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LUCIANA ARANHA BERTO

EFICÁCIA ANESTÉSICA DA FORMULAÇÃO LIPOSSOMAL DE ARTICAÍNA EM RATOS

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

Orientador: Prof. Dr. Francisco Carlos Groppo

Co-orientadoras: Profa. Dra. Maria Cristina Volpato

Profa. Dra. Eneida de Paula

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RESUMO

O presente estudo teve como objetivo avaliar a eficácia anestésica de duas formulações lipossomais injetáveis de articaína (3% e 4%), em dois modelos animais: bloqueio do nervo infra-orbital (BNIO) e bloqueio do nervo alveolar inferior (BNAI). Para cada experimento, 48 animais foram divididos em 6 grupos (n=8), que receberam a injeção de uma das seguintes formulações, no lado direito: Grupo 1: articaína 4% com epinefrina 1:100,000; Grupo 2: articaína 3% lipossomal; Grupo 3: articaína 4% lipossomal; Grupo 4: articaína 4%; Grupo 5: articaína 3% e Grupo 6: lipossomas 4mM sem anestésico local. O lado contralateral recebeu NaCl 0,9% (controle). Para BNIO, 0,1 mL da preparação foi injetado próximo ao forame infra-orbitário. Foi avaliada a duração da anestesia em tecido mole por pinçamento vigoroso do lábio superior, a cada 5 minutos, até que fosse obtido o primeiro sinal de resposta aversiva, indicando o final da anestesia. Para BNAI, 0,2 mL da preparação foi depositado próximo ao forame mandibular e os parâmetros latência e duração da anestesia pulpar foram avaliados por estímulo elétrico. Os dados foram submetidos ao teste de ANOVA, com nível de significância 5%. Para duração de anestesia em tecido mole, os grupos 1, 2 e 3 não diferiram entre si ($p>0,05$) e promoveram duração maior que os grupos 4 e 5 ($p<0,05$). O grupo 1 obteve menor latência da anestesia pulpar que os grupos 2, 3, 4 e 5 ($p<0,05$), que não diferiram entre si ($p>0,05$). O grupo 1 apresentou a maior duração de anestesia pulpar, seguido pelos grupos 2 e 3. Com relação à duração da anestesia pulpar, não houve diferença entre os grupos 2 e 3 e entre os grupos 4 e 5 ($p>0,05$). Grupo 6 não obteve efeito anestésico. A encapsulação em

lipossomas permitiu aumento na duração da anestesia da articaína quando comparada à solução pura. Entretanto, a solução de articaína 4% com epinefrina promoveu maior duração de anestesia pulpar que as formulações lipossomais.

Palavras-chave: lipossoma, articaína, anestésicos locais, rato, bloqueio.

ABSTRACT

Liposome encapsulation has been found to enhance the clinical efficacy of local anesthetics. This study evaluated the anesthetic efficacy of two liposomal formulations of articaine (3% and 4%) by means of two animal models: infraorbital nerve block (IONB) and inferior alveolar nerve block (IANB) in rats. For each experiment, 48 animals were divided in 6 groups (n=8), which received the injection of one of the following formulations, in the right side: Group 1: 4% articaine with 1:100,000 epinephrine; Group 2: 4% liposomal articaine; Group 3: 3% liposomal articaine; Group 4: 4% articaine; Group 5: 3% articaine and Group 6: 4mM liposome. The left side received NaCl 0.9% (control). For the IONB, 0.1 mL of the tested formulation was injected near the rat infraorbital foramen. The duration of soft tissue anesthesia was evaluated by pinching the upper lip, every 5 minutes, until the first sign of aversive response was observed. For the IANB, 0.2 mL of the tested formulations was injected near the rat mandible foramen and the parameters onset and duration of pulpal anesthesia were evaluated by means of an electrical pulp tester. Data were submitted to ANOVA test and significance level was set at 5%. Concerning soft tissue anesthesia, groups 1, 2 and 3 showed similar duration ($p>0.05$) which were longer than that of the groups 4 and 5 ($p<0.05$). Group 1 showed the shortest onset of pulpal anesthesia ($p<0.05$) and there was no difference among groups 2, 3, 4 and 5 ($p>0.05$). Group 1 promoted longer duration of pulpal anesthesia ($p<0.05$) followed by groups 2 and 3. Concerning duration of pulpal anesthesia, no significant difference was found between groups 2 and 3 and groups 4 and 5 ($p>0.05$). Liposome encapsulation prolonged anesthetic effects of articaine when compared to plain solution. However, the epinephrine-containing solution showed longer pulpal anesthesia duration than liposomal formulations.

Key words: liposome, articaine, local anesthetic, rat, nerve block.

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INTRODUÇÃO

Apesar dos avanços recentes em pesquisas clínicas e laboratoriais com relação a agentes terapêuticos, o manejo da dor ainda se mostra um desafio (de Paula *et al.*, 2010). A anestesia local é o método mais utilizado para o controle da dor em Odontologia. Por este motivo é importante o estudo constante acerca do assunto, em busca de formas melhores e mais seguras de efetuar este procedimento.

