo ($P > .05$).

The use of EMLA promoted significant lower VAS values of pain during needle insertion in female than in male volunteers ($P < .05$).

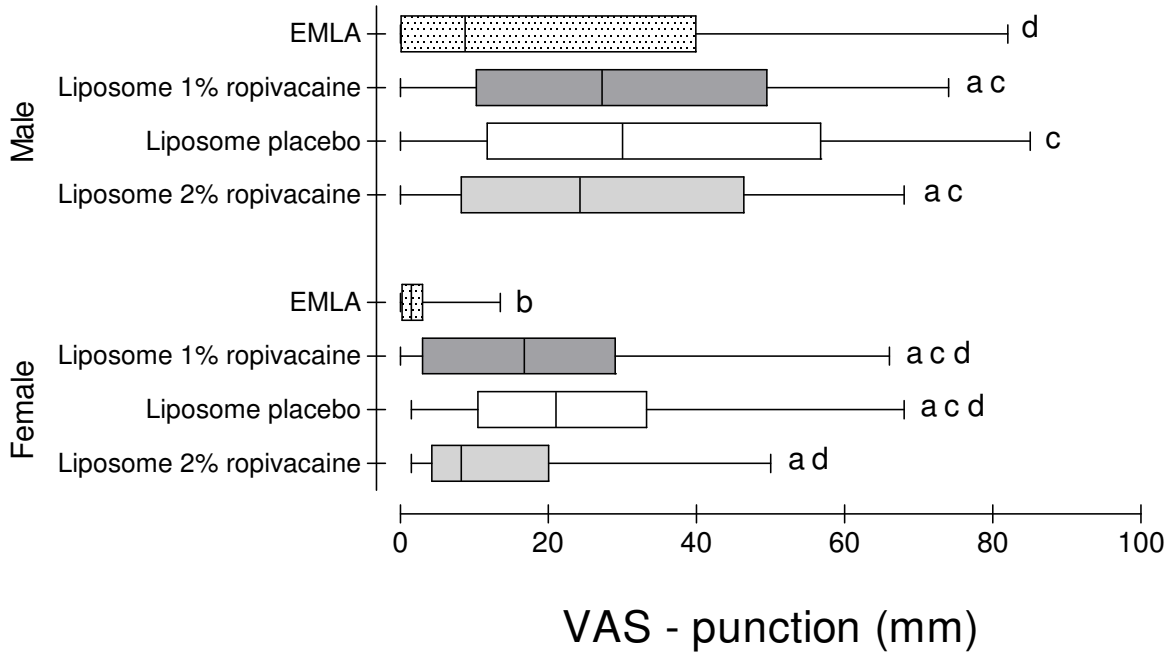

Figure 1. VAS scores rated by volunteers during needle insertion (Central line: median; Box: lower and upper quartiles; Whisker: maximum and minimum values). Different letters represent statistically significant differences - p<0.05.

Regarding local anesthetic injection, there was no statistical difference among topical anesthetics used ($P > .05$). Figure 2 shows medians of VAS for all groups concerning pain during local anesthetic injection.

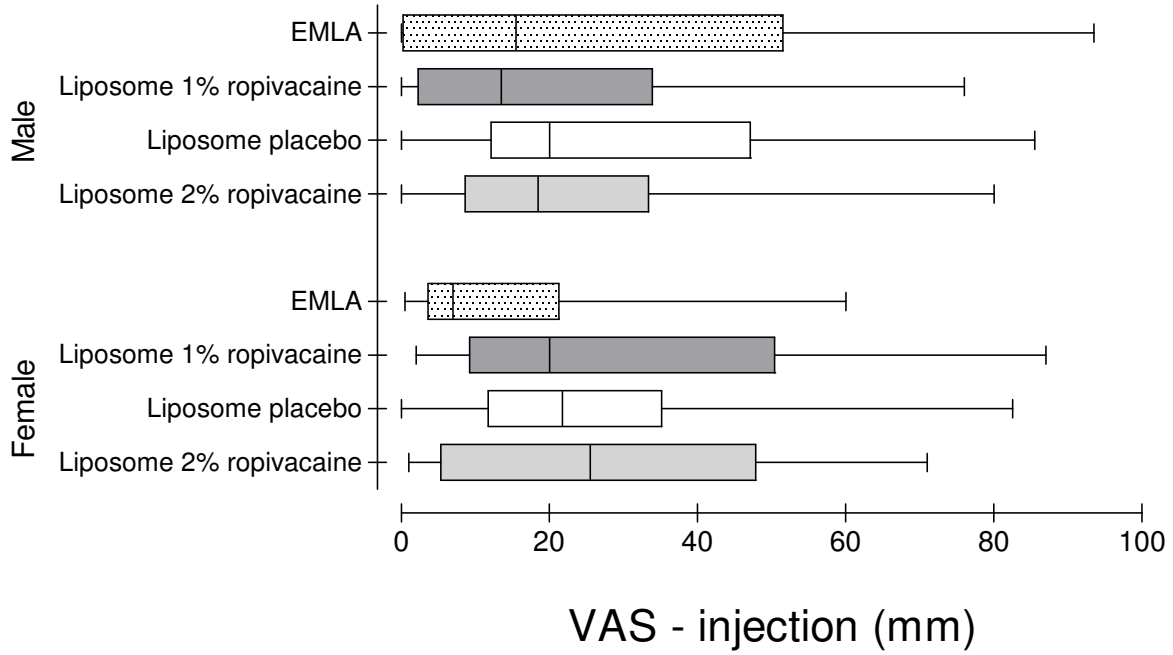

Figure 2. VAS scores rated by volunteers after local anesthetic injection (Central line: median; Box: lower and upper quartiles; Whisker: maximum and minimum values).

Discussion

Topical anesthetics are commonly used by dentists to reduce pain during dental anesthesia. The results in the literature concerning topical anesthetics efficacy are contradictory. The efficacy depends on the topical anesthetic agent, the site and the duration of application (Meechan, 2002).

According to Meechan et al. (2005) pain during needle insertion is more intense in the anterior region of the palate in comparison with the posterior region. Harker (1997) attributes the pain associated to local anesthetic administration to the dislocation of the muco-periosteum. In agreement with the latter statement,

Hutchins et al. (1997) stated that a topical anesthetic is better evaluated concerning its efficacy if an injection is performed rather than only simulated.

In the present study we decided to test liposomal ropivacaine in a very strict model for oral topical anesthetic: local anesthetic injection in the anterior palate region.

Recently, it was shown that liposome encapsulated 1% ropivacaine was equivalent to EMLA in reducing pain during needle insertion, (no anesthetic solution was injected) in the maxillary buccal fold after a 2-minute application (Franz-Montan, et al., 2007). In the present study, however, even in double concentration (2%) and with a longer application time (5min) liposome-encapsulated ropivacaine was not effective in reducing pain to needle insertion, as recorded by VAS.

EMLA, on the contrary, was effective in reducing pain during needle penetration in both genders. This result is in agreement with other authors (Holst & Evers, 1985; Svenson et al. 1992; Al-Melh et al. 2007, Al-Melh & Andersson, 2008) that observed a superiority of EMLA over other topical anesthetics or placebo in reducing pain related to needle penetration in the palate.

Interestingly, no difference was observed between genders in the present study, with the exception of EMLA groups. These results are in agreement with that of Meechan et al. (2005) who found no difference between men and women concerning VAS scores after needle penetration in the anterior and posterior region of the palate.

Liposome encapsulated local anesthetics have been related as having equal or superior performance in comparison with EMLA (Fisher et al. 1998, Friedman et al. 1999) and non encapsulated tetracaine (Geztes & Mezei, 1988; Hung et al., 1997) in reducing pain to needle insertion in skin after 30 and 60 minutes of application. Differences in the methodology such as patient age, number of volunteers and also the inclusion of a placebo group could explain the difference in results between the present study and these ones. Other possible causes for the difference in the results are the size of liposome used and the percentage of local

anesthetic encapsulated, which are not mentioned in the majority of the studies, except for the Geztes & Mezei (1988) in which multilamelar liposomes were used. In the present study ropivacaine was encapsulated in unilamelar liposome with 24% of encapsulation.

Two studies have evaluated liposomal local anesthetics in oral mucosa (Zed et al. 1996, Franz-Montan et al. 2007). In the former study liposome amethocaine was effective in reduce needle penetration and anesthetic injection pain (no mention is made in relation to the exact site and time of application). In the later study it was observed a reduction in the pain due to needle insertion after liposomal ropivacaine application in the buccal fold mucosa, a region known as less painfull than the palate (Meechan, 2002).

However, as shown in the present study, after application in the palate the results were disappointing. The hypothesis of enhanced penetration through the keratinized palatal mucosa of liposome-encapsulated ropivacaine was not confirmed here.

These findings confirm that topical anesthesia is more effective in the buccal sulcus than in the palatal mucosa (Hutchins et al. 1997, Meechan, 2002) and that the best way to evaluate the topical anesthetic efficacy is by performing an injection.

None of the preparations were able to reduce the pain related to local anesthetic injection. These results are in agreement with that of Hutchins et al. (1997) who did not find difference between 20% benzocaine and placebo application before anesthetic injection in the palate.

Although some studies have related reduction in scores of injection pain (Meechan & Winter 1996) after the use of EMLA and even a case report of soft tissue lesion removal (Meechan, 2001), there must be emphasized the differences in amount of topical anesthetic applied and application time as observed in the second study. Specifically in the latter study an amount of 0.5g of EMLA was applied during 15 minutes. For the purpose of obtaining mucosal anesthesia previous to local anesthetic injection this application time is too long for clinical use.

In addition, longer times may cause mucosa necrosis, as observed by Franz-Montan et al. (2008) with a 30 minute of EMLA application to buccal mucosa.

In the study of Meechan & Winter (1996) EMLA was more effective than placebo and TENS (transcutaneous electronic nerve stimulation) in reducing the injection pain in the palate. Although the apparent great number of patients (100) used to compare the treatments, the study was not designed as a crossover and palatal injections were performed in the anterior and posterior region of the palate, according to the tooth to be treated. The authors did not find difference in pain perception between the anterior and posterior region of the palate, what in a more recent crossover study (Meechan et al., 2005) was shown to be different, with higher degree of discomfort during needle insertion in the anterior than in the posterior palatal region.

