Dental Traumatology 2008; **24**: 27–31; doi: 10.1111/j.1600-9657.2006.00482.x

Healing pattern of experimental soft tissue lacerations after application of novel topical anesthetic agents – an experimental study in rabbits

Adel Al-Asfour[1], Manal Al-Melh[2], Lars Andersson[1], Bobby Joseph[3]

[1]Oral and Maxillofacial Surgery, Department of Surgical Sciences, Faculty of Dentistry, Kuwait University, Kuwait City; [2]Faculty of Dentistry, Kuwait University, Kuwait City; [3]Oral Medicine and Oral Pathology, Department of Diagnostic Sciences, Faculty of Dentistry, Kuwait University, Kuwait City, Kuwait

Correspondence to: Dr Adel A. Al-Asfour, Faculty of Dentistry, Kuwait University, PO Box 24923 Safat, 13110 Kuwait
Tel.: (965) 4986768
Fax: (965) 5347948
e-mail: adelalasfour@hsc.edu.kw

Accepted 13 December, 2005

Abstract – Topical anesthetics based on a combination of 2.5% lidocaine and 2.5% prilocaine are efficient in eliminating pain from needle stick when placed on skin and oral mucosa. This suggests their application in soft tissue lacerations before suturing to enable pain-free exploration and suturing of traumatic lacerations without prior injection needle stick. The aim of the present study was to study the healing of experimental oral lacerations after topical anesthetic substances were placed in the lacerations. Thirty-six standardized incisions were made bilaterally in the lower and the upper labial mucosa of nine white New Zealand rabbits. All wounds were intentionally contaminated with saliva to simulate laceration wounds in trauma situation. EMLA cream and Oraqix thermosetting gel were applied into 30 lacerations and six lacerations were left untreated as control. In some lacerations the topical anesthetic agent was left in the wound, while in others they were rinsed off by saline before suturing the laceration wound. The rabbits were then killed after 3 days, 2 weeks and 4 weeks of healing and the lips were processed for histological evaluation. Similar normal histological healing patterns were seen in wounds in which EMLA and Oraqix were applied compared with control lacerations at all stages of healing. No adverse tissue or foreign body reactions were seen in any of the lacerations. We conclude that EMLA and Oraqix can be used in oral mucosal lacerations prior to suturing without the risk of adverse tissue reaction.

Soft tissue injuries are seen in one-third of all patients seeking emergency treatment for oral injuries (1). One of the soft tissue injuries is the laceration injury. Laceration injuries are usually treated by careful debridement of the wound and proper suturing, which are painful procedures that cannot be carried out without the use of anesthetic agents. One way of obtaining local anesthesia is by using needles for the administration of local anesthesia. Although local anesthesia is achieved, the needle stick can also induce the pain experienced by the patient leading to more anxiety and an uncooperative behavior.

To avoid the pain from the needle stick, topical anesthetics can be used. However, some of these agents are not effective in reducing or eliminating pain from the needle stick. With the introduction of more efficient topical anesthetic agents in a mixture of 2.5% lidocaine and 2.5% prilocaine, it has been possible to provide a better pain control.

In medical practice, EMLA (AstraZeneca, Södertälje, Sweden), which is a creamy preparation of lidocaine and prilocaine, was used as a topical skin analgesic agent to eliminate pain during venous cannulation procedures in children (2). Other applications in the medical field include both maxillary sinus puncture procedures and minor gynecologic procedures (3, 4). In dentistry, the use of EMLA has been documented in various procedures, such as the excision of gingival tissues, scaling and root planing, and in other procedures in pediatric dentistry (5–9). In a recent study, EMLA was able to eliminate or reduce the pain from the anesthetic needle stick (10). Because EMLA is not registered for intra-oral use, a new topical anesthetic agent, known as Oraqix (Dentsply Pharmaceutical, York, PA, USA), has been introduced in the dental field for intra-oral application (11). This new non-injection local anesthetic agent, Oraqix, which is made up of 5% lidocaine/prilocaine gel, provides pain relief during periodontal probing and scaling and root planing (12–15). Oraqix offers several advantages over EMLA, such as the quick onset of action, its higher C_{max} values after absorption, and its ability to harden and stay in place after application (6, 7). Unlike Oraqix, EMLA is diluted by saliva and dispersed due to its creamy nature. Therefore, Oraqix may offer an injection-free alternative for patients with lacerated mucosa.