Dentre as medidas para que a anestesia local seja efetuada com segurança é importante ressaltar a escolha correta da solução anestésica, priorizando-se aquela que é mais efetiva com a menor concentração tanto do sal anestésico como do vasoconstritor, reduzindo-se, desta forma, a possibilidade de reações tóxicas (Tófoli *et al.*, 2003).

O sal anestésico cloridrato de articaína foi introduzido em 1976 na Alemanha e, posteriormente, em outros países como Canadá em 1984, Brasil em 1998 e Estados Unidos em 2000. Atualmente já está disponível em 135 países sendo que no Canadá já é o anestésico mais vendido para uso odontológico (Malamed, 2008).

Como a maioria dos anestésicos locais atualmente em uso, a articaína pertence ao grupo amida. Entretanto, ao contrário dos outros compostos pertencentes a este grupo que contêm um anel benzeno, a articaína tem em sua estrutura química um anel tiofeno, que lhe confere maior lipossolubilidade. Outra particularidade é a presença de um grupamento éster em sua molécula, permitindo que sua biotransformação ocorra por duas vias, plasmática e hepática, enquanto as outras amidas são biotransformadas essencialmente no fígado. Isso implica um tempo de meia vida plasmática menor, de cerca de 27 minutos, quando comparada aos demais anestésicos do grupo (aproximadamente 90 minutos), e confere ao paciente menor risco de intoxicação em

caso de sobredosagem (Malamed, 2008). Os adequados tempos de latência e de anestesia pulpar, o baixo risco de toxicidade e a superioridade deste anestésico local em certas situações clínicas são responsáveis pela sua grande utilização (Ferger *et al.*, 1973; Vree *et al.*, 2005; Malamed, 2008).

A articaína tem também alto grau de ligação protéica (95%) e excelente difusão pelos tecidos devido à sua maior solubilidade em lipídios. Por suas características, o cloridrato de articaína é considerado um anestésico local potente, com baixo nível de toxicidade e de rápida metabolização, observados em animais (Leuschner *et al.*, 1999) e em humanos (Pitkanen *et al.*, 1999; Hersh *et al.*, 2006). Por outro lado, sua duração de ação sem a presença de vasoconstritor é muito pequena, não sendo recomendado seu uso nesta forma (Winther & Nathalang, 1972).

Devido às suas propriedades vasodilatadoras, os anestésicos locais são freqüentemente associados a vasoconstritores, o que possibilita vantagens clínicas como aumento da duração e da qualidade anestésica, diminuição dos níveis plasmáticos do agente anestésico e, conseqüentemente, da probabilidade de ocorrência de efeitos sistêmicos adversos decorrentes de sobredose do agente anestésico, além do controle da hemorragia durante procedimentos cirúrgicos (Sisk, 1993). Entretanto, os vasoconstritores, principalmente aqueles similares à epinefrina, também podem ocasionar efeitos indesejáveis ao paciente (Hoffman & Lefkowitz, 1996). O uso de doses excessivas ou a injeção intravascular acidental da solução anestésica local podem causar manifestações tais como taquicardia, arritmias, hipertensão arterial, tremores e cefaléia (Cassidy *et al.*, 1986; Yagiela, 1999), podendo levar, em alguns casos, ao infarto do miocárdio, acidente vascular cerebral e morte (Tomlin, 1974; Pearson, 1987). Estes riscos

fazem com que as soluções anestésicas que contêm vasoconstritor do tipo amina simpatomimética sejam utilizadas com certa precaução pelo cirurgião-dentista.

Tendo em vista a melhoria na segurança do procedimento anestésico, a associação de anestésicos locais e novos sistemas de liberação controlada tem sido alvo de estudos. Dentre estes novos carreadores de drogas, os lipossomas têm alcançado resultados promissores em medicina. Estas esferas lipídicas vêm sendo amplamente utilizadas como sistema de liberação controlada para vários fármacos, incluindo antineoplásicos, antibióticos, antifúngicos e também anestésicos locais para uso médico (Bucalo et al., 1998).

Descobertos por Bangham em 1963, os lipossomas consistem de moléculas esféricas, que medem entre 50 a 1000nm de diâmetro, formadas pela interação de lipídios suspensos numa fase aquosa que, devido à diferença de polaridade com o meio, tendem a se agrupar, formando vesículas (Banerjee, 2001; Grant, 2002). Podem ser constituídos por uma ou mais bicamadas lipídicas, sendo assim classificados em unilamelares ou multilamelares, respectivamente. Além disso, os lipossomas são estruturas anfipáticas, ou seja, possuem uma região hidrofílica e uma região hidrofóbica e podem carregar tanto substâncias hidrossolúveis como lipossolúveis em suas diferentes fases (Grant, 2002).

