Original Article

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Efficacy of anesthetic rice nanogel on pain reduction in human oral cavity

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Summary **The aim of this study was to determine the efficacy of two local anesthetic rice nanogels (RNG) on pain reduction from needle insertion in oral cavity. Nanogel base was prepared using modified rice as gelling agent. The average particle size of RNG determined by photon correlation spectrophotometer was 485 ± 70 nm. Lidocaine hydrochloride (LH) and prilocaine hydrochloride (PH) were incorporated into RNG to obtain anesthetic RNG containing 5% and 20% LH or PH. Clinical efficacy test of each gel was performed in oral cavity of 100 healthy volunteers (25-60 years old). Evaluation was done by recording different pain measurements after inserting a needle into buccal mucosa after applying 5% and 20% anesthetic RNG. RNG base (placebo) and commercial anesthetic gels were used as negative and positive controls, respectively. It was found that the pain level in the negative control group was significantly higher than those of the anesthetic groups. Moreover, the pain level of the anesthetic RNG groups were lower than that of the commercial groups, especially in 20% anesthetic groups. For patient's satisfaction, most of the volunteers were appreciated with the anesthetic RNG as well as the commercial gels. They preferred to use high drug content RNG more than those with low drug content or placebo. It can be concluded that the anesthetic RNG has potential clinical efficacy in pain reduction during needle insertion in oral cavity.**

Keywords: Nanogel, lidocaine, prilocaine, anesthetic efficacy, pain relief

1. Introduction

Nobody likes going to the dentist, especially for some people, the problems run deeper and form a phobia. The causes of dental phobia can be many and varied but one of those is painful from needle insertion. The anxiety and fear often occurred in preoperative patients (*1*). Especially in children, anxiety and fear caused negative impression to dental treatment and reflecting in avoidance to dental attendance. However, properly dental management could reduce such a fear (*2*). It was reported that the anxiety was increased according to the poor oral hygiene of the patients and related to low quality of life (*3*). In addition, Armfield and his coworkers found that not only children are afraid of dentists but also adults (*4*). The local anesthetic gel is, therefore, introduced to oral mucosa prior to injection in order to reduce the pain from the needles. However, the efficacy of the available anesthetic gels are not high enough, which might be due to the delivery systems are not well suitable.

Nanoparticle delivery systems have been shown to be effective in protecting drugs from degradation, overcoming biological barriers, and controlling the rate and duration of drug release (*5-7*). Various types of nanoparticle systems have been developed using polymer based nanoparticles (*8-10*). Recently, natural polymers such as chitosan and starch have been used

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instead of synthetic polymers (*11,12*). Hydrogels are cross-linked networks of hydrophilic polymers containing a large amount of water. This structure can be used for loading and release of drugs and natural bioactives (*13,14*). Nanogel is an advanced formulation of nano-sized hydrogel particles. Nanogel possesses advantage above its original macroscopic hydrogel that it can be injected in the circulation directly to target tissues and can better deliver their payloads for both local and systemic applications (*15*). It has been reported that nanogels not only protect the drugs from degradation and elimination but also participate actively in the delivery process due to their characteristic properties like adhesive, stimuli-responsive behaviour, softness and swelling to help achieve a controlled response at the target tissues (*16-18*).

Lidocaine and prilocaine are amino amide class local anesthetics (*19*). They are nowadays widely used in dental treatment for pain protection and elimination during treatment and minor surgery (*20,21*). Commercial available semisolid products are gel, cream, and ointment. The gels usually contain only lidocaine at various concentrations of 2-10% as hydrochloride salt form. The creams and ointments usually contain lidocaine alone or in the combination with prilocaine as the base form. However, the commercial available anesthetic gels have poor property on mucoadhesion. Moreover, most of gelling agents commonly used in the gels are of chemical synthetic polymers. We previously reported the advantages of modified rice on high mucoadhesive property and suitable for using as filming or gelling agent in drug delivery systems *via* oral mucosa (*22,23*). We have also developed rice nanogel (RNG) using modified rice as gelling agent and found that type of rice affect the properties including drug release behavior of RNG (*24,25*). Importantly, it has been reported that RNG containing local anesthetics causes no toxicity to oral epithelial cells and no inflammatory effect to oral tissues (*26*). We hypothesized that RNG could increase anesthetic efficacy and patient satisfactory during dental treatment. Therefore, in the present study, efficacy of RNG containing lidocaine or prilocaine at different drug concentrations on pain reduction and patient's satisfaction were investigated.