© 2007 Blackwell Munksgaard

Because of the efficiency of these new topical substances in reducing and eliminating pain, we intended to try the use of these substances directly in soft tissue lacerations to see whether it was possible to clean and suture without using injection anesthesia in the area of laceration. Before this can be carried out in humans, we investigated whether there are any adverse tissue reactions from these substances and whether there is a delay in healing when compared with lacerations where topical anesthetics have not been placed.

Hence, the aim of this study was to compare the tissue reactions and healing patterns during the first month of healing of experimental lacerations in rabbits where topical anesthetics had been applied before suturing of the laceration.

Material and methods

Animals and sedation

Nine 4- to 5-month-old white New Zealand rabbits, weighing 3–4 kg, were obtained from the Central Animal House, Kuwait University. The animals were caged in a room and fed food and water *ad libitum*. The rabbits were sedated by intramuscular injection of xylazine HCl 50 mg ml^{-1}, 5 mg kg^{-1} body weight (Rompun; Bayer, Leverkusen, Germany) and anesthetized by intramuscular ketamine HCl 35 mg kg^{-1} body weight (Tekam; Hikma Pharmaceutical, Amman, Jordan).

Design of the study and experimental laceration

Using a scalpel, a standardized labial incision was made in the labial vestibule on the left- and right-hand sides of the upper and lower lips of each rabbit. The incisions were standardized, i.e. approximately 5 mm long and applied at the same sites in all rabbits. The incisions were made deep through the epithelium and connective tissue reaching into the muscular layer (Fig. 1). To simulate the conditions of an accidental laceration, all lacerations were intentionally contaminated by saliva. In 30 lacerations, various topical anesthetic substances were applied and six lacerations were left untreated and served as controls. In some lacerations, the topical agents were left in the lacerations whereas in others the topical substances were rinsed off by saline from the lacerations before suturing. All lacerations were closed in the middle with one resorbable 3-0 suture (Vicryl; Ethicon, Somerville, NJ, USA) extending into the connective tissue. The rabbits were killed after 3 days, 2 weeks and 4 weeks of healing, and the lips were processed for histological evaluation (Table 1).

Histologic preparation and evaluation

The rabbits were killed by an intravenous overdose of ketamine HCl (Tekam; Hikma Pharmaceutical). The lips were dissected out, fixed in 10% neutral buffered formalin overnight and prepared for histologic evaluation. Tissues were washed in distilled water, dehydrated in alcohol and were embedded in paraffin by a standard histological procedure (16). Longitudinal serial sections (5 μm) were cut for hematoxylin and eosin staining.

Evaluation was carried out blindly by one of the authors (BJ) so that the evaluator had no information about the rabbits, treatment of laceration or healing time. The evaluator graded the inflammation based on the intensity of inflammatory infiltrate in the tissue. The inflammation was graded as follows: 0 = no inflammation, 1 = mild inflammation, 2 = moderate inflammation, 3 = severe inflammation. The evaluator also gave a description of the histologic picture in the section with regard to the site of laceration, intensity of the inflammatory infiltrate, presence of granulation tissue, fibroblast proliferation and increased collagen deposition. The presence of macrophages and foreign bodies in the tissues was also noted.

Statistics

The Wilcoxon test was used to test for significant differences between the groups. $P < 0.05$ was considered as significant.