Os lipossomas são biocompatíveis, biodegradáveis, com risco reduzido de toxicidade sistêmica ou local, imunogenicidade e antigenicidade, principalmente pela semelhança de seus monômeros constituintes (fosfatildilcolina e colesterol) com os das membranas biológicas (Malinovsky *et al.*, 1997; Grant, 2002).

O uso clínico do sistema lipossomal para anestesia local em medicina vem confirmando as vantagens terapêuticas desta associação. Em Odontologia, o uso de

preparações lipossomais traz a possibilidade de eliminação dos vasoconstritores, sem redução de duração e efetividade anestésica, o que pode representar um avanço significativo na segurança do tratamento odontológico.

O uso de anestésicos locais encapsulados em lipossomas tem como vantagens a liberação lenta da droga, prolongando a duração da anestesia e reduzindo a toxicidade para o sistema cardiovascular e o sistema nervoso central (Gesztes & Mezei, 1988; Langerman *et al.*, 1992; Boogaerts *et al.*,1993 a,b; Mowat *et al.*, 1996; Bucalo *et al.,* 1998; Araujo *et al.*, 2003), tanto em relação ao sal anestésico, quanto ao vasoconstritor. Além disso, pacientes submetidos a intervenções que necessitem de controle de dor pós-operatória seriam beneficiados pela liberação prolongada da droga (Kuzma *et al.*, 1997).

Já foi demonstrado que a bupivacaína lipossomal apresenta reduzida toxicidade para os sistemas nervoso central e cardiovascular, quando injetada intravascularmente em coelhos (Boogaerts *et al.*, 1993b). Posteriormente, realizou-se o primeiro estudo em humanos, no qual a preparação lipossomal de bupivacaína injetada pela técnica epidural promoveu maior duração do alívio da dor, com concentrações plasmáticas reduzidas e constantes, em comparação à forma pura do anestésico local (Boogaerts *et al.*, 1994).

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; Hung *et al.*, 1997; Bucalo *et al.*, 1998; Fisher *et al.*, 1998; Friedman *et al.*, 1999).

Com relação a estudos em Odontologia, em técnica infiltrativa na maxila 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.

Da mesma forma, outros autores verificaram que a encapsulação em lipossomas dos anestésicos lidocaína, mepivacaína e prilocaína promoveu um aumento na duração da anestesia em tecido mole de ratos (de Araújo *et al*., 2004; Cereda *et al*., 2004; 2006).

Estes resultados demonstram que o uso destas formulações poderia representar uma nova alternativa aos anestésicos locais para uso em odontologia, com prolongada duração e elevada segurança. Desta forma, o objetivo deste estudo foi avaliar a eficácia anestésica da formulação lipossomal de articaína.

Esta dissertação 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. Desta forma, a referida dissertação é composta de um capítulo contendo um artigo científico, intitulado "*Anesthetic efficacy of liposomal articaine in rats",* submetido para publicação à revista científica "Journal of Dental Research" (Anexo 2).

CAPÍTULO 1

Anesthetic Efficacy of Liposomal Articaine in Rats

Authors: Luciana Aranha Berto[1], DDS

Eneida de Paula[2], PhD

Maria Cristina Volpato[1], PhD

Francisco Carlos Groppo[1]*, PhD

[1]Department of Physiological Sciences, Piracicaba Dental School, State University of Campinas – Unicamp, Av. Limeira, 901, Piracicaba, 13414-903, SP, Brazil. [2]Department of Biochemistry, Institute of Biology, State University of Campinas – Unicamp, Campinas, SP, Brazil. *corresponding author: fcgroppo@fop.unicamp.br

Short tittle: Efficacy of Liposomal Articaine in Rats

Key words: liposome, articaine, local anesthetic, rat, nerve block

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ABSTRACT

Liposome encapsulation has been found to enhance local anesthetic efficacy. This study evaluated the anesthetic efficacy of liposomal 3% and 4% articaine, in two animal models: infraorbital nerve block (soft tissue anesthesia) and inferior alveolar nerve block (onset and duration of pulpal anesthesia) in rats. Six formulations were tested: 4% articaine with 1:100,000 epinephrine (commercial solution), 4% and 3% liposomal articaine, 4% and 3% plain articaine and liposomal suspension (control). Control presented no anesthetic effect. Liposomal formulations and commercial solution provided the longest duration of soft tissue anesthesia. The shortest onset of pulpal anesthesia was obtained with commercial solution. Duration of pulpal anesthesia was longer for commercial solution, followed by liposomal articaine formulations. 3% and 4% plain articaine showed the shortest duration of pulpal anesthesia. Liposome encapsulation prolonged the duration of anesthesia of articaine. However, commercial solution showed longer duration of pulpal anesthesia than liposomal formulations.