These results clearly show that palatal injection is a very painful stimulus and a strict model to evaluate topical anesthetic efficacy. The ideal intra-oral topical anesthetic is not presently available.

In conclusion liposome-encapsulated ropivacaine formulation although effective in the buccal mucosa did not reduce pain related to needle insertion nor to anesthetic injection in the palatal mucosa. EMLA was the only effective topical anesthetic to reduce pain during needle insertion, but none of the anesthetic formulation tested were effective in reducing pain related to local anesthetic injection as compared to a placebo. There is still a need to develop newer and better topical anesthetics for palatal mucosa application.

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CAPÍTULO 3: Efficacy of liposome-encapsulated 0.5% ropivacaine in maxillary dental anesthesia.

Short title: Liposome-ropivacaine in local anesthesia.

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Implication Statement

Liposome- encapsulated ropivacaine was not able to improve the anesthetic properties of ropivacaine for infiltration in maxillary dental local anesthesia.

Abstract

Background. Ropivacaine, a long acting amide-type local anesthetic, has been reported as an effective local anesthetic for maxillary infiltration and inferior alveolar nerve block in dentistry. Liposome encapsulation has been found to increase local anesthetic efficacy. Therefore, the aim of this study was to evaluate the effectiveness of liposome-encapsulated ropivacaine (0.5%) in dental anesthesia.

Methods. This randomized, double-blind, crossover, four-period treatment study included 40 volunteers receiving 1.8 mL of the following local anesthetics in the buccal sulcus at the right level of the upper canine: a) 0.5% ropivacaine (plain ropivacaine); b) 0.5% ropivacaine with 1:200,000 epinephrine (ropivacaine-epi); c) liposome-encapsulated 0.5% ropivacaine (liposome-ropi); and d) 2% lidocaine with 1:100,000 epinephrine (lidocaine-epi). Onset of pulpal anesthesia, anesthesia success, and duration of labial, gingival and pulpal anesthesia involving the lateral incisor, canine, and first and second premolars (teeth 12, 13, 14 and 15) were evaluated. At the end of each injection, volunteers rated anesthetic injection pain on a visual analogue scale (VAS). Blood pressure (systolic and diastolic) and heart rate were measured before, during and after anesthesia injection.

Results. Teeth 12 and 15 presented low anesthesia success. Both ropivacaine-epi and lidocaine-epi showed higher incidence of anesthesia success than liposome-ropi and plain ropivacaine concerning teeth 13 and 14 ($P < 0.05$). No statistically significant difference was observed between ropivacaine-epi and lidocaine-epi or between liposome-ropi and plain ropivacaine ($P > 0.05$). In relation to the onset of pulpal anesthesia, no statistical difference was observed among the anesthetic preparations for teeth 13 and 14 ($P > 0.05$). Ropivacaine-epi and lidocaine-epi showed a significantly longer duration of pulpal anesthesia for these teeth. VAS showed no statistically significant difference among the groups tested. Cardiovascular parameters remained within a physiological range.

Conclusion. Liposome-encapsulated ropivacaine was considered ineffective as a dental local anesthetic.

Introduction

Most conventional procedures in dentistry are of short duration and do not result in postoperative pain, however there are some specific dental procedures that lasts longer requiring a long lasting local anesthesia to prevent the need for reinjection (1).

Long-acting local anesthetics are also indicated in dentistry to avoid severe postoperative pain, thus reducing the need for other analgesic drugs (2). It was demonstrated in previous studies that 0.5% bupivacaine significantly reduced the pain experience after third molar surgery when compared to 2% lidocaine (3, 4).

Ropivacaine, a long acting amide-type local anesthetic, chemically homologous to bupivacaine and mepivacaine (5), has been reported as having lower toxic effect than bupivacaine on central nervous and cardiovascular system (6-9).

In addition, several *in vivo* studies have reported ropivacaine as an effective local anesthetic for maxillary infiltration and inferior alveolar nerve block in dentistry (10-13). This local anesthetic has also been proven effective in patients undergoing many oral surgical procedures, such as cystectomy, apicoectomy, and extraction of lower and upper impacted third molars involving maxillary sinus with oro-antral communication (14).

Previous animal and human studies showed that local anesthetics associated with liposomal formulations were effective to prolong the duration of local anesthesia, as well as to reduce nervous and cardiac toxicity (15-24).

It was demonstrated in volunteers that maxillary infiltration of liposome-encapsulated 3% mepivacaine promoted longer pulpal anesthesia when compared to the plain solution of the same concentration. In addition, even in a 50% lower concentration (2%), the liposome-encapsulated mepivacaine was similar to 3% plain mepivacaine concerning pulpal anesthesia (25).

Therefore, the aim of the present study was to evaluate the efficacy of liposome-encapsulated ropivacaine as an alternative for local anesthesia in dentistry.

Methods

This study was approved by the Ethics Committee at Piracicaba Dental School, University of Campinas, São Paulo, Brazil (#164/2006). Informed written consent was obtained from each volunteer.

In a randomized, double-blind, crossover design, forty healthy volunteers (20 men and 20 women), age 18–44 years (22.6 ± 4.5), received 1.8 mL of four different local anesthetics at the apex of the maxillary right canine, in four different appointments spaced one week apart.

Through oral questioning, the subjects reported no history of allergy to any of the local anesthetics tested or use of any medication that would alter their pain perception. The teeth tested had no history of trauma or sensitivity and were free of caries, large restorations, or periodontal diseases.

Local Anesthetic Formulations, Infiltration Anesthesia, and Parameters of Local Anesthesia

All subjects received four local anesthetic formulations: a) 0.5% ropivacaine (plain ropivacaine), b) 0.5% ropivacaine with 1:200,000 epinephrine (ropivacaine-epi), c) liposome-encapsulated 0.5% ropivacaine (liposome-ropi), and d) 2% lidocaine with 1:100,000 epinephrine (lidocaine-epi). The liposomal formulation, consisting of large unilamellar vesicles (LUV) of homogenous size (400nm), was prepared at the Department of Biochemistry, Institute of Biology, University of Campinas, SP, Brazil, based on a previously described method (24, 26). Samples of each anesthetic formulation were tested to determine pH values using a pH meter (Orion Research, Boston, MA).

Because it is not available in dental cartridges, ropivacaine was obtained in clinical vials (Naropin® 10mg mL AstraZeneca, Sao Paulo, SP, Brazil). The concentration of 0.5% ropivacaine was prepared under sterile conditions, using a simple dilution as follows: 5 mL of 1% ropivacaine was drawn from a 10-mL vial and 5 mL of a sterile saline solution was added and then 1.8 mL of this final

solution was placed into a sterile 3-mL syringe (Luer-Lok, Becton Dickinson, Curitiba, Brazil) with a 30 G x 1" needle (Becton Dickinson and Company, Franklin Lakes, NJ, 07417) immediately before application. To prepare the epinephrine-containing ropivacaine solution, 0.05 mL of 1:1,000 epinephrine was added to 5 mL of 1% ropivacaine and 4.95 mL of sterile saline solution. Lidocaine solution was commercially obtained (Alphacaine DFL, Ind. Com. Ltda, Rio de Janeiro, RJ, Brazil). To allow blindness of the experiment, all the anesthetic preparations were injected by using sterile 3-mL Luer-Lok syringes with 30 G x 1" needles.

To reduce pain during needle insertion, 20% benzocaine gel (Benzotop® - DFL Ind Com Ltda, Rio de Janeiro, Brazil) was applied during 2 min at the apex of the maxillary right canine (27).

The anesthetic formulations were injected into the buccal sulcus at the right level of the upper canine at an injection rate of 1mL/min. The needle was inserted up to periosteum of the apex of the canine and withdrawn 1mm prior to injection. All the injections were performed by the same operator. Right after local anesthesia infiltration, the volunteers were asked to rate anesthesia-related pain on a 0 (indicating "no pain") to 10 ("unbearable pain") visual analogue scale (VAS).

An electric pulp tester (Analytic Technology Corp., Redmond WA) was used to evaluate pulpal anesthesia (28). The pulp tester has a voltage output which ranges from 0 to 300 V (0-80 units on a digital scale) at 0.08 mA (10 pulses every 6 ms).

At the beginning of every session, and before any anesthetic procedure, the lateral incisor (12), canine (13), first pre-molar (14) and second pre-molar (15) were tested three times (two-minute intervals) using the pulp tester to obtain the baseline tooth vitality. The probe tip of the pulp tester was placed in the center of the buccal side of each tooth, using fluoride gel as a conductive substance (29). The contralateral canine was also tested and used as a control to confirm that the pulp tester was operating properly and to certify that the subjects were responding accurately during the study.

After injection, the teeth (12; 13; 14; 15) were tested every 2 minutes until

there was no response to the maximum output of the pulp tester (80 reading). After this, these teeth were tested every 10 minutes until two positive responses of stimulus perception were obtained. All the pulp testing was performed by a trained person who was blinded to the anesthetic formulations administered.

Gingival and lip anesthesia was evaluated by pinprick test and palpation, respectively, every 10 minutes up to cessation of numbness (27, 30).

The parameters evaluated were: duration of soft tissue anesthesia (time from beginning to end of lip and gingival numbness); onset of pulpal anesthesia (time from end of injection to the first two consecutive readings of 80 without response); duration of pulpal anesthesia (time from the first two readings of 80 without response to the time recorded before 2 consecutive positive responses to the pulp tester); and anesthesia success (a minimum of 10 minutes of pulpal anesthesia).

Cardiovascular parameters

A wrist blood pressure monitor (HEM 610 INT- Omron, China) was used to measure the blood pressure (systolic and diastolic) and the heart rate of all subjects in four different periods: 1) 5 min before anesthesia infiltration; 2) at the beginning of the anesthetic injection; 3) immediately after and 4) 5 min after anesthetic injection. All the subjects were asked to lie down in a dental chair comfortably at a supine position for 5 minutes prior to cardiovascular monitoring.

Statistical analysis

Onset and duration of pulpal anesthesia, duration of soft tissue anesthesia and VAS data were submitted to Kruskal-Wallis and Student Newman Keuls test. Anesthesia success results were compared using the chi-square test. The significance level for the statistical analyses was set at 5%.

