Journal of Oral Biology and Craniofacial Research 13 (2023) 791–795

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Encapsulation of Lidocaine nanoparticles in Gadus morhua derived lipoic acid

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ARTICLE INFO

Keywords:
Local anesthetics
Liposomal encapsulation
Pharmacokinetics

ABSTRACT

Local Anesthetics are used clinically for anesthesia and analgesia either following surgery or for management of acute and chronic pain conditions. Liposomal Encapsulation aids in improved delivery at the tissue level. This paper deals with formulation and characterization of Gadus morhua derived liposome encapsulated Lidocaine nanoparticles.

Materials and methods: Water Soluble liposomes were synthesized and encapsulated to lidocaine. The prepared liposomes were assessed using field emission scanning electron microscope, TEM, FTIR, Zetapotential, Anti-inflammatory property and Drug release kinetics.

Results: The structural and morphological characters of the conjugated liposomes were studied using SEM & TEM, surface charge Zetapotential. The cumulative drug release was studied for up to 72 h in which more than 70 % of the drug was released from the Liposomal nanoparticles. FTIR revealed similar functional groups like the control. Stability of the drug was superior than the control.

Conclusion: Liposomal conjugation delays the drug release which can be used in slow release applications. Improving the drug release kinetics can be advantageous in many chronic pain conditions. Additionally, the changes in the functional groups can also aid in reduction or masking of bitterness.

1. Introduction

Local anesthesia are crucial in pain management in oral and maxillofacial surgery (LA). Perfect pain control is necessary for the healing process to go on without any hindrance and produce a satisfactory outcome.[1] Local anesthetics effectively stop nociceptive afferents from transmitting pain. Direct application of local anesthetics exerts their action by nerve depolarization blocking the inward Na + current at the sodium ionophore. In addition to blocking Na + channels, LAs also block Ca2+, K+[2] and other ligand-gated receptors as well as transient receptor potential vanilloid-1 receptors.[3] The connection between certain G proteins and the corresponding receptors is also broken down by local anesthetics. LAs have strong anti-inflammatory effects through this process, especially on neutrophil priming reactions.[4] LAs that are currently available have wide range of variations in terms of onset, duration, and applications. The biggest challenge till date is that there is no single local anesthetic that is advantageous in all ideal requisites needed. Therefore, creation of a long-acting LAs with predictable onset, delivery and duration of action is the need of the hour.[5]

Lidocaine is one of the most widely used and well-known local anesthetic agents. It can be used for epidural or spinal blocking, local or regional anesthesia, and a variety of other uses in anesthesia and pain management. It is also administered parenterally to treat neuropathic pain conditions.[6] Limitations of Lidocaine include reduced

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https://doi.org/10.1016/j.jobcr.2023.10.005
Received 24 May 2023; Received in revised form 13 August 2023; Accepted 16 October 2023

bioavailability and liver first pass effect which necessitates frequent small doses of lidocaine and increased blood concentration. Urge to create a sustained release version with limited dosage have been attempted with liposomes, implants and microspheres. However such attempts are still in research and have not been translated, thus compelling to attempt newer formulations. Modifications of the local anesthetics to derive improved characteristics is vital as they belong to the most important group of drugs in dentistry.

Liposomes are often studied as drug carriers to improve the delivery of therapeutic drugs to specific areas of the body since they are non-immunogenic, biodegradable, and non-toxic. Liposomes are phospholipid bilayers, composed of phospholipids, fatty acids, and other compounds. Because of their amphiphilic properties, liposomes are advantageous as carriers for medications, vaccinations, and macromolecular treatments for sickness, cosmetics, diagnosis, and immunization.[7] To distribute drugs, liposomes encase both hydrophilic and hydrophobic compounds. Due to concentration gradients between the internal and external "nanocage," drugs that are encapsulated with channel proteins are successfully protected from early breakdown by proteolytic enzymes and are able to diffuse via the channel. Numerous forms of liposomes can be produced depending on the number of lipid layers, size, surface charge, lipid content, and vesicle manufacturing methods. In liposome-based devices, the encapsulated medication releases from these lipid bilayers at a slower rate, which improves pharmacological potency.[8,9]

Cod liver oil has been in use as a nutritional supplement from time immemorial. Cod fish (Gadus Morhua) liver is the common source from which the lipoic acids are extracted. The oil is abundant in lipoic acids omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The present study aims to formulate liposome encapsulated Lidocaine and to assess the characteristics for improved performance and therapeutic action.