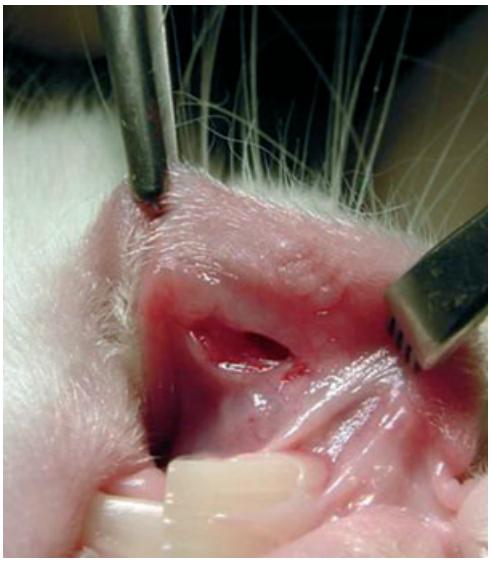

Fig. 1. Experimental laceration in the lower left vestibular mucosa of the rabbit.

Table 1. Distribution of application of substances and controls in 36 lip lacerations of nine rabbits with healing times of 3 days, 2 weeks and 4 weeks

No	Healing time	Right side upper lip	Left side upper lip	Right side lower lip	Left side lower lip
1	3 days	EMLA	Control	Oraqix	Oraqix + rinse
2	3 days	EMLA + rinse	Oraqix + rinse	Control	EMLA + rinse
3	3 days	Oraqix	EMLA	EMLA + rinse	Oraqix + rinse
4	2 weeks	Oraqix + rinse	Control	EMLA	EMLA + rinse
5	2 weeks	Oraqix	Oraqix + rinse	Oraqix	EMLA
6	2 weeks	EMLA + rinse	Oraqix + rinse	Control	Oraqix + rinse
7	4 weeks	EMLA	Control	Oraqix	EMLA + rinse
8	4 weeks	EMLA	Control	Oraqix	EMLA + rinse
9	4 weeks	EMLA + rinse	EMLA	Oraqix + rinse	Oraqix

© 2007 Blackwell Munksgaard

Results

The animals tolerated the experimental procedures well and the healing period was uneventful.

Histologic pattern after 3 days of healing

With a few exceptions, inflammation was graded as moderate or severe with a picture of an early wound healing. The experimental cut was visible (Fig. 2a) and an acute inflammatory infiltrate was present around the wound. Leukocytes of several types could be seen including numerous neutrophils (Fig. 2b). Immediately adjacent to these areas, vascularity was prominent. These areas were highly cellular and loosely textured. In some sections on the margins of incision of the tissues, a 'beading' of nuclei of the muscle could be noticed, suggesting tissue regeneration. Epithelial proliferation was also seen along with macrophages adjacent to hemosiderin pigments. Most of the tissues that were examined contained suture material. However, there were no multinucleate giant cells to suggest features of a foreign body-type reaction. Regardless of the type of topical anesthetic agent used, a similar histologic pattern was seen, and control sites showed a similar histologic picture.

Histologic pattern after 2 weeks of healing

Inflammation was graded as mild and less than the 3-day group with a picture of healing. The experimental cut was not visible and tissues were less inflamed, although chronic inflammatory cells were still present. There was a continued collagen accumulation and a fibroblast proliferation (Fig. 3). The leukocyte infiltrate and increased vascularity were substantially diminished. This histologic picture was seen regardless of the substance group, and control sites showed a similar histologic picture.

Histologic pattern after 4 weeks of healing

Inflammation was mild and similar to that observed in the 2-week group. The experimental cut was not visible and the fibroblastic tissue was mature or showed complete healing. Tissues were more fibrous and in some parts the collagen was quite mature, the fibroblasts appeared stable, the blood vessels well established and overall the features in these parts indicated successful repair. The scar comprised a cellular connective tissue largely devoid of inflammatory cells and covered by an essentially normal epithelium (Fig. 4). No foreign bodies could be seen in this group. This histologic picture was seen regardless of the substance group, and control sites showed a similar histologic picture.