Results

The pH values concerning all solutions were: 5.5 for 0.5% ropivacaine; 4.7 for 0.5% ropivacaine with 1:200,000 epinephrine; 6.2 for liposome-encapsulated 0.5% ropivacaine; and 4.1 for 2% lidocaine with 1:100,000 epinephrine.

Figure 1 shows the incidence of anesthesia success concerning the formulations and teeth tested. Lidocaine-epi showed higher incidence of anesthesia success for teeth 13, 14 and 15 ($P < 0.05$), followed by ropivacaine-epi, with no significant difference between them ($P > 0.05$). Liposome-ropi and plain ropivacaine were the least effective anesthetic formulations. Lidocaine-epi was observed to be the most effective formulation for tooth 12 ($P < 0.05$) with no significant difference among the others ($P > 0.05$).

Insert figure 1

Because of the low incidence of pulpal anesthesia observed for teeth 12 and 15, especially with liposome-ropi and plain ropivacaine, these teeth were not evaluated in relation to onset and duration of pulpal anesthesia.

Figure 2 shows results for pulpal anesthesia onset (teeth 13 and 14). No significant differences concerning onset of pulpal anesthesia were observed among the anesthetic formulations tested.

Insert figure 2

Figure 3 shows results for duration of pulpal anesthesia (teeth 13 and 14). Ropivacaine-epi and lidocaine-epi promoted a significantly longer duration of pulpal anesthesia when compared to liposome-ropi and plain ropivacaine. No significant difference was observed between ropivacaine-epi and lidocaine-epi or between liposome-encapsulated ropivacaine and plain ropivacaine ($P > 0.05$).

Insert figure 3.

Figure 4 shows results for soft tissue anesthesia (gingival and lips). Ropivacaine-epi promoted the longest gingival anesthesia ($P < 0.05$), followed by plain ropivacaine and lidocaine-epi; although not statistically different from lidocaine-epi ($P > 0.05$), liposome-ropi provided the shortest gingival anesthesia.

Concerning lip numbness, ropivacaine-epi and plain ropivacaine promoted longer duration of anesthesia when compared to lidocaine-epi and liposome-ropivacaine ($P < 0.05$). There was no significant difference between ropivacaine-epi and plain ropivacaine or between lidocaine-epi and liposome-ropi ($P > 0.05$).

Insert figure 4.

There was no significant difference ($P > 0.05$) among all the groups tested concerning pain during anesthetic injection. Figure 5 shows medians of VAS (mm) for all groups.

Insert figure 5.

Cardiovascular parameters

Figure 6 summarizes changes in blood pressure (systolic and diastolic) considering the four periods evaluated (5 min before, at the beginning; immediately after and 5 min after anesthetic injection). The results are described as median and inter-quartile range values.

A statistically significant increase in the values concerning systolic and diastolic blood pressure was observed for all anesthetic formulations during local anesthetic injection ($P < 0.05$); right after the anesthetic injection, such values were observed to return to those obtained initially ($P > 0.05$).

Insert figure 6.

Figure 7 shows median (interquartile range) values for heart rate considering all the periods tested. A decrease in heart rate was observed for plain ropivacaine during local anesthetic injection ($P < 0.05$); right after the anesthetic injection, such values were observed to return to those obtained initially. However, an increase in heart rate was observed right after the anesthetic injection for both epinephrine formulations ($P < 0.05$) maintaining the same levels in the 5 minutes after anesthetic injection period. Liposome-ropi induced an increase in heart rate just after the anesthetic injection ($P < 0.05$) returning to the pre-anesthetic values 5 minutes after local anesthesia.

Insert figure 7.

Discussion

The first study to test ropivacaine for use in dental anesthesia reported a higher anesthetic success rate and longer anesthesia for 1.8 mL of 0.5% ropivacaine associated with 1:200,000 epinephrine for maxillary infiltration in the lateral incisor (target tooth) region, when compared to plain ropivacaine (10).

In the present study, the anesthetic success observed for canine (72.5%) and lateral incisor (75%) was similar to that reported by Kennedy et al. (10), investigating the same teeth. In both studies, epinephrine-containing ropivacaine was more effective than plain ropivacaine (50% in the present study and 68% in the study of Kennedy et al. (10). However, Ernberg & Kopp (11) observed only 25% success for pulp anesthesia involving the maxillary lateral incisor, which could be due to the low volume injected (0.5 to 1 mL). More recently, Oliveira et al. (31) found no difference between plain ropivacaine and epinephrine-containing ropivacaine for inferior alveolar nerve block. These findings could be explained by the fact that long acting local anesthetics such as bupivacaine are more effective for nerve block than for infiltration anesthesia.

The onset of pulpal anesthesia observed for plain ropivacaine was longer in the present study than that observed by Kennedy et al. (10). Not only the differences involving the methodology but also the great result variability obtained by these authors could explain the results (onset of pulpal anesthesia) obtained in the present study.

Pulpal anesthesia duration observed for the target tooth (tooth 13) in the present study was similar to that obtained by Kennedy et al. (10), reporting 12 min for ropivacaine and 33 min for epinephrine-containing ropivacaine. An increased duration of anesthesia concerning epinephrine-containing ropivacaine for soft tissue anesthesia was reported in both studies.

A previous study involving mepivacaine and lidocaine in a rat infraorbital nerve block model reported that the encapsulation of local anesthetics into large unilamellar vesicles (LUVs) intensified the analgesic effects of such anesthetics and that mepivacaine was affected to the greatest extend, probably due to the

greater vasodilatory property of lidocaine (23).

Although previous *in vivo* studies have reported that ropivacaine has vasoconstriction properties (32-34), the results obtained in the present study indicate that epinephrine should be associated with ropivacaine to achieve anesthetic efficacy for dental use.

The encapsulation of ropivacaine into large unilamellar vesicles (LUVs) has been reported to increase the duration and intensity of the anesthetic for either sciatic or infraorbital nerve blockade in rats (24). Tofoli et al. (25) reported an improved duration of pulpal anesthesia in human canine after maxillary infiltration of 2% liposome-encapsulated mepivacaine (LUV) in comparison to 2% plain mepivacaine. In addition, this 2% liposome-encapsulated mepivacaine was equivalent to 3% mepivacaine, a commercially available solution.

The anesthetic properties of mepivacaine have been reported to improve with liposomal encapsulation; however, such finding was different from that observed for ropivacaine in the present study, using the same size and composition of vesicles. This finding was not expected since positive results with liposomal ropivacaine were previously shown in animal studies (24) and most of the characteristics of ropivacaine such as long-acting local anesthetic and vasoconstrictive properties (32-34) lead to the hypothesis that ropivacaine effectiveness could be improved by liposome encapsulation.

According to Barenholz (35), a high level of loading into the liposome and a slow release profile are important factors to prolong the effect of an encapsulated drug. In a study mentioned above (24), even though the release profile of ropivacaine was observed to be decreased by liposome encapsulation, the loading efficiency of the local anesthetic was only 24%. The authors (24) suggested that with an enhanced encapsulation efficiency or chemical alterations in liposome composition, controlling both its size (to avoid fast clearance or delayed onset) and anesthetic release rate, it would be possible to achieve a prolonged analgesic effect, with lower cytotoxicity. These changes could improve the clinical efficacy of ropivacaine in dentistry.

Tofoli et al. (25) observed a significant reduction in injection pain (VAS values) with the liposomal formulation compared to the vasoconstrictor-associated anesthetic solution; this finding was different from that observed in the present study.

According to Meechan & Day (36), differences found in perception of pain during dental local anesthesia considering different solutions may be attributed to their different pHs. Oikarinen et al. (37) reported that local anesthetic solutions with a low pH were susceptible to pain than those with a high pH. Higher concentrations of the local anesthetic were also found to lead to higher pain susceptibility.

In the present study, the pH of ropivacaine formulations ranged from 4.7 (ropivacaine-epi) to 6.2 (liposome-ropi). Anesthetic solutions containing epinephrine have a pH that varies from 3.5 to 4.5. The higher pH observed for ropivacaine-epi might have been responsible for the absence of difference in pain perception between ropivacaine-epi and the other formulations tested. It is also important to emphasize that the onset of anesthesia, which is also expected to be affected by pH, did not differ among the groups in the present study.

The baseline values of the cardiovascular parameters (systolic and diastolic blood pressure and heart rate) were compatible with the good physical condition of the volunteers, all of whom were young and healthy and were classified as ASA 1 according to the American Society of Anesthesiology. However, all the formulations induced slight alterations in heart rate and blood pressure which, although statistically significant, remained within the normal accepted physiological values (38).

In conclusion, liposome-encapsulated ropivacaine was ineffective for maxillary infiltration in humans. Further studies are needed to investigate the liposome-encapsulated ropivacaine as to enhance its anesthetic effect in dentistry.

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Figure legends

Figure 1. Percentage of volunteers and incidence of pulpal anesthesia (teeth 12, 13, 14 and 15) determined by the lack of response to electrical pulp testing at the maximum reading of 80.

Figure 2. Onset of pulpal anesthesia (median and interquartile range, in minutes) for teeth 13 and 14 after infiltrations of 0.5% ropivacaine, 0.5% ropivacaine with 1:200,000 epinephrine (ropivacaine-epi), liposome-encapsulated 0.5% ropivacaine (liposome-ropi), and 2% lidocaine with 1:100,000 epinephrine (lidocaine-epi).

Figure 3. Duration of pulpal anesthesia (median and interquartile range, in minutes) for teeth 13 and 14 after anesthetic infiltrations of 0.5% ropivacaine, 0.5% ropivacaine with 1:200,000 epinephrine (ropivacaine-epi), liposome-encapsulated 0.5% ropivacaine (liposome-ropi), and 2% lidocaine with 1:100,000 epinephrine (lidocaine-epi).

Figure 4. Duration of soft tissue anesthesia (median and interquartile range, in minutes) for gingiva and lip after anesthetic infiltrations of 0.5% ropivacaine, 0.5% ropivacaine with 1:200,000 epinephrine (ropivacaine-epi), liposome-encapsulated 0.5% ropivacaine (liposome-ropi) and 2% lidocaine with 1:100,000 epinephrine (lidocaine-epi).