INTRODUCTION

Articaine hydrochloride is a widely used local anesthetic in dentistry. Commercially available since 1976, this most recent acquisition for the dental anesthetic armamentarium has become the most used local anesthetic in several countries, such as Germany and Canada (Malamed, 2008). Adequate onset of the block, satisfactory quality of anesthesia, intermediate anesthesia duration and low toxicity have been pointed out as reasons for this wide employment (Ferger and Marxkors, 1973; Vree and Gielen, 2005).

In recent years, several studies have demonstrated the superiority of articaine in relation to other local anesthetics in certain clinical situations. Previous works showed that 4% articaine with 1:100,000 epinephrine overmatched 2% lidocaine with the same epinephrine concentration regarding pulpal anesthesia success after buccal infiltration in the mandibular first molar (Kanaa *et al*., 2006; Robertson *et al*., 2007). These results are probably due to the singular molecular structure of articaine, especially its thiophene ring, which increases the diffusion of the anesthetic throughout tissues (Vree and Gielen, 2005; Potocnik *et al*., 2006; Robertson *et al*., 2007).

New controlled-release systems, such as liposome encapsulation, enhance the clinical efficacy of local anesthetics (Boogaerts *et al*., 1993, 1994; Grant *et al*., 1994, 2001, 2004; Mowat *et al*., 1996; Yu *et al*., 2002; Cereda *et al*., 2004, 2006; de Araujo *et al*., 2004, 2008). Liposomes, extensively described as effective drug-carriers, enhance drug bioavailability and reduce systemic toxicity (Boogaerts *et al*., 1993; Grant *et al*., 2001; Cereda *et al*., 2004).

Previous studies have demonstrated that the widely used local anesthetics (prilocaine, mepivacaine and lidocaine) encapsulated into liposomes showed higher

duration of anesthesia when compared to their respective plain solutions, after infraorbital nerve block in rats (Cereda *et al*., 2006).

Liposome-encapsulated 3% prilocaine induced similar anesthetic effects when compared to the 3% prilocaine with felypressin, in the infraorbital nerve block model (Cereda *et al*., 2004). In addition, other authors showed that liposome-encapsulated 2% mepivacaine provided similar anesthetic efficacy to 3% plain mepivacaine, after human canine maxillary infiltration (Tófoli *et al*., 2008). Therefore, the association of local anesthetics and liposomes could allow the reduction of the anesthetic concentration or become an alternative to vasoconstrictor usage.

The present study is the first attempt to verify the anesthetic efficacy of liposome encapsulated articaine.

MATERIAL AND METHODS

Material and animals

Two animal models (infraorbital nerve block and inferior alveolar nerve block) were used to evaluate the formulations. Ketamine hydrochloride (Sespo Ind. Com. Ltda, Paulinia, SP, Brazil) and xylazine hydrochloride (Bayer S.A., Sao Paulo, SP, Brazil) were used for general anesthesia. Sodium thiopental (Cristália Produtos Químicos e Farmacêuticos Ltda, SP, Brazil) was used to sedate the animals. The liposomal formulations, consisting of large unilamellar vesicles (LUV) of homogenous size (400nm), and the plain articaine solutions, were prepared at the Department of Biochemistry, Institute of Biology, University of Campinas, SP, Brazil, based on a previously described method (de Araujo et al., 2004; 2008; Cereda *et al*., 2004; 2006). Prior to the administration, samples of each anesthetic formulation were tested to determine pH values

using a pHmeter (Orion Research, Boston, MA). Male Wistar SPF rats (90 days old; 300-350g) were obtained from the Multidisciplinary Center for Biological Investigation of the University of Campinas (CEMIB/Unicamp, Campinas, SP, Brazil). Animals were submitted to a 12-hour day/night cycle and were given free access to water and food during the study. The experiment was approved by the Institutional Committee for Ethics in Animal Research of the University of Campinas (CEEA-Unicamp/Protocol Number 1341-1), and carried out in accordance with the norms of the Brazilian College of Animal Experimentation. The tested formulations were injected by means of a 1mL-syringe (Luer Slip, Becton Dickinson, Curitiba, Brazil) and a 0.45X13 (26G ½") needle (PrecisonGlide, Becton Dickinson, Curitiba, Brazil).

Groups and testing preparations

For each experimental model, 48 rats were randomly distributed into six groups (n=8). Each group received one of the following formulations: 3% plain articaine (ART3); 4% plain articaine (ART4); lipossomal 3% articaine ($ART3_{LUV}$); lipossomal 4% articaine ($ART4_{LUV}$); 4% articaine with 1:100,000 epinephrine (Articaine 100, DFL Ind Com Ltda, Rio de Janeiro, RJ, Brazil) ($ART4_{EPI}$) and 4mM liposome suspension ($LUV_{LA\text{-}FREE}$). All the experiments were performed by the same investigator using coded formulations. The formulation codes were kept until the completion of the study.