Grading of inflammation

Inflammation was more significant after 3 days when compared with the 2- and 4-week groups ($P < 0.05$) (Table 2). There was no significant difference between the 2- and 4-week groups. There was no significant difference between the treatment or control groups.

Discussion

The present study showed that two topical anesthetic agents, EMLA and Oraqix, both based on a combination of lidocaine and prilocaine, did not seem to impair wound healing when placed in the laceration. A normal healing pattern was seen in the experimental and control groups without any adverse foreign body reactions. Oral laceration wounds in which EMLA and Oraqix were applied showed normal healing similar to laceration wounds where no topical anesthetic agents applied (control). Rinsing off the topical anesthetic agents from

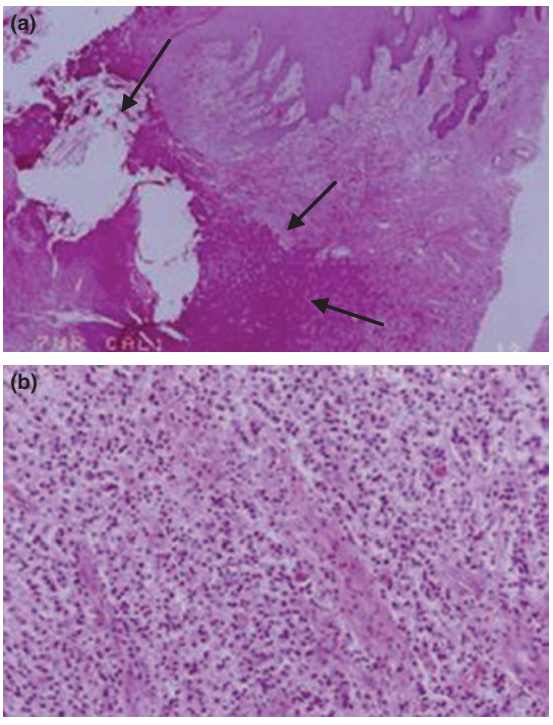

Fig. 2. (a) Hematoxylin and eosin-stained sections show healing after 3 days. Arrows show a pool of acute inflammatory cells and the experimental cut (×10). (b) High-power view showing highly cellular and loosely textured areas with large number of blood vessels (×20).

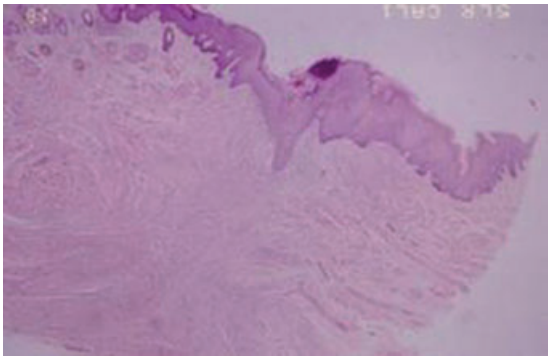

Fig. 3. Histologic pattern after 2 weeks of experimental laceration. The tissues are less inflamed with collagen accumulation and epithelial proliferation. The experimental cut is not visible (×10).

© 2007 Blackwell Munksgaard

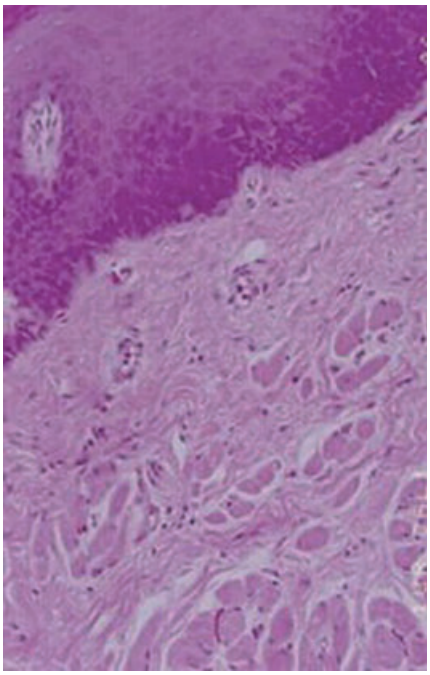

Fig. 4. Histologic pattern after 4 weeks of experimental laceration. There are very few inflammatory cells with evidence of mature collagen – features of a successful repair (×20).