Figure 5. VAS scores (in mm) rated by the volunteers after anesthetic infiltration of 0.5% ropivacaine, 0.5% ropivacaine with 1:200,000 epinephrine (ropivacaine-epi), liposome-encapsulated 0.5% ropivacaine (liposome-ropi), and 2% lidocaine with 1:100,000 epinephrine (lidocaine-epi). (Central line: median; Box: lower and upper quartiles; Whisker: maximum and minimum values).

Figure 6. Median (interquartiles range) values of systolic (continuous line) and

diastolic (dashed lines) blood pressures (in mmHg), after injection of 0.5% ropivacaine, 0.5% ropivacaine with 1:200,000 epinephrine (ropivacaine-epi), liposome-encapsulated 0.5% ropivacaine (liposome-ropi) and 2% lidocaine with 1:100,000 epinephrine (lidocaine-epi) in the evaluated periods (5 min before, at the beginning of the anesthetic injection; immediately after and 5 min after anesthetic procedure).

Figure 7. Median (interquartiles range) values of heart rate (in beats per minute) after injection of 0.5% ropivacaine, 0.5% ropivacaine with 1:200,000 epinephrine (ropivacaine-epi), liposome-encapsulated 0.5% ropivacaine (liposome-ropi) and 2% lidocaine with 1:100,000 epinephrine (lidocaine-epi) in the evaluated periods (5 min before, at the beginning of the anesthetic injection; immediately after and 5 min after anesthetic procedure).

Figures

Figure 1

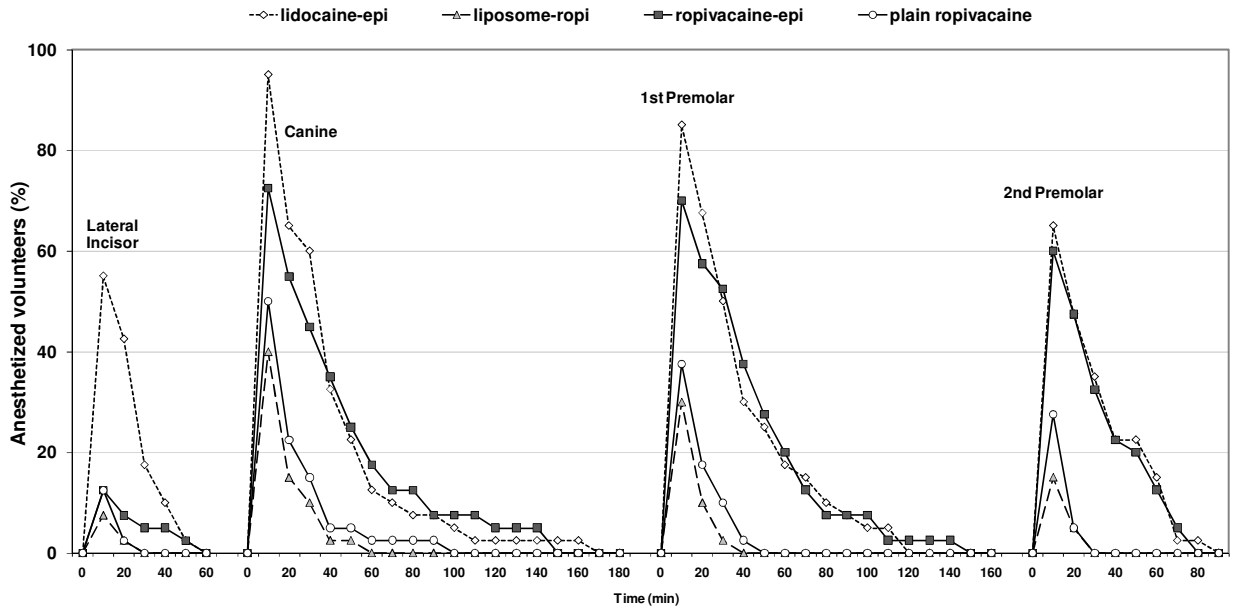


Figure 2

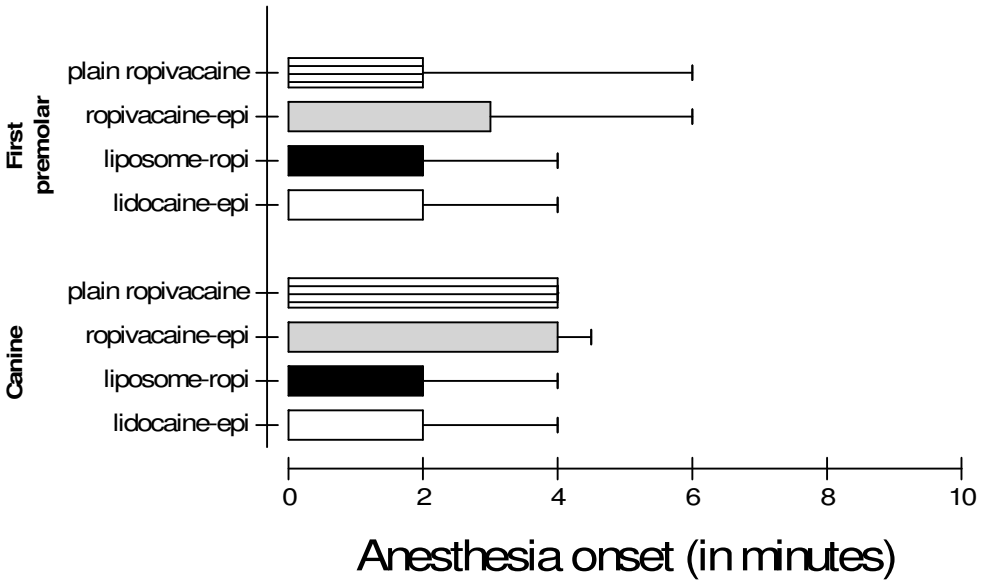


Figure 3

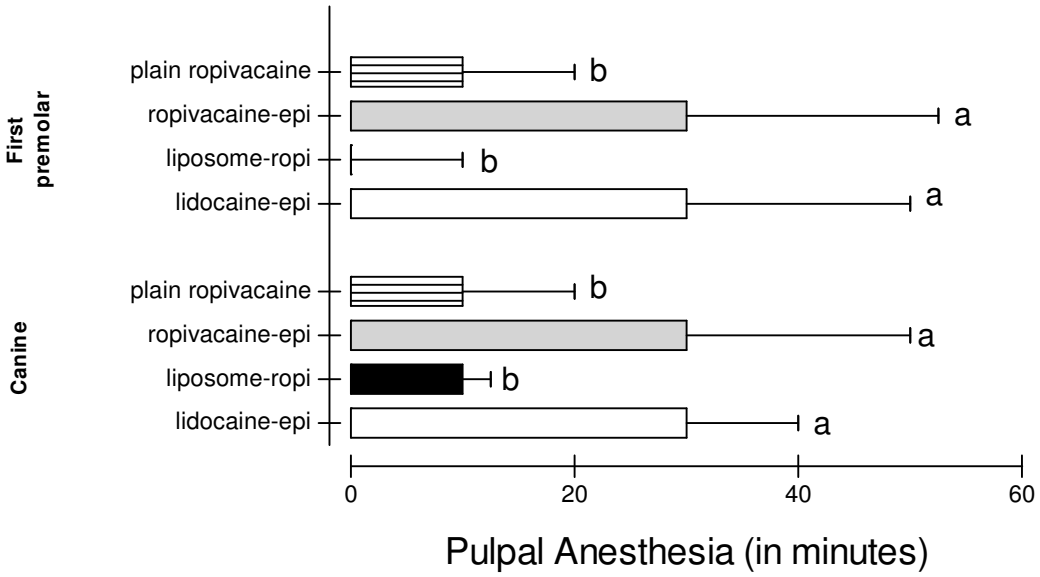


Figure 4.

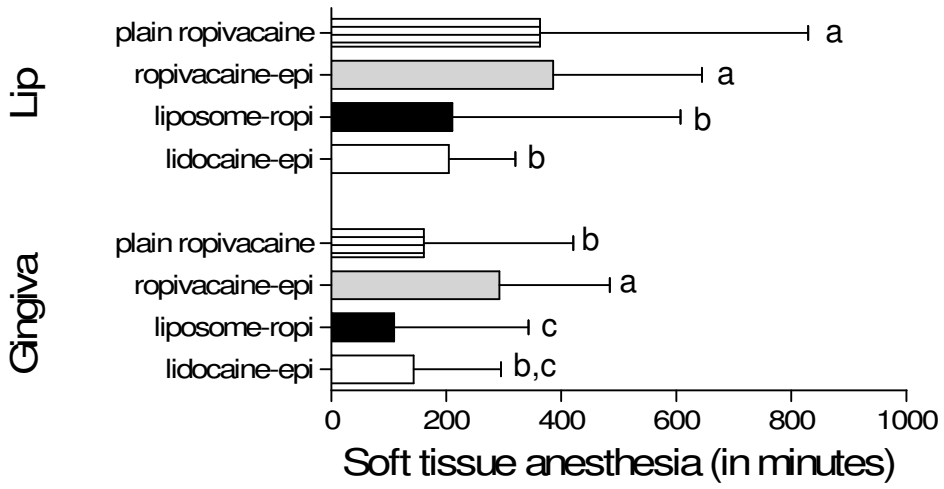


Figure 5.

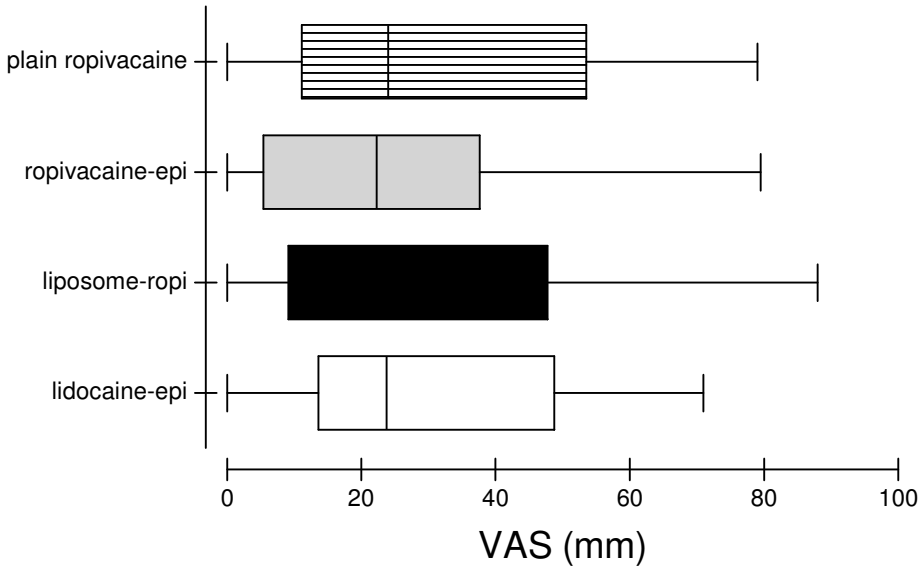

plain ropivacaine
ropivacaine-epi
liposome-ropi
lidocaine-epi
0
20
40
60
80
100
VAS (mm)

Figure 6.