Infraorbital nerve block in rats

The anesthetic effect of the articaine preparations was analyzed by using infraorbital nerve block technique (IONB), adapted from Fink *et al.* (1975) and used in previous studies (Cereda *et al.*, 2004; 2006). The infraorbital nerve supplies the upper lip (buccal fold and skin) and whisker area. This technique is based on the observation and

classification of the aversive response to the upper lip pinching with surgical tweezers according to the scores: 0 (aversive response) or 1 (no aversive response). After slight sedation with 25 mg/kg intraperitoneal sodium thiopental, 0.1mL of the tested preparations was injected near the right infraorbital notch, which is situated above a gap between the upper first molars and the incisor. The left side received 0.1 mL of 0.9% NaCl solution (control), in order to compare the response of the two sides. All sedated animals were capable of responding to the upper lip pinching. Animals were tested every five min up to the time when the first aversive sign in the injected side was detected. The efficacy of infraorbital nerve block was analyzed by duration of anesthetic effect.

Inferior alveolar nerve block in rats

The inferior alveolar nerve block (IANB) model was based on previous reports describing the anatomy of the rat mandible and the trajectory of the nerves that supply the rat inferior teeth (Naftel et al., 1999; Silva et al., 2009). IANB and, consequently, mandible molar anesthesia were achieved by depositing anesthetic solution near the mandible foramen of the rats.

After general anesthesia, induced with ketamine (90mg/kg) and xylazine (10mg/kg), two pieces of PVC coated copper wire, 0.3 mm in diameter and 20 cm in length, were attached to the occlusal surface of the right and left mandible molars (1st, 2nd, and 3rd), using dental resin (Z100, 3M ESPE Dental Products, St. Paul, MN, USA). After general anesthesia recovering (approximately three hours), verified by tail flick reflex, the animals were slightly sedated with sodium thiopental (25mg/kg) (Cereda *et al.,* 2006). All sedated animals had the ability to respond to the electric stimulus performed by an electric pulp tester (Vitality Scanner model 2006 - Analytic Technology, Redmond, WA,

USA). Before the local anesthetic injection, the pain threshold for the lower molars was obtained by the average of three measurements with the pulp tester. The electrical stimulus was applied on the copper wire attached on inferior molars with a two-minute interval between each measurement. The animal responses to the electrical stimulus were biting, itching their cheeks or flinching their heads (Silva *et al.,* 2009)

After pain threshold was established, 0.2 mL of the tested preparations was deposited near the mandible foramen, by an extraoral injection. Nearly 13 mm of the needle was inserted perpendicularly to the mandible body and tangentially to the mandible ramus through the internal face, with the bevel oriented towards the ramus (figure 1). The opposite side received the same volume (0.2 mL) of 0.9% NaCl solution (control side), in order to verify that the animals were responding accurately and the pulp tester was functioning properly. After the injections, inferior molars in both sides, were electrically stimulated every two minutes until the absence of response to the maximum stimulus of the pulp tester (80 mA). This condition determined teeth anesthesia (Certosimo and Archer, 1996). The electric stimulation was then applied every five minutes until two consecutive aversive responses to the pulp tester were obtained, indicating the end of pulpal anesthesia. Onset of pulpal anesthesia was considered as the time from the end of the injection until the first absence of aversive response to the maximum stimulus of the pulp tester. Duration of pulpal anesthesia was the time in which the animal did not respond to the electric stimulus.

Statistical analysis

Data were submitted to ANOVA test, Tukey test (IONB) and t test (IANB). Significance level was set at 5%. Sample size calculation (n=8 animals/group) was

performed considering previous literature reports (Ready and Fink, 1980; Hassan *et al*., 1985a,b; Cereda *et al*., 2004). The software BioEstat 5.0 (Instituto Mamiramuá, Belém, PA) was used for statistical analysis.

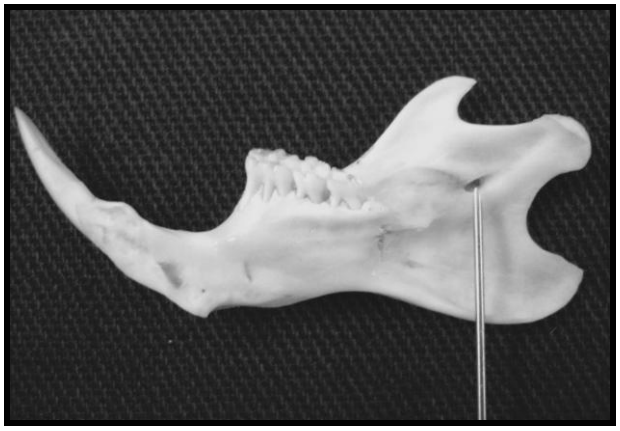

Figure 1. Needle position during the injection of the local anesthetic formulation.

RESULTS

The pH values of formulations were: 5.6 for ART3; 5.8 for ART4; 6.5 for $ART3_{LUV}$; 6.3 $ART4_{LUV}$; 2.9 for $ART4_{EPI}$ and 6.7 for $LUV_{LA\text{-}FREE}$.