Table 2. Grading of inflammation. Mean values of experimental groups, control groups and for different healing times for 36 lacerations

Substance	3 days	2 weeks	4 weeks
EMLA	2.0	1.0	0.3
EMLA + rinse	1.3	1.0	1.3
Oraqix	3.0	1.0	1.3
Oraqix + rinse	2.0	1.6	1.0
Control	2.0	1.0	1.0
All lacerations	2.1* (n = 12)	1.3 (n = 12)	1.0 (n = 12)

*Indicates significantly ($P < 0.05$) more inflammation compared with 2 and 4 weeks.

the wound after application seemed to be of no benefit for wound healing.

This is a study simulating a real-life oral soft tissue trauma scenario in which lacerations of sufficient length and depth were experimentally created in the mucosa. The lacerations were intentionally contaminated by saliva, a situation similar to that seen in a trauma patient. The application of topical anesthetics, rinsing, and suturing of the wounds can be compared with situations occurring in humans, making it a good study model. Evaluation of the healing pattern was carried out blindly. This is very important to minimize bias when looking at and interpreting the histologic findings of the different grades of the inflammatory processes for the three different groups during healing. The design of the study was time-related by using different evaluation times for healing after application and suturing of the wounds. Healing was studied in both short- and long-term perspectives.

A rabbit model may differ from a human model in many ways, e.g., composition of tissues and speed of healing process. For this reason, we used different stages of healing by studying different time intervals. In all the groups normal healing was seen with no difference between the groups. Due to ethical reasons the number of rabbits that was used was minimal and this may be a limitation of the present study. However, the results in this study showed conclusive evidence that normal healing was not affected by using these agents.

Besides prilocaine and lidocaine, there are components in EMLA and Oraqix that may hypothetically interfere with healing if placed in a wound. EMLA contains carbomer to adjust viscosity, and macroglycerolhydroxystearat which is a surfactant. Oraqix contains poloxamer which is a thermosetting surfactant making Oraqix liquid at room temperature and gel in oral cavity temperature. We hypothesize that these ingredients might potentially cause foreign body reactions or delay healing; however, this was not seen in any of the groups.

In our experimental model the topical anesthetic was left in the laceration before suturing. Although there was no evidence of any foreign body reactions, we recommend irrigating the wound before closure of the suture.

Healing after EMLA and Oraqix application in lacerations was similar to that observed in control lacerations. This is of great value in emergency clinical situations where laceration wounds are closed after local anesthesia with a needle in an apprehensive patient. The needle anesthesia will be painful and will evoke fear and anxiety, especially in children. The topical anesthetic agent will reduce or eliminate pain from the needle stick (10). Hence, it is tempting to draw a conclusion that if these new generation topical anesthetics can eliminate pain from needle stick anesthesia, it should also reduce or eliminate pain from the suture needle.

Given this, in the future we may not have to give local anesthesia injection when treating some soft tissue lacerations, and it may be possible to close some soft tissue lacerations with sutures without injecting anesthetics. This would relieve anxiety in young patients in an emergency situation. Clinical studies are now needed to test the efficiency of topical anesthetic agents placed directly in laceration wounds, and there is also a need for further investigation into clinical differences regarding the use of EMLA or Oraqix with regard to taste, smell, applicability, and patient perception. Currently these studies are already underway in our center.

Conclusion

The topical anesthetics EMLA and Oraqix can be used in soft tissue lacerations in the oral mucosa without adverse tissue reactions.