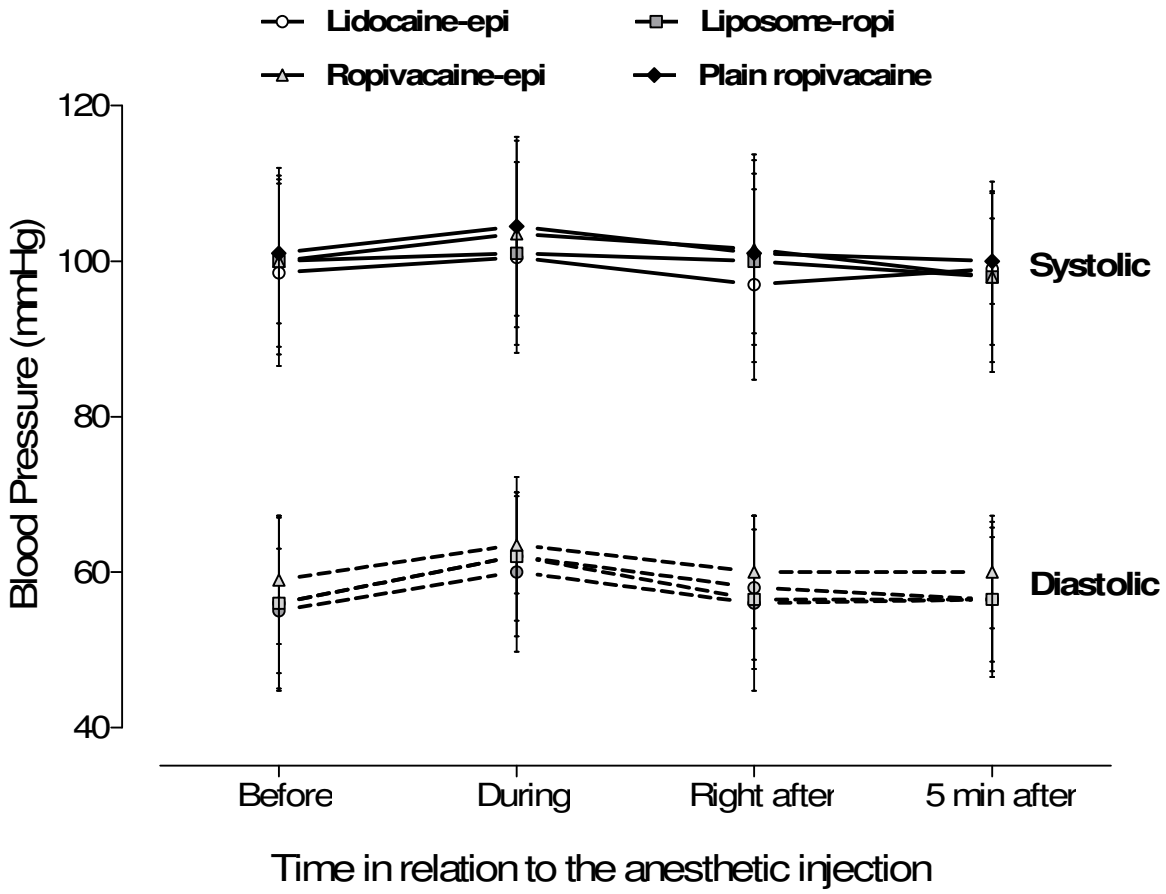
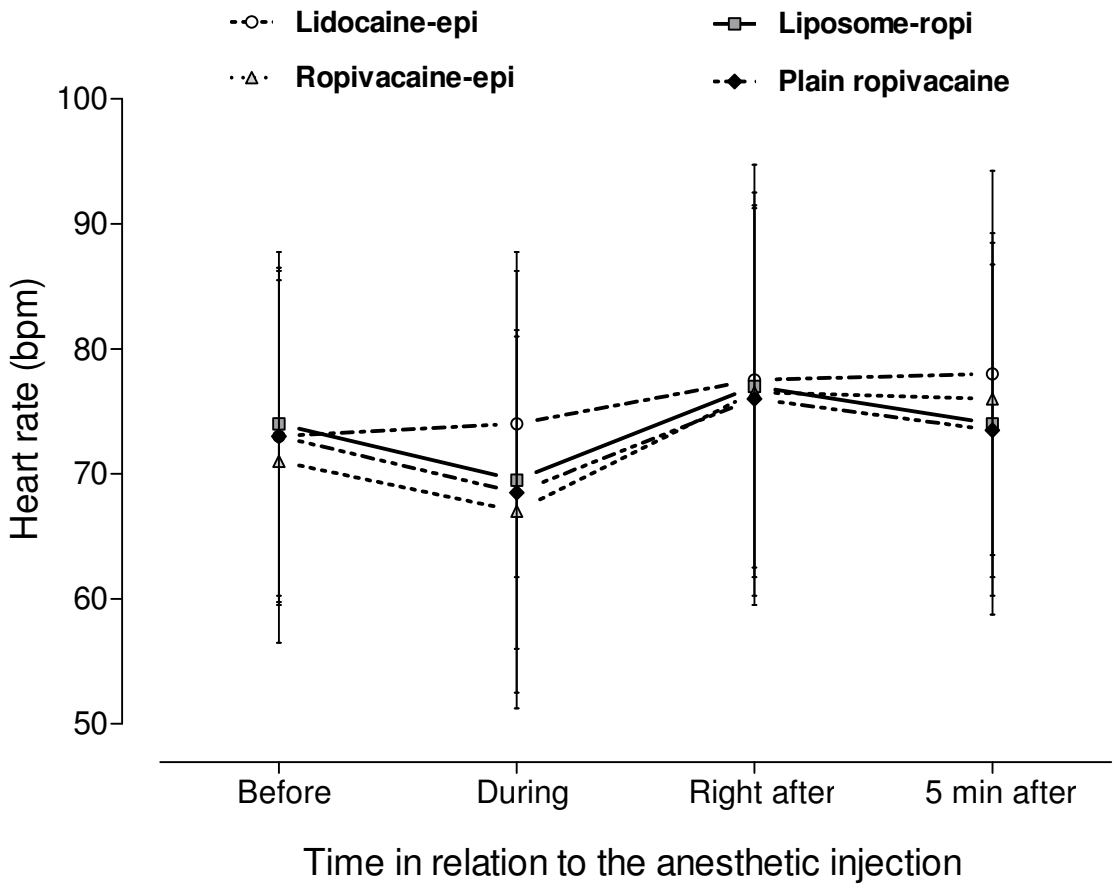

Lidocaine-epi
Liposome-ropi
Ropivacaine-epi
Plain ropivacaine
120
100
80
60
40
Blood Pressure (mmHg)
Systolic
Diastolic
Before
During
Right after
5 min after
Time in relation to the anesthetic injection

Figure 7.


Lidocaine-epi
Liposome-ropi
Ropivacaine-epi
Plain ropivacaine
100
90
80
70
60
50
Heart rate (bpm)
Before
During
Right after
5 min after
Time in relation to the anesthetic injection

CAPÍTULO 4: Pharmacokinetics of ropivacaine with epinephrine or encapsulated in liposome after dental anesthesia.

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Abstract

The aim of this study was to compare the pharmacokinetic parameters of ropivacaine with epinephrine and encapsulated in liposome, after dental anesthesia in 14 healthy volunteers. In this randomized, double-blind and crossover study, the volunteers received maxillary infiltration of 0.5% ropivacaine with 1:200,000 epinephrine (RopiEpi) and liposome-encapsulated 0.5% ropivacaine (RopiLipo), in two different sessions spaced one week apart. Blood samples were collected before and 15, 30, 45, 60, 75, 90, 120, 240, 420, 600 and 1440 minutes after the administration of either ropivacaine formulations. HPLC-UV detection was used to quantify plasmatic ropivacaine concentrations. The pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, CL, T_{max} and VD) were analyzed by Wilcoxon signed-rank test. For RopiLipo the median (1^{st} and 3^{rd} quartiles) were C_{max} 92.9 (82.7 - 97.7) ng/mL; T_{max} 30.0 (15.0 - 56.3) min ; AUC_{0-t} 40.4 (26.3 - 55.2) ng-min/mL; $AUC_{0-\infty}$ 71.9 (28.1 - 138.6) ng-min/mL; Vd 2.6 (1.5 - 4.4) mL/kg; CL 0.07 (0.05 - 0.28) mL/min. Considering RopiEpi the values were C_{max} 93.4 (63.2 - 114.7) ng/mL; T_{max} 37.5 (30.0 - 45.0) min ; AUC_{0-t} 32.4 (20.1 - 44.0) µg-min/mL; $AUC_{0-\infty}$ 78.5 (4.9 - 102.6) ng-min/mL; Vd 2.8 (1.5 - 13.8) mL/kg; CL 0.08 (-0.11 - 0.11) mL/min. No differences ($p>0.05$) were observed between the formulations for all the pharmacokinetic parameters evaluated. In addition, no differences (t test, $p>0.05$) were observed between ropivacaine concentrations of both formulations considering each period of time. In conclusion, RopiLipo and RopiEpi showed similar pharmacokinetic.

Introduction

Long-acting local anesthetic is required when postoperative pain and discomfort are expected especially after major surgical procedures (Marković & Todorović, 2006). In many countries, bupivacaine, the racemic mixture of S- and D-bupivacaine, is the only long-acting local anesthetic available in dental cartridges.