$LUV_{LA\text{-}FREE}$, used as control, presented no anesthetic effect in both infraorbital nerve and inferior alveolar nerve blocks.

Figure 2 shows results for duration of anesthetic effect in soft tissues of rats obtained after IONB with the tested formulations. There were no significant differences among $ART4_{EPI}$, $ART3_{LUV}$ e $ART4_{LUV}$ ($p>0.05$), and these formulations provided longer duration of anesthesia than ART4 and ART3 ($p<0.01$). There was no difference between these two last formulations ($p>0.05$).

Figure 3 shows the results for pulpal anesthesia onset after NAI block. $ART4_{EPI}$ presented faster onset of pulpal anesthesia when compared to the other preparations tested ($p<0.05$). No significant difference was found among $ART3_{LUV}$, $ART4_{LUV}$, ART3 and ART4 ($p>0.05$).

Figure 4 shows the results for duration of pulpal anesthesia in the lower molars after IANB in rats. $ART4_{EPI}$ provided a significantly longer duration of pulpal anesthesia when compared to all other tested preparations ($p<0.05$). $ART3_{LUV}$ and $ART4_{LUV}$ induced longer duration of pulpal anesthesia when compared to ART3 and ART4 ($p<0.05$). No significant difference was found between $ART3_{LUV}$ and $ART4_{LUV}$ or between ART3 and ART4 ($p>0.05$).

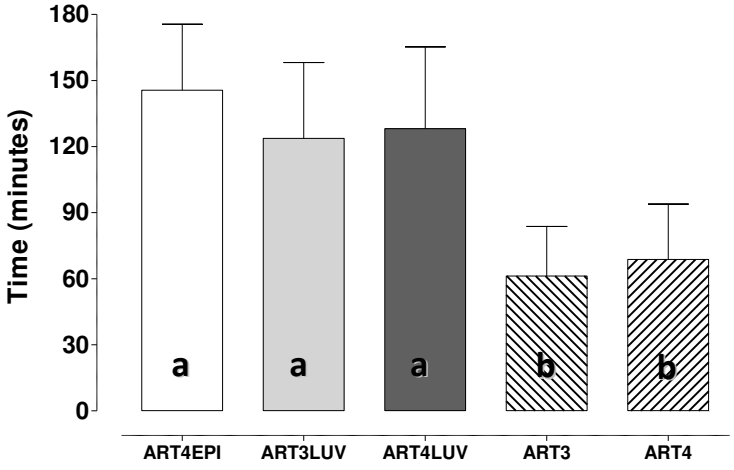

Figure 2. Duration of soft tissue anesthesia (mean and standard deviation, in minutes) for IONB model after anesthetic injection of 3% plain articaine (ART3), 4% plain articaine (ART4), lipossomal 3% articaine ($ART3_{LUV}$), lipossomal 4% articaine ($ART4_{LUV}$) and 4% articaine with 1:100,000 epinephrine ($ART4_{EPI}$). $LUV_{LA\text{-}FREE}$, used as control, presented no anesthetic effect. Different letters indicate statistical significance ($p<0.05$).

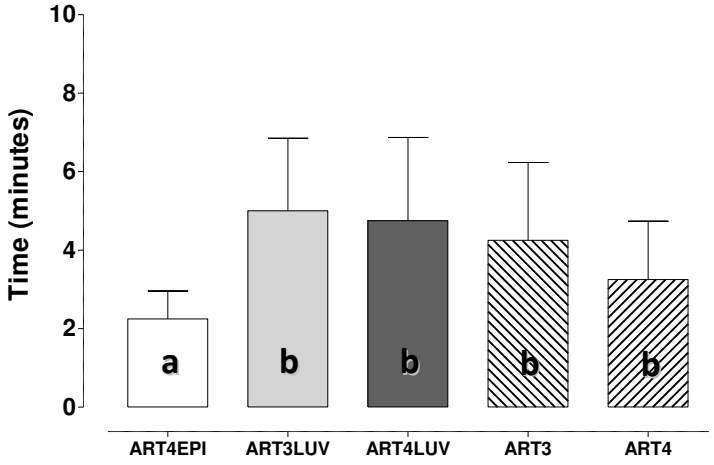

Figure 3. Onset of pulpal anesthesia (mean and standard deviation, in minutes) for IANB model after anesthetic injection of 3% plain articaine (ART3), 4% plain articaine (ART4), lipossomal 3% articaine ($ART3_{LUV}$), lipossomal 4% articaine ($ART4_{LUV}$) and 4% articaine with 1:100,000 epinephrine ($ART4_{EPI}$). $LUV_{LA\text{-}FREE}$, used as control, presented no anesthetic effect. Different letters indicate statistical significance ($p<0.05$).