2. Materials and Methods

2.1. *Materials*

Pharmaceutical-grade lidocaine hydrochloride (LH) and prilocaine hydrochloride (PH) were obtained from Gufic Bioscince Ltd. (Mumbai, India). Sodium hydroxide and glacial acetic acid were from RCI Labscan Co., Ltd. (Bangkok, Thailand). Commercial gel A containing 5% LH was from Septodont Ltd. (Kent ME16 0JZ, UK). Commercial gel B containing 20% benzocaine was from Ultradent Products Inc. (South Jordan, USA). Commercial gel C containing combination of 14% benzocaine, 2% butamben, and 2% tetracaine hydrochloride was from Hager Worldwide Inc. (Maidstone, UK). All other chemicals and solvents were of AR grade or the highest grade available unless otherwise stated.

2.2. *Anesthetic RNG preparation*

Modified rice powder was prepared according to the previous method (*24*) and used as gelling agent. RNG base was prepared by mixing suitable amount of modified rice powder with purified water. The particle size of the obtained RNG base was determined using photon correlation spectroscopy (PCS). The anesthetic RNG containing of LH (LH-RNG) or PH (PH-RNG) were prepared according to the method previously described (*25*). Briefly, exact amount of LH or PH was incorporated into certain amount of RNG base and mixed well until the drug was completely dissolved. Subsequently added with NRG base until the desired concentration of drug was reached. The mixture was further triturated until the obviously transparent anesthetic RNG was obtained.

2.3. *Volunteers and ethical considerations*

One hundred healthy volunteers were recruited from Faculty of Dentistry and Faculty of Pharmacy, Chiang Mai University. The exclusion criteria included having a systemic disease, bleeding disorders, drug allergy, pregnancy, breastfeeding, habits of smoking or alcohol consumption, being under medical treatment with drugs or having acute or chronic infection in oral and maxillofacial region. The study was approved by the Human Experiment Committee of the Faculty of Dentistry, Chiang Mai University (Process No. 26/2556). Written informed consent was obtained from all volunteers prior to study.

2.4. *In vivo study of pain reduction*

The study was designed to be double-blind randomized trial. The LH-RNG and PH-RNG were prepared by an independent researcher who was not involved in this *in vivo* research procedure. Both LH-RNG and PH-RNG were similar in appearance. In 5% drug content gel group, RNG and placebo were prepared as a clear gel similar to a commercial gel A whereas in 20% group, RNG and placebo were prepared in the same color as the commercial gels B and C. The pain reduction measurement was performed twice in the same 100 volunteers, the first test was done with the use of 5% anesthesia for 1 week prior to the second test with 20% anesthetic. RNG base (placebo) was used as a negative control. Commercial gel A (5% LH) was used as a positive control in case of 5% anesthetic test whereas

20% commercial gel B and C were used as positive controls in case of 20% anesthetic test. The allocated oral area of the volunteers was divided into four parts, including upper right, upper left, lower right, and lower left buccal vestibules (Figure 1). Neither the dentist nor the volunteers knew which product was applied to each area. Exact amount (0.2 mL) of the anesthetic product was applied on the selected area. After 1 min, the anesthetic product was removed and a sterile dental 27-gauge, 1.5-inch needle was inserted into the mucosa by only one dentist.

The anesthetic efficacy was obtained from each volunteer using visual analogue scale (VAS) and numerical rating scale (NRS) as described by Ferreira-Valente and coworkers (*27*) as well as Wong-Baker faces pain rating scale (WPS) (*28*). Briefly, the VAS is a 100 mm horizontal line; left end (0 mm) represents no pain and right end (100 mm) represents the most imaginable pain. The NRS is a line with 10 score; 0 on the left end represents no pain whereas 10 on the right end represents the most severe pain. The volunteers were asked to check a mark on the line of VAS and NRS and the pain intensity was measured. For WPS, 6 facial expressions reflect 6 pain levels. Level 0 or happiest face represents no pain whereas level 5, the saddest face, represents the highest pain (Figure 2). All variables were recorded and analyzed by the same investigator.