Ropivacaine, another long-acting local anesthetic, of the cyclic aminoamide family is synthesized in the S-enantiomer form and presents a lower toxicity to the cardiovascular and the central nervous systems when compared to bupivacaine (Leone et al., 2008).

Drug delivery systems, such as liposomes, have been used to prolong the duration of action of many drugs, including local anesthetics (de Araújo et al., 2008). Liposomes are phospholipid vesicles that were demonstrated to be effective drug carriers, improving anesthetic effectiveness and reducing its toxicity in both cardiovascular and central nervous systems (Geztes & Mezei, 1988; Boogaerts et al., 1993a; Boogaerts et al., 1994). These vesicles are nontoxic and nonimmunogenic because their components (phosphatidyl choline and cholesterol) are also found in biological membranes (Langer, 1990).

Some important features of effective drug carriers are the ability to encapsulate high concentrations of the transported drug, the slow removal from the injection site, the gradual release of the drug and the ability to significantly prolong its action with a reduced toxicity (Mowat et al., 1996; Grant & Bansinath, 2001). These characteristics were demonstrated *in vivo* (animal models) for liposome-encapsulated bupivacaine using multilamellar vesicles (Grant et al., 1994; Grant et al., 1997; Malinovsky et al., 1997; Yu et al., 2002; Grant et al., 2003) and large unilamellar vesicles (Mowat et al., 1996).

Previous authors showed that liposomal encapsulation of bupivacaine altered its pharmacokinetic profile after extradural injection in rabbits resulting in lower concentrations of the drug in plasma, liver and myocardium (Boogaerts et al., 1995). Grant et al. (2003) observed that bupivacaine, when encapsulated in

liposomes, remained at the injection site for a significant longer period of time, after subcutaneous injection in mice.

Attempting to simulate an accidental intravascular injection of a local anesthetic, Boogaerts et al. (1993a) accessed the acute CNS (central nervous system) and cardiac toxicities induced by intravenous infusion of 0.25% bupivacaine with and without epinephrine (1:200,000) in comparision to liposome-encapsulated bupivacaine in rabbits. They demonstrated a reduction of the CNS and cardiac toxicities of liposome-encapsulated bupivacaine. The addition of epinephrine to the plain solution did not decrease the CNS and cardiac toxicities induced by bupivacaine.

It was recently demonstrated in animal studies, which used sciatic and infraorbital nerve blockades, that ropivacaine encapsulated into large-unilamellar vesicles increased the duration and the intensity of analgesic effects (de Araújo et al., 2008).

Although long acting local anesthetics are used in low doses in dentistry, high doses of local anesthetic may be required for removal of four impacted third molar in one session (Eickbohm et al., 1991). According to Zink & Graf (2008) ropivacaine seems to have the greatest margin of safety of all long-acting local anesthetics and it could be useful in long lasting dental procedures.

The present study is the first attempt to access the pharmacokinetic parameters of ropivacaine after maxillary infiltration anesthesia of liposome encapsulated ropivacaine and ropivacaine with epinephrine formulations in healthy volunteers.

Materials and Methods

Subjects

The Ethical Committee of Piracicaba Dental School, University of Campinas approved this research (approval # 164/2006). Fourteen healthy volunteers (seven males) aging 24 (± 3.1) years old were selected and signed a written informed consent.

The volunteers presented no systemic or oral disorders, had no history of allergy to any of the local anesthetics used, and were not taking any medication, as determined by oral questioning and written health history.

Previously to the beginning of the study, all the subjects were submitted to laboratory tests which included cross-reactive protein, blood-hemoglobin, lymphocyte count, platelet count, erythrocyte sedimentation rate, serum (S)-sodium, S-potassium, S-chloride, S-albumin, S-alkaline phosphate, S-gamma-glutamyl-transferase, S-aspartate transaminase, S-alanine transaminase, S-creatine, plasma-glucose, urea, cholinesterase, total protein, bilirubin, uric acid, urine glucose, urine leukocyte count, urine protein, and urine hemoglobin. Serology tests of human immunodeficiency virus and hepatitis B and C were also performed. Female subjects had a urine βHCG pregnancy test performed. All laboratory testing was performed to confirm that the subjects were in good health and the females were not pregnant.

AMBULATORY PROCEDURES

Anesthetic procedures

In this double-blind and crossover study, the volunteers randomly received 1.8mL of 0.5% ropivacaine with 1:200,000 epinephrine and liposome-encapsulated 0.5% ropivacaine for infiltration anesthesia at the apex of the maxillary right canine in two different sessions spaced one week apart.

Liposome-encapsulated 0.5% ropivacaine was prepared at the Department of Biochemistry, Institute of Biology, University of Campinas, SP, Brazil. Ropivacaine used was kindly donated by Cristália Prod. Quím. Farm. Ltda (Itapira, SP, Brazil). The liposomes consisted of large unilamellar vesicles of homogenised sizes (400nm), prepared by a previously described method (de Araújo et al., 2008).

Ropivacaine with 1:200,000 epinephrine was achieved by a simple dilution of the commercially available solution of ropivacaine (Naropin® 10mg/mL, AstraZeneca, São Paulo, Brazil) immediately before application. Under sterile conditions, 5mL of 1% ropivacaine was diluted with 5mL of 1:100,000 (v/v)

epinephrine (Drenalin®, Ariston Ind. Quim. Farm. Ltda, São Paulo, SP, Brazil).

The local anesthetics (1.8mL) were placed into coded sterile 3mL Luer-Lok syringes (Becton Dickinson Curitiba, Brazil) with disposable needles (30G, one-inch, Becton-Dickinson Company, Franklin Lakes, NJ, USA). After topical anesthesia on the injection site with 20% benzocaine, the formulations were injected at the maxillary buccal fold of the right-canine region at an injection rate of 1mL/min. The maxillary infiltration anesthesia in all the subjects was performed by the same operator.

Blood sampling and drug analysis

Blood samples (4.5 mL) from a forearm vein were collected with a heparinized cannula before and 15, 30, 45, 60, 75, 90, 120, 240,420, 600 and 1440 minutes after the administration of either ropivacaine formulations. A heparinized saline solution (0.9% NaCl and heparin, 9.8:0.2) was injected (0.4 mL) into the cannula to prevent blood clotting after each blood sampling. The last sampling was obtained using a sterile syringe and needle. Immediately after each blood collection, the samples were centrifuged at 3000×*g* for 15 min and plasma was removed and stored at –70°C.

Detection of ropivacaine concentrations in the plasma samples was performed by high-performance liquid chromatography (HPLC) and a method adapted from Kawata et al. (2005). Briefly, chromatographic separations were carried out using a ODS column (TSK-GEL, 4.6 i.d. 150 mm, TOSOH) at room temperature. The detection wavelength was set at 215 nm. The mobile phase consisted of acetonitrile, methanol and 0.05 M phosphate buffer adjusted to pH 4.0 (10:30:60, v/v) pumped at a 1.0 mL/min of flow rate. The HPLC system consisted of Varian 9012 pump, a Varian diode-array detector (ProStar 335 DAD) coupled with Galaxie software integrator and a Varian autosampler (ProStar 410).

Plasma samples (250 µL) were extracted by adding 125 µL of 0.1 M sodium hydroxide in a 2.0 mL tube. The mixture was submitted to agitation and addition of 1 mL ethylacetate in order to extract ropivacaine. The 2.0 mL tube was vortexed for

1.5 min and centrifuged at 1500×*g* for 6 min. The upper organic phase was transferred to another 2.0 mL tube, and 1 mL of ethylacetate was added. The upper organic phase was removed to a new 2.0 mL tube. After evaporation to dryness at room temperature the residue was dissolved in 30 µL of the mobile phase and injected into the HPLC system.

A calibration curve was performed by diluting ropivacaine (Cristália Prod. Quím. Farm. Ltda) in drug-free human plasma samples in concentrations ranging from 0.03 µg/mL to 10 µg/mL.

Pharmacokinetic and statistical analyses

The following pharmacokinetic parameters: C_{max} (maximum drug concentration); T_{max} (maximum drug concentration time); AUC_{0-24}, (area under the plasma concentration- time curve from baseline to 24 h); $AUC_{0-\infty}$ (the area under the plasma concentration–time curve from baseline to infinity); CL (renal clearance) and VD (volume of distribution) were evaluated by a computer software (PK Solutions, non-compartmental pharmacokinetics data analysis, 2001; Summit Research Services, Montrose, CO, USA)

Statistical analysis was performed by using the Student *t* test in order to compare the ropivacaine concentrations between the groups at each period of time. Pharmacokinetic parameters were compared by Wilcoxon signed-rank test. The significance level was set at 5% and the tests were performed by BioEstat 5.0 (Fundação Mamirauá, Belém, PA, Brazil) software.

Results

Adverse events were not observed during research period. The detection limit of ropivacaine in plasma was 30 ng/mL. The calibration curve for determining plasma ropivacaine was linear at the concentration of 30-250 ng/ml, showing that HPLC was sensitive in quantifying ropivacaine in plasma. Figure 1 shows the calibration curve ($R^2 = 0.9991$) for HPLC method.

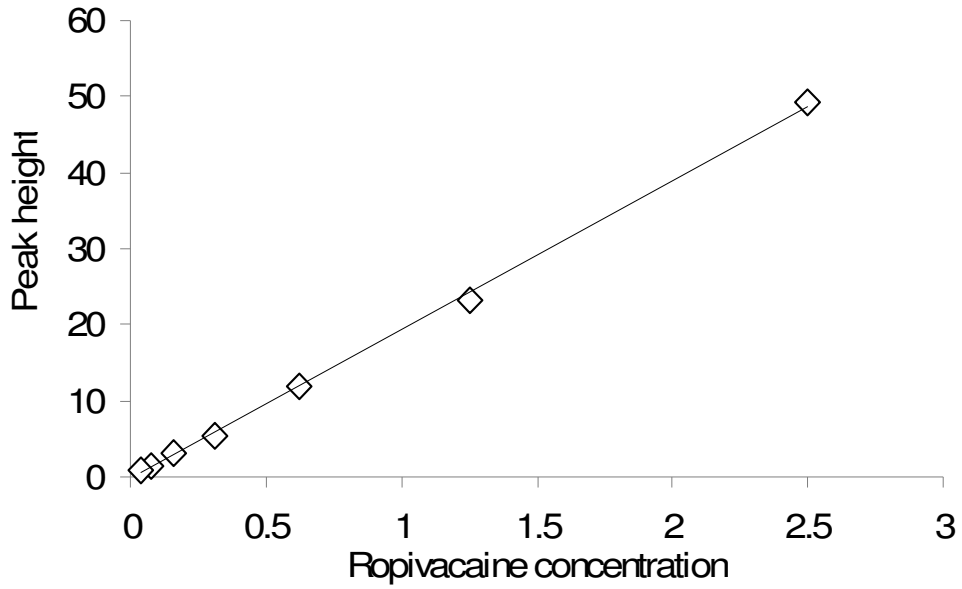

Figure 1. Calibration curve of plasma concentration of ropivacaine and peak height. as measured by HPLC (see text for details).