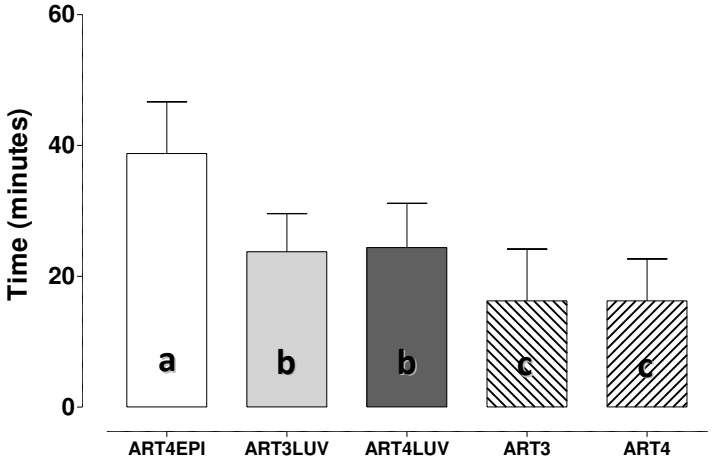

Figure 4. Duration of pulpal anesthesia (mean and standard deviation, in minutes) for IANB model after anesthetic injection of 3% plain articaine (ART3), 4% plain articaine (ART4), lipossomal 3% articaine ($ART3_{LUV}$), lipossomal 4% articaine ($ART4_{LUV}$) and 4%

articaine with 1:100,000 epinephrine ($ART4_{EPI}$). $LUV_{LA\text{-}FREE}$, used as control, presented no anesthetic effect. Different letters indicate statistical significance ($p<0.05$).

DISCUSSION

The two animal models used in the present study involve intraoral anesthesia. These techniques were chosen to simulate the conditions of a dental anesthetic procedure. The IONB model was previously used to test other local anesthetic solutions and liposomal formulations (Cereda *et al*, 2004; Ready and Fink, 1980; Hassan *et al*., 1985a;b). It is a simple and reproducible method to measure the soft tissue anesthetic efficacy. However, in most dental procedures pulpal anesthesia is demanded. Therefore, the evaluation of the pulp anesthesia by using the IANB model (Silva *et al*., 2009) was more appropriated than IONB model to reproduce dental procedures.

The association of commonly used local anesthetics and new controlled-release systems, especially liposome, has been studied in search of a more effective, safe and comfortable dental treatment. These systems prolong the anesthetic duration and reduce toxicity, due to the slow release of the drug (Boogaerts *et al*., 1993, 1994; Grant *et al*., 1994, 2001, 2004; Mowat *et al*., 1996; Yu *et al*., 2002; Cereda *et al*., 2004, 2006; de Araujo *et al*., 2004, 2008).

The pH of the formulation is a factor that affect onset of anesthesia. Low pH usually induces slow onset (Malamed, 2005). However, previous studies found no significant differences concerning onset of pulpal anesthesia when comparing liposomal formulations and epinephrine-containing solutions after maxillary infiltration in humans (Tófoli *et al.,*

2008; Franz-Montan, 2009). In the present study, liposomal formulations presented higher pH (6.3 and 6.5) and slower onset of pulpal anesthesia than epinephrine-containing articaine (pH=2.9). This fact could be explained by the profile of interaction between articaine and liposome, which probably is different from the interaction with other local anesthetic molecules and is not well established. The onset of pulpal anesthesia after IANB presents a considerable variation among studies, from 4.2 to 9.7 minutes for epinephrine-containing solutions (Tófoli *et al.,* 2004; Jung *et al*., 2008; Moore *et al.,* 2006). The IANB model used must have influenced the difference in the anesthesia onset observed in the present study (Figure 2). Fast onset (4.8 minutes) was also observed by other authors for 2% lidocaine with 1:100,000 epinephrine in the same animal model (Silva *et al*., 2009).

The present study is the first attempt to e the anesthetic properties of liposome encapsulated articaine. Liposomal formulations of the amide-type local anesthetics prilocaine, mepivacaine and lidocaine provided longer anesthesia duration than their respective plain solutions, with the same anesthetic concentration, in rat IONB model (Cereda *et al*., 2006). Our study also showed that liposomal articaine induced longer duration of anesthesia when compared to the plain solution, by using the same *in vivo* model.

The present investigation showed that liposomal 3% articaine presented similar duration of soft tissue anesthesia in comparison with 4% articaine with 1:100,000 epinephrine in the IONB model. The slow release of the local anesthetics provided by the liposome encapsulation could explain these findings (Barenholz, 2003).

Articaine hydrochloride is commercially available at 4% concentration with 1:100,000 or 1:200,000 epinephrine. An increased number of paresthesia cases, mainly after inferior alveolar/lingual nerve block, has been attributed to articaine when compared to other anesthetics. The high concentration of articaine (4%) is related as one of the causes of the neural cytotoxicity (Gaffen and Haas, 2009). A less concentrated articaine formulation showing similar anesthetic activity could be an important alternative to 4% articaine.