2. Materials and methods

2.1. Preparation of liposome-encapsulated lidocaine nanoparticles

Preparation of liposomes involved drying of lipids from organic solvents, dispersing the lipids in aqueous media, purifying the resultant liposome and analyzing the final product. Further lidocaine was loaded to liposomes synthesized from fresh liver oil from Gadus morrhua in the proportion of (diethyl ether: methanol) in (1:1) ratio and stirred for 2 h to allow the encapsulation of the liposomes to the local anesthetics. Water soluble liposomes that were synthesized were then studied in-vitro for drug release kinetics. The cumulative drug release percentage of the local anesthetic drug encapsulated with liposome was studied for up to 72 h.

2.2. Material characterization

2.2.1. Surface and functional characterization

Hydrodynamic size distribution and surface charge (ζ-potential, −20.0 mv) of encapsulated lidocaine nanoparticles were done with Horiba, Japan. Scanning electron microscopy (FE-SEM, JSMIT800) and FEI Tecnai G 20 S-TWIN TEM were used to investigate the morphology and shape. FTIR spectra was done with Bruker, United States, were recorded in the range of 4000 to 400 cm^{-1}.

2.2.2. In vitro anti-inflammatory studies

2.2.2.1. Nitric oxide (NO) scavenging activity. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH was measured by Griess reaction (Marcocci et al., 1994). The reaction mixture (3 ml) containing sodium nitroprusside (10 mm) in phosphate buffer saline and the test extract (10, 25, 50 and 100 μg/ml) was incubated at 25 deg C for 150 min, after incubation 1.5 ml of the reaction mixture was removed and 1.5 ml of the Griess reagent (1 % sulphanilamide, 2 % orthophosphoric acid and 0.1 % Napthylethyline diamine hydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. Percent inhibition of nitric oxide scavenging was calculated using the formula.

$$\text{Percentage Inhibition} = (\text{A of Control} - \text{A of Sample})/\text{A of Control} \times 100.\text{A-absorbance.}$$

2.2.2.2. Protein denaturation inhibition assay. Inhibition of albumin denaturation was done with reference to Mizushima et al. with minor modifications 24. The reaction mixture consisted of test extracts at different concentrations and 1 % aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using a small amount of 1 N HCl. Diclofenac sodium was taken as a standard drug. The samples were incubated at 37 °C for 20 min and then heated at 57 °C for 30 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\text{Percentage Inhibition} = (\text{A of Control–A of Sample}) \,/\, \text{A of Control x100}$$

2.2.2.3. Proteinase inhibitory activity. Proteinase inhibitory activity was performed according to the modified method of Oyedepo*et al.* (1995). The reaction mixture (2 ml) contained 0.06 mg trypsin, 1 ml 20 mMTrisHCl buffer (pH 7.4) and 1 ml test sample of different concentrations. The mixture was incubated at 37 °C for 5 min. The 1 ml of 0.8 % (w/v) casein was added. The mixture was incubated for an additional 20 min. Then 2 ml of 70 % perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was read at 210 nm against the buffer as blank. The experiment was performed in triplicate. The percentage of proteinase inhibitory activity was calculated using the following formula.

$$\text{Percentage Inhibition} = (\text{A of control–A of sample}) \,/\, \text{A of control x100}$$

2.2.3. In Vitro drug release study of Liposomes loaded with anesthetic drug

Releasing profile of Liposomes loaded with anesthetic drug in PBS medium were estimated using UV–Vis spectrophotometer at 208 nm. For every 1 h time intervals the release profile of drug loaded Liposomes were taken up to 72 h and their cumulative drug release was calculated with the following equation.

$$\text{Drug releasing } (\%) = [\text{Initialconc(drug)-Finalconc(drug)} \,/\, \text{Initialconc(drug)}]\text{x } 100$$

2.2.4. HRBC membrane stabilization test

HRB membrane stabilization test was performed by the following described method proposed by Sadique et al. (1989). Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re-constituted as 10 % v/v suspension with normal saline. The reaction mixture 2 ml consists of 1 ml of test sample

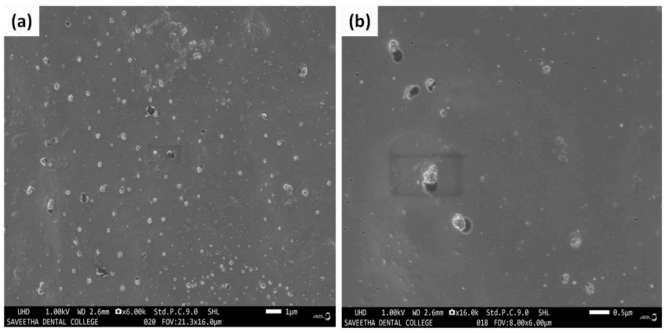

Fig: 1. a, b Scanning electron microscopy reveals particles were homogenous and uniform in size ranging 10–20 nm. Morphology was spherical shaped.