2.5. *Side effects & satisfactory level*

After applying the anesthetic RNG, systemic side effects such as nausea, vomiting, dizziness, and palpitation and local side effects such as erythema, irritation, swelling, and color change of the tissue were recorded. In addition, all volunteers were recalled after 24 h and were asked whether they had any delayed side effects. The responses of volunteers on satisfaction with the use of anesthetic products were evaluated with the help of rating scale of 1-5 for bad, fair, good, very good, and excellent, respectively.

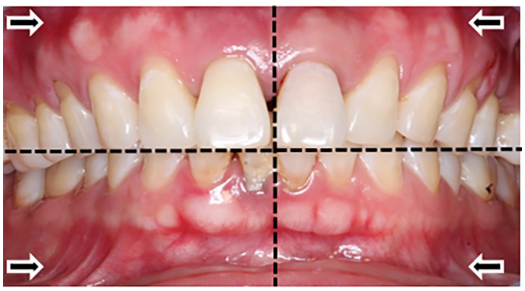

Figure 1. Illustration of the area where the test products were applied.

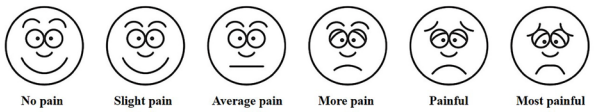

Figure 2. Wong-Baker FACES Pain rating scale.

2.6. Statistical analysis

The VAS and NRS were presented as mean and standard deviation (SD). Statistical analysis was performed by independent *t*-test or a one-way ANOVA with Dunnett C. Kolmogorov-Smirnov's test was used as normality of data evaluation. The statistical significance was considered as p-value < 0.05. The WPS and satisfactory levels were reported as frequencies.

3. Results

3.1. *RNG preparation and characterization*

RNG base was successfully prepared using modified rice powder of approximately 8-10% in water. The outer appearance of RNG was transparent and colorless having average particle size measured by PCS, after 1000-fold water dilution, of 485 ± 70 nm with a polydispersity index (PdI) of approximately 0.3. Incorporation with LH or PH to obtained 5% and 20% of either LH or PH gave the LH-RNG and PH-RNG with the same outer appearance as RNG base.

3.2. *Efficacy of LH-RNG and PH-RNG on pain reduction*

One hundred volunteers were recruited in this study (51 females, 49 males, age ranged between 25-60 years, average age is 37 ± 2.54 years). Significant difference (p < 0.01) in terms of VAS was found between the negative control group and all anesthetic groups as seen in Figure 3. The highest VAS pain score was 3.37 ± 2.41 in negative control group. Comparison among the 5% anesthetic gel groups, it was found that commercial gel A group showed slightly higher VAS than LH-RNG and PH-RNG groups with no significant differences (p = 0.35 and 0.25, respectively). Among 20% anesthetic groups, both RNG groups showed significantly lower VAS values (0.68 ± 1.29 and 0.38 ± 0.72, respectively)

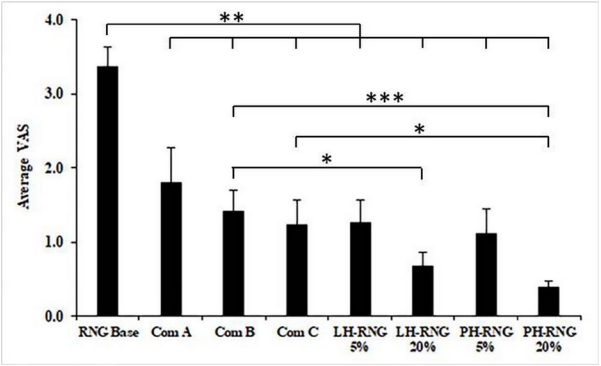

Figure 3. VAS scores between groups; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