Considering pulpal anesthesia, the liposomal 3% articaine provided longer duration than plain 4% and 3% articaine, similar duration when compared to liposomal 4% articaine, but shorter duration when compared to 4% articaine with 1:100,000 epinephrine. Our results are in agreement with a previous investigation, which found similar duration of pulp anesthesia in human maxillary canine for liposomal 2% mepivacaine and 3% mepivacaine solution (Tofoli *et al*., 2008).

Epinephrine-containing local anesthetics provide longer duration of pulpal anesthesia when compared to liposome-encapsulated anesthetics, as previously observed with 2% mepivacaine with 1:100,000 epinephrine and liposomal 2% mepivacaine after maxillary infiltration in humans (Tófoli *et al.,* 2008). Epinephrine-containing ropivacaine, a long-action local anesthetic, also provided longer duration of pulpal anesthesia when compared to liposomal ropivacaine after maxillary infiltration (Franz-Montan, 2009). The slow release and prolonged action of the local anesthetics encapsulated in liposomes (Barenholz, 2003) have been insufficient to achieve the same anesthesia duration of the vasoconstrictor-associated solutions. In the studies mentioned above (Tófoli *et al.* 2008; Franz-Montan, 2009), large 400nm unilamellar liposomes were used. Enhanced encapsulation efficiency or chemical alterations in liposome composition, in order to

control size and anesthetic release rate, could result in a more prolonged effect of liposomal anesthetic formulation (de Araújo *et al.*, 2008). The addition of epinephrine to articaine solutions is essential for consistent and profound pulpal anesthesia (Moore et al, 2006). The properties of liposomal formulations could be enhanced with the addition of vasoconstrictor, but this association was not tested yet.

In conclusion, liposome encapsulation prolonged the anesthetic effects of articaine when compared to plain solution. However, the epinephrine-containing solution showed longer pulpal anesthesia duration than liposomal formulations. Further studies must be performed in order to improve the anesthetic efficacy of liposomal 3% articaine formulation.

ACKNOWLEDGMENTS

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CONCLUSÃO

A encapsulação em lipossomas permitiu aumento na duração da anestesia da articaína quando comparada à solução pura. Entretanto, a articaína 4% com epinefrina 1:100.000 obteve maior duração da anestesia pulpar que as formulações lipossomais.

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ANEXOS

Anexo 1

UNICAMP

CEEA/Unicamp

Comissão de Ética na Experimentação Animal
CEEA/Unicamp

C E R T I F I C A D O

Certificamos que o Protocolo nº **1341-1**, sobre **"Estudo da eficácia da preparação anestésica local lipossomal de articaína em ratos"**, sob a responsabilidade de **Prof. Dr. Francisco Carlos Groppo / Luciana Aranha Berto**, está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética na Experimentação Animal – CEEA/Unicamp em **26 de setembro de 2007**.

C E R T I F I C A T E

We certify that the protocol nº **1341-1**, entitled **"Efficacy of a liposomal articaine hydrocloride formulation in rats"**, is in agreement with the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation (COBEA). This project was approved by the institutional Committee for Ethics in Animal Research (State University of Campinas - Unicamp) on **September 26, 2007**.

Campinas, 26 de setembro de 2007.

Profa. Dra. Ana Maria A. Guaraldo
Presidente

Fátima Alonso
Secretária Executiva

CEEA/IB – Unicamp
Caixa Postal 6109
13083-970 Campinas, SP – Brasil

Telefone: (19) 3521-6359
Telefax: (19) 3521-6356
E-mail: comisib@unicamp.br
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Anexo 2

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Detailed Status Information

Manuscript #	10-0074
Current Revision #	0
Submission Date	2010-02-04
Current Stage	Under Consideration
Title	Anesthetic Efficacy of Liposomal Articaine in Rats
Running Title	Efficacy of Liposomal Articaine in Rats
Manuscript Type	Research Report
Special Section	N/A
Category	Clinical
Manuscript Comment	Number of words in the abstract: 147 Number of words in the abstract and the text: 2,805 Number of figures: 3 Number of cited references: 34
Corresponding Author	Francisco Groppo (State University of Campinas, Piracicaba Dental School)
Contributing Authors	Luciana Berto , Eneida de Paula , Maria Volpato

Anexo 3

Folha_____
Processo_____
Rubrica_____

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

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 de Mestrado intitulada "EFICÁCIA ANESTÉSICA DA ARTICAÍNA LIPOSSOMAL EM RATOS", não infringem os dispositivos da Lei nº 9.610/98, nem o direito autoral de qualquer editora.

Piracicaba, 24 de Novembro de 2009.

LUCIANA ARANHA BERTO
RG: 43970992-1
Autor(a)

FRANCISCO CARLOS GROPPO
RG: 15.432.620
Orientador(a)