Acknowledgements

We wish to thank Mrs Preethi John for her excellent technical assistance, Dr Severino Gabato and Shebeer Shamsudeen for the excellent anesthetic techniques, and Farah Nejad for the excellent care of animals.

© 2007 Blackwell Munksgaard

References

1. Petersson EE, Andersson L, Sörensen S. Traumatic oral vs non-oral injuries. Swed Dent J 1997;21:55–68.
2. Speirs AF, Taylor KH, Joanes DN, Girdler NM. A randomized, double-blinded, placebo-controlled, comparative study of topical skin analgesics and the anxiety and discomfort associated with venous cannulation. Br Dent J 2001;190:444–9.
3. Joki-Erkkilä V-P, Kääriainen J, Penttilä M, Rautiainen M. Local anesthesia with EMLA cream for maxillary sinus puncture. Ann Otol Rhinol Laryngol 2002;111:80–2.
4. Zilbert A. Topical anesthesia for minor gynecological procedures: a review. Obstet Gynecol Surv 2002;57:171–7.
5. McMillan AS, Walshaw D, Meechan JG. The efficacy of Emla® and 5% lignocaine for anesthesia of human gingival mucosa. Br J Oral Maxillofac Surg 2000;38:58–61.
6. Friskopp J, Huledal G. Plasma levels of lidocaine and prilocaine after application of Oraqix®, a new intrapocket anesthetic, in patients with advanced periodontitis. J Clin Periodontol 2001;28:425–9.
7. Friskopp J, Nilsson M, Isacsson G. The anesthetic onset and duration of a new lidocaine/prilocaine gel intra-pocket anesthetic (Oraqix®) for periodontal scaling/root planing. J Clin Periodontol 2001;28:453–8.
8. Munshi AK, Hegde AM, Latha R. Use of EMLA®: is it an injection free alternative? J Clin Pediatr Dent 2001;25:215–8.
9. Jeffcoat MK, Gerurs NC, Magnusson I, MacNeill SR. Intrapocket anesthesia for scaling and root planing: results of a double-blind multicenter trial using lidocaine prilocaine dental gel. J Periodontol 2001;72:895–900.
10. Al-Melh M, Andersson L, Behbehani E. Reduction of pain from needle stick in the oral mucosa by topical anesthetics: a comparative study between lidocaine/prilocaine and benzocaine. J Clin Dent 2005;16:1–4.
11. Meechan JG. Effective topical anesthetic agents and techniques. Dent Clin North Am 2002;46:759–66.
12. Steenberghe DV, Bercy P, Boever JD, Adriaens P, Geers L, Hendrickx E et al. Patient evaluation of a novel non-injectable anesthetic gel: a multicenter crossover study comparing the gel to infiltration anesthesia during scaling and root planing. J Periodontol 2004;75:1471–8.
13. Magnusson I, Jeffcoat MK, Donaldson D, Otterbom IL, Henriksson J. Quantification and analysis of pain in nonsurgical scaling and/or root planing. J Am Dent Assoc 2004;135:1747–54.
14. Donaldson D, Gelskey SC, Landry RG, Matthews DC, Sandhu HS. A placebo-controlled multi-centered evaluation of an anesthetic gel (Oraqix®) for periodontal therapy. J Clin Periodontol 2003;30:171–5.
15. Herdevall BM, Klinge B, Persson L, Huledal G, Abdel-Rehim M. Plasma levels of lidocaine, o-toluidine, and prolicaine after application of 8.5 g Oraqix in patients with generalized periodontitis: effect on blood methemoglobin and tolerability. Acta Odontol Scand 2003;61:230–4.
16. Joseph BK, Gobe GC, Savage NW, Young WG. Expression and localization of sulfated glycoprotein-2 mRNA in the rat incisor tooth ameloblast: relationship with apoptosis. Int J Exp Pathol 1994;75:313–20.

© 2007 Blackwell Munksgaard

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.