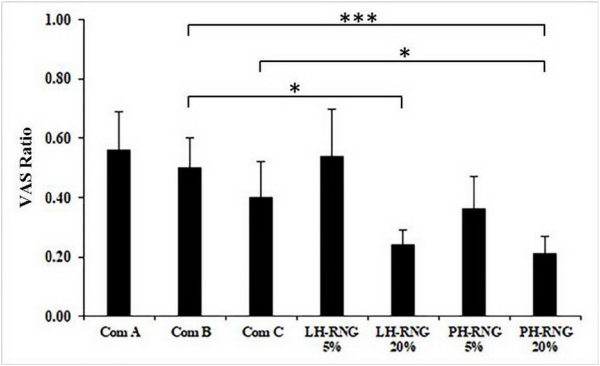

Figure 4. VAS ratio based on VAS placebo group; $^{*}p < 0.05$, $^{***}p < 0.001$.

Table 1. Average numerical rating scale (NRS) of the test products

Drug content	Test products	Average NRS*
5%	Commercial gel A	1.43 ± 0.90d
	LH-RNG	0.97 ± 0.83c
	PH-RNG	0.85 ± 0.74bc
20%	Commercial gel B	0.60 ± 0.65b
	Commercial gel C	0.62 ± 0.68b
	LH-RNG	0.24 ± 0.47a
	PH-RNG	0.21 ± 0.43a
0%	RNG base	2.81 ± 0.73e

*Values are mean ± SD followed by different lowercase letters imply the significant differences ($p < 0.001$) between values in the same column.

than those of commercial products B and C (1.42 ± 1.73 and 1.23 ± 1.28, respectively). Significant difference between 20% LH-RNG and commercial product B was found ($p = 0.03$) whereas 20% PH-RNG showed significant differences from both commercial products B and C ($p < 0.001$ and $p = 0.03$, respectively). However, similar results of LH-RNG and PH-RNG were obtained in both concentrations.

Further data analysis was presented as VAS ratio based on VAS of the negative control group (VAS ratio = VAS anesthetics/VAS placebo). Efficacy of the anesthetics on pain reduction is addressed when the ratio was less than 1 as shown in Figure 4. It was found that all anesthetic products showed effective reduction of pain, particularly 20% LH-RNG and 20% PH-RNG which exhibited significant differences from the commercial products.

The NRS is presented in Table 1. The highest NRS value was found in the negative control group and significant difference from other anesthetic groups was shown ($p < 0.001$). Among 5% anesthetic group, the significant differences between a commercial gel A and both rice gels were presented. Furthermore, 5% LH and PH showed no significant difference. In 20% anesthetic group, NRS scores of LH-RNG and PH-RNG were significantly lower than a commercial gels B and C. No significant difference between LH-RNG and PH-RNG was found in both concentrations. It was noted that higher anesthetic content caused the significant

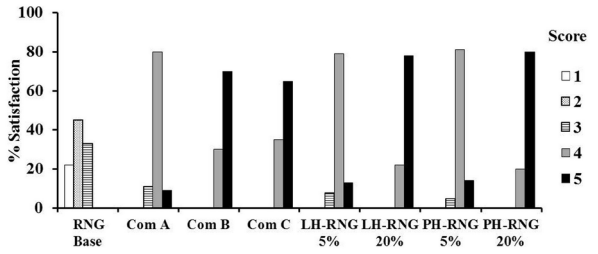

Figure 5. Percentage of satisfaction in each level to the test products.

reduction of pain.

For WPS, the negative control group showed the highest frequency of facial pain expression at level 3 (49%), followed by at level 2 (32%). Considering the 5% anesthetic group, the highest frequency of facial pain expression of the commercial gel was found at level 2 (50%) whereas that of LH-RNG and PH-RNG was found at level 1 (37% and 43%, respectively), followed by level 0 (34% and 36%, respectively). Among the 20% anesthetic group, all gels showed facial pain expression at level 0 but the percentage frequency at this level was different. The extremely high percentage of frequency of facial pain expression at level 0 was found in LH-RNG and PH-RNG (78% and 80%, respectively), whereas that of the commercial gels B and C was only 49% and 47%, respectively.