No statistically significant differences ($p>0.05$) were observed between the formulations considering all the pharmacokinetic parameters evaluated (C_{max}; T_{max}; AUC_{0-24}; $AUC_{0-\infty}$; CL and VD). Mean plasma concentrations of ropivacaine in liposomal formulation and ropivacaine with 1:200,000 epinephrine are shown in figure 2.

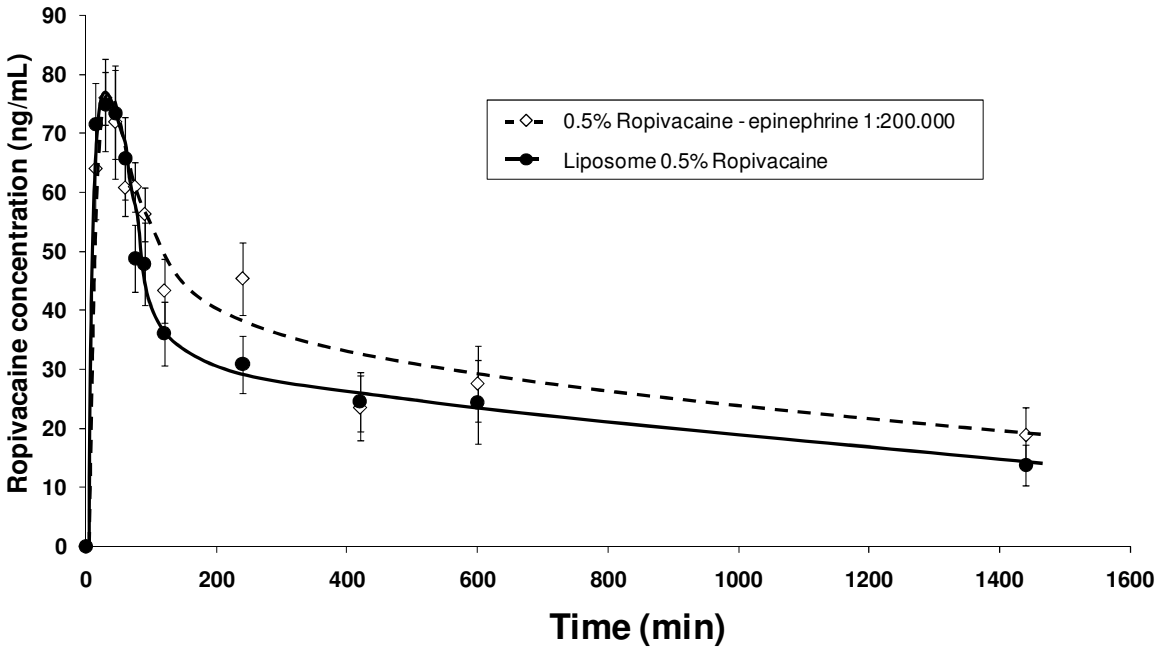

Figure 2. Mean (± SEM) values and regression curve for plasma concentration of ropivacaine after maxillary infiltration of liposome-encapsulated 0.5% ropivacaine (continuous line) and 0.5% ropivacaine with 1:200,000 epinephrine (dashed line).

The median values for pharmacokinetic parameters of ropivacaine, C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, VD and CL are listed in Table 1.

Table 1. Median pharmacokinetic parameters following maxillary infiltration of liposome-encapsulated 0.5% ropivacaine and 0.5% ropivacaine with 1:200,000 epinephrine.

Pharmacokinetic parameters	Groups	Median	Quartiles		*p* value
			First	Third	
C_{max} (ng/mL)	liposome-encapsulated 0.5% ropivacaine	92.9	82.7	97.7	0.6378
	0.5% ropivacaine with 1:200,000 epinephrine	93.4	63.2	114.7	
T_{max} (min)	liposome-encapsulated 0.5% ropivacaine	30.0	15.0	56.3	0.9645
	0.5% ropivacaine with 1:200,000 epinephrine	37.5	30.0	45.0	
AUC_{0-t} (ng-min/mL)	liposome-encapsulated 0.5% ropivacaine	40.4	26.3	55.2	0.6378
	0.5% ropivacaine with 1:200,000 epinephrine	32.4	20.1	44.0	
$AUC_{0-\infty}$ (ng-min/mL)	liposome-encapsulated 0.5% ropivacaine	71.9	28.1	138.6	0.7794
	0.5% ropivacaine with 1:200,000 epinephrine	78.5	4.9	102.6	
Vd (mL/kg)	liposome-encapsulated 0.5% ropivacaine	2.6	1.5	4.4	0.5754
	0.5% ropivacaine with 1:200,000 epinephrine	2.8	1.5	13.8	
CL (mL/min)	liposome-encapsulated 0.5% ropivacaine	0.07	0.05	0.28	0.4008
	0.5% ropivacaine with 1:200,000 epinephrine	0.08	-0.11	0.11	

Discussion

The method of ropivacaine quantification in plasma samples used in the present study showed selectivity and sensitivity as previously reported by Kawata

et al. (2005). The detection limit of ropivacaine observed in our study (30 ng/mL) was close to the limit observed by these authors (25 ng/mL).

Kawata et al. (2005) studied the topical application of 5 mL of 0.5% ropivacaine viscous that was held in the mouths of only two volunteers for 10 min. They observed a C_{max} of 107 (± 25.5) ng/mL and a T_{max} 50 (± 14.1) min and in spite of the methodological differences these results are similar to the ones observed in the present study.

Many substances are added to improve local anesthetics efficacy modifying their pharmacodynamic and pharmacokinetic properties, being epinephrine the most commonly used (Lee et al., 2002). These authors demonstrated that the addition of epinephrine significantly reduced the concentration of ropivacaine after epidural anesthesia in humans, during the first hour in both arterial and venous blood. In the present study, there were no differences between the pharmacokinetic profiles of both formulations, showing that the liposome encapsulation of ropivacaine was as effective as epinephrine in reducing ropivacaine absorption.

Several animal studies also demonstrated that liposomal encapsulation of long acting local anesthetics was able to change their pharmacokinetics resulting in lower plasma concentrations and toxicity when compared to the plain solution (Boogaerts et al., 1993b; Grant et al., 1997; Yu et al., 2002; Grant et al., 2003).

Despite differences in liposolubility, partition coefficient, and some other physico-chemical/pharmacokinetics parameters, ropivacaine and bupivacaine has some similarities, such as pka, protein binding and molecular weight. In addition, they have similar onset time and duration of the block, when used in epidural blockade (Leone et al., 2008). No differences in anesthetic efficacy parameters after maxillary infiltration were found between these two local anesthetics (Kennedy et al., 2001).

Grant et al. (1997) compared 0.5% plain bupivacaine with 2% liposomal bupivacaine, and even with a 4-fold higher concentration of bupivacaine in the liposomal formulation, the plasmatic levels of bupivacaine decreased when the

liposomal formulation was used for wound analgesia in rats. In the present study, the pharmacokinetics of liposome-encapsulated ropivacaine was comparable to the epinephrine-associated ropivacaine, suggesting the same profile observed by Grant et al. (1997), i.e., the encapsulation into liposome vesicles can delay the anesthetic absorption into the blood.

According to Grant & Bansinath (2001) liposome structure affects the release kinetics of encapsulated drugs. Drugs tend to be released more rapidly from liposomes composed of a single lipid bilayer while the release tends to be retarded from multilamellar vesicles (Grant, et al., 1997; Yu et al., 2002). In our study, the unilamellar vesicles were able to delay the ropivacaine absorption since both formulations presented similar pharmacokinetic profile. Further studies are necessary to evaluate how the changes in liposome composition affect the absorption of ropivacaine from the injection site and its plasmatic concentration after dental anesthesia.

Another factor that could maintain a low constant plasma concentration for hours resulting in a prolonged effect is the percentage of encapsulated drug (Barenholz, 2003). According to a previous study (de Araújo et al., 2008) that used the same liposome used in the present study, the encapsulation efficiency of ropivacaine was 24%, while reports in the literature have shown higher encapsulation efficiency values (Grant et al., 2001; Grant et al., 2003; Grant et al., 2004). Ostergaard et al. (2008) showed that ropivacaine had less liposome affinity than bupivacaine. De Araújo et al. (2008) also suggested that enhancement of the liposome encapsulation could prolong the analgesic effect and decrease the cytotoxicity.

In conclusion, liposome-encapsulated ropivacaine showed a similar pharmacokinetic profile when compared with ropivacaine associated with epinephrine.

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CONSIDERAÇÕES FINAIS

Em estudo prévio (Franz-Montan *et al.,* 2007b) a ropivacaína a 1% encapsulada em lipossomas não foi capaz de promover anestesia pulpar quando aplicada na mucosa vestibular por 2 minutos. A fim de melhorar sua eficácia, o mesmo foi testado na concentração de 2% aplicado por 30 minutos na mucosa vestibular (capítulo 1) e por 5 minutos na mucosa palatina (capítulo 2).

Na mucosa vestibular o objetivo era reproduzir os resultados já publicados anteriormente (Vickers & Punnia-Moorthy, 1993; Vickers *et al.*, 1997; Munshi *et al.*, 2001) de obtenção de anestesia pulpar clinicamente útil por meio da aplicação tópica do EMLA. No entanto, mesmo sendo um carreador altamente lipossolúvel, o anestésico encapsulado não conseguiu alcançar a região apical do dente e promover anestesia pulpar, a despeito do aumento da concentração e do tempo de aplicação (30 minutos).

Apesar da alta penetração de anestésicos lipossomais na pele, com eficácia já demonstrada (Gesztes & Mezei, 1988; Singh & Vyas 1996; Hung *et al.*, 1997; Bucalo *et al.*, 1998; Fisher *et al.*, 1998; Friedman *et al.*, 1999; Grant *et al.*, 2001; Yu *et al.*, 2002), o mesmo não foi observado para a formulação lipossomal de ropivacaína ao ser aplicada na região palatina. Tanto a ropivacaína a 1% quanto a 2% não foram diferentes do gel placebo em reduzir dor à punção e à injeção. Mesmo o anestésico EMLA, considerado por muitos autores como o que apresenta melhor eficácia anestésica nessa região em comparação ao placebo ou a outros anestésicos tópicos (Holst & Evers,1985; Svensson & Petersen, 1992; Hutchins *et al.*, 1997; Meechan & Winter, 1996; Al-Melh & Andersson, 2007; Al-Melh & Andersson, 2008) não reduziu a dor à injeção.

Esses resultados mostram que a formulação lipossomal utilizada não permitiu a difusão do anestésico local mais profundamente na mucosa, o que pode estar relacionado ao tamanho dos lipossomas utilizados (400nm), conforme relatado no capítulo 1.

Outro fator que poderia explicar essa ausência de efetividade na palatina e a ineficácia da formulação lipossomal em aumentar a duração da

anestesia promovida pela ropivacaína em técnica infiltrativa é a porcentagem de anestésico efetivamente encapsulado que para a ropivacaína nesse tipo de lipossoma é baixa (24%).

A eficácia de anestésicos na forma lipossomal em modelo animal de bloqueio infraorbitário, no qual é avaliado bloqueio sensitivo para tecidos moles, não reproduz de fato o que ocorre na técnica infiltrativa para anestesia pulpar. Assim, de Araújo *et al.* (2008) demonstraram que formulações lipossomais de ropivacaína apresentaram duração e intensidade anestésica superiores em comparação à forma pura tanto em bloqueio do nervo ciático, quanto do infraorbitário, o que não foi observado no presente estudo.

Os resultados obtidos por esses autores, juntamente com o fato de a ropivacaína ser mais efetiva em técnica de bloqueio do que em infiltração (Ernberg & Kopp, 2002) levam à suposição de que essa formulação possa apresentar resultados mais satisfatórios em técnica de bloqueio, como é o caso do bloqueio do nervo alveolar inferior. Outra possibilidade, que abre perspectiva maior é a utilização de lipossomas diferentes, tanto no tamanho (menores), quanto na composição, como é o caso dos lipossomas flexíveis, com maior poder de penetração.

Por fim, o estudo relatado no capítulo 4 mostra que a preparação lipossomal proporciona efetividade semelhante à da epinefrina em relação à absorção do anestésico local para a corrente sangüínea, não havendo diferença nos parâmetros farmacocinéticos da ropivacaína nas duas formulações testadas. Entretanto, quando esses resultados são somados aos relatados no capítulo 3, observa-se que, embora a preparação lipossomal diminua a passagem do anestésico local para a corrente sangüínea, o mesmo não consegue atingir a região apical do dente em concentração suficiente para promover aumento da duração da anestesia, como ocorre com a solução contendo epinefrina.

Em conjunto, esses resultados mostram que a formulação testada não é eficaz para promover anestesia tópica na região palatina e nem para uso em técnica infiltrativa, devendo ser testadas outras formas de encapsulação.

CONCLUSÕES

De acordo com os resultados obtidos no presente estudo conclui-se que:

- A ropivacaína encapsulada em vesículas unilamelares de tamanho grande - LUV (400nm), avaliada a 1 e 2% em administração tópica palatina e a 0,5% em técnica infiltrativa na maxila não apresentou eficácia anestésica comparável ou superior às preparações não-lipossomais, não havendo vantagem no seu uso.
- O aumento do tempo de aplicação e da concentração da ropivcaína encapsulada em lipossomas não foi suficiente para promover anestesia pulpar por meio da aplicação tópica no fundo de sulco vestibular.
- A ropivacaína encapsulada em vesículas unilamelares de tamanho grande - LUV (400nm) na concentração de 2%, por apresentar eficácia semelhante à da benzocaína 20% em aplicação tópica na mucosa vestibular, pode ser uma opção a esse anestésico.
- A encapsulação em lipossomas fez com que a formulação apresentasse perfil farmacocinético semelhante ao da preparação de ropivacaína associada à epinefrina.

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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 "**Atividade anestésica de formulações lipossomais de anestésicos locais em odontologia**", protocolo nº **093/2006**, dos pesquisadores **MARIA CRISTINA VOLPATO, ENEIDA DE PAULA, FRANCISCO CARLOS GROPPO e MICHELLE FRANZ MONTAN**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 02/08/2006.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Local anesthetic activity of lipossomal formulations in dentistry**", register number **093/2006**, of **MARIA CRISTINA VOLPATO, ENEIDA DE PAULA, FRANCISCO CARLOS GROPPO and MICHELLE FRANZ MONTAN**, 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 02/08/2006.

Profa. Cecília Gatti Guirado
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
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CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Avaliação da eficácia anestésica da ropivacaína encapsulada ou não em lipossomas, em anestesia odontológica**", protocolo nº **164/2006**, dos pesquisadores **MARIA CRISTINA VOLPATO, ENEIDA DE PAULA, FRANCISCO CARLOS GROPPO e MICHELLE FRANZ MONTAN**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 08/11/2006.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Anesthetic efficacy of ropivacaine and liposomal ropivacaine formulations in local dental anesthesia**", register number **164/2006**, of **MARIA CRISTINA VOLPATO, ENEIDA DE PAULA, FRANCISCO CARLOS GROPPO and MICHELLE FRANZ MONTAN**, 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 08/11/2006.

Profa. Cecilia Gatti Guirado
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: 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 "**Avaliação da anestesia tópica em mucosa oral de géis de ropivacaína lipossomal em diferentes concentrações**", protocolo nº **059/2008**, dos pesquisadores **MARIA CRISTINA VOLPATO, ENEIDA DE PAULA, FRANCISCO CARLOS GROPPO e MICHELLE FRANZ MONTAN**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 11/06/2008.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project "**Topical anesthesia in oral mucosa of liposomal ropivacaine gel at different concentrations**", register number **059/2008**, of **MARIA CRISTINA VOLPATO, ENEIDA DE PAULA, FRANCISCO CARLOS GROPPO and MICHELLE FRANZ MONTAN**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 11/06/2008.

Prof. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

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

ANEXO 2

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.

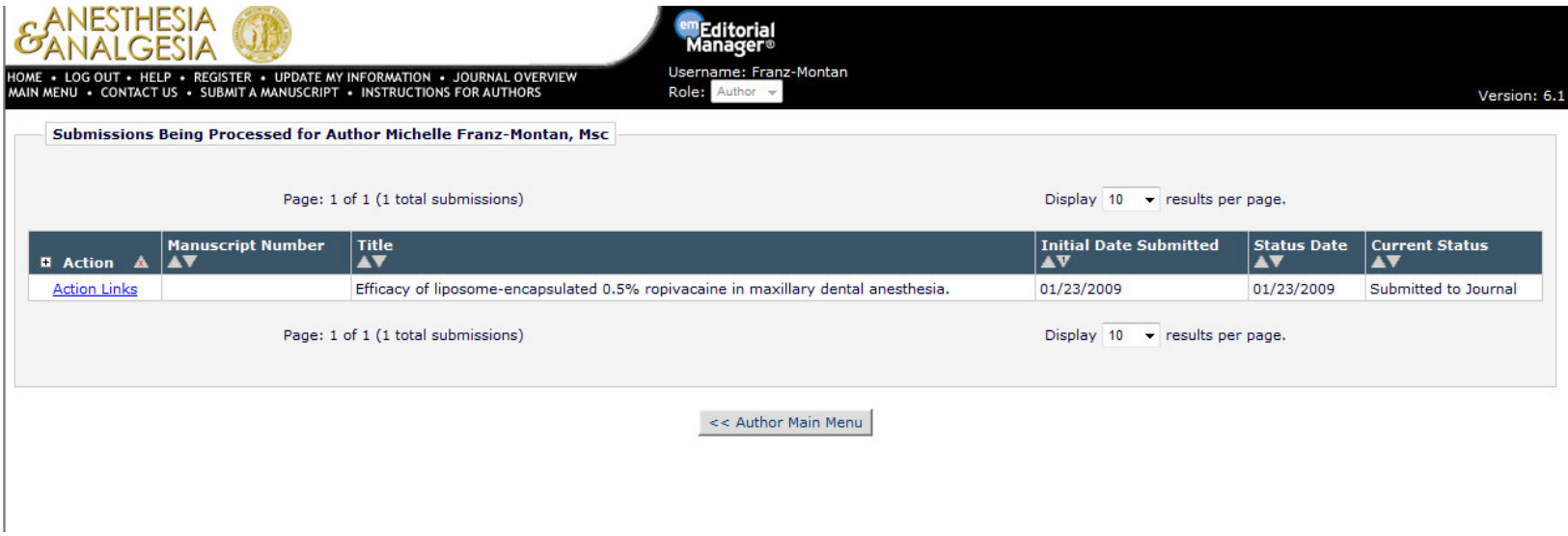
§ 7º - O custo, em reais, de cada exemplar produzido pela gráfica será definido pela Administração Superior da Universidade.

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

ANEXO 3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ANEXO 4

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
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DECLARAÇÃO

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Doutorado, intitulada "AVALIAÇÃO DA EFICÁCIA ANESTÉSICA E DA CONCENTRAÇÃO PLASMÁTICA DA ROPIVACAÍNA ENCAPSULADA EM LIPOSSOMAS, EM ANESTESIA ODONTOLÓGICA", não infringem os dispositivos da Lei nº 9.610/98, nem o direito autoral de qualquer editora.

Piracicaba, 10 de março de 2009.

MICHELLE FRANZ MONTAN BRAGA LEITE
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AUTOR

MARIA CRISTINA VOLPATO
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ORIENTADOR