3.3. *Side effects & satisfactory level*

No sign of side effects was found in all volunteers. The satisfactory results are shown in Figure 5. The number indicates the level of satisfaction, *e.g.*, level 1 represents the least satisfaction whereas level 5 is the highest satisfaction. Higher satisfaction was found with the use of the anesthetic gel than the placebo. Moreover, the volunteers preferred to use 20% to 5% anesthetic gels.

4. Discussion

Nanogel is defined as a three-dimensional hydrogel that possesses particle size in the nanoscale size range (*29*). Many kinds of polymers particularly from natural sources have been used for producing nanogels (*22*). In the present study, the nanogel was prepared from the modified rice starch. The particles size of RNG obtained in this study was 485 ± 70 nm indicating that the nanogel from rice starch could be formed. A polydispersity index (PdI) of approximately 0.3 of the obtained RNG indicates that its particle size distribution is in moderate range. The cross-linked swellable polymer networks in nanogels possess high capacity to hold water (*30,31*), therefore, the outer appearance of the RNG obtained is transparent. For a local anesthetic to be dental use, it should be compatible with the oral mucosal tissues, *e.g.*, not irritating, and its action

should be temporary and completely reversible. It should be effective in doses far below its toxic level, it should be hypoallergenic and have a rapid onset of anesthesia with a duration of action sufficient to complete the dental procedure comfortably (*32*). From our experience, lidocaine and prilocaine are effective local anesthetic with no toxicity to oral epithelial tissues (*26*). LH and PH molecules were used in the study because they have higher hydrophilicity than their base form (lidocain and prilocaine). Both LH and PH can be easily incorporated into RNG to obtained LH-RNG and PH-RNG because the nanogel also possess high hydrophilicity. Moreover, many researches have shown that the desirable features of the nanogels have high loading capacity for hydrophilic therapeutics, and their network protects the encapsulated drug molecules against degradation as enzymes cannot penetrate into the particles (*33-35*). Moreover, nanogel also show high mucoadhesive property (*23*). The commercial dental anesthetic gels that are most commonly used can be divided into 2 groups, one is low drug content and another is high drug content gels. The low drug content gels usually contain 5% LH. For the high drug content group, the gels contain 20% benzocaine and a combination of 14% benzocaine, 2% butamben, and 2% tetracaine hydrochloride are generally used. From the best of our knowledge, there is no 20% LH or PH gel available in the market. Therefore, the commercial products containing 5% LH was used as a positive control for the low drug content group. For the high drug content group, as there is no commercial anesthetic gels containing 20% LH or PH, the gels with other anesthetics having the same drug concentration of 20% were used as positive controls to the main aim of pain reduction and satisfaction of volunteers.

Evaluation of pain is one of the most difficult challenges for researchers. Many measurement tools, including color scales, pain thermometers, VAS, NRS, and WPS have been developed to elicit self-reports of pain from volunteers (*27,28,36*). In the present study, VAS, NRS, and WSP have been selected for evaluation of the favorable results of LH-RNG and PH-RNG on pain reduction. From VAS and NRS, anesthetic gel groups showed significantly less pain than placebo group after needle insertion into buccal mucosa. Both developed RNG could reduce pain in all concentrations. The 5% anesthetic RNG was comparable to commercial product, whereas 20% anesthetic RNG presented superior results over the two commercial gels. Confirming with the VAS ratio analysis, the developed 20% anesthetic gels significantly exhibited the better pain reduction results than the commercial products, especially PH-RNG. However, no significant differences in efficacy between lidocaine and prilocaine for both concentrations was found. The WPS evaluation of patient's satisfaction demonstrate that the anesthetic gels are more preferable than placebo, especially those gels with the higher concentration of anesthesia. Taken together, it can be concluded that the developed anesthetic gels can potentially reduce pain from needle injection in oral cavity. The patient's satisfaction will reflect the attitude of dental procedures which injection is needed. The efficacy of both anesthetic rice gels will not only reduce the pain from injection, but also can reduce patient's dental fear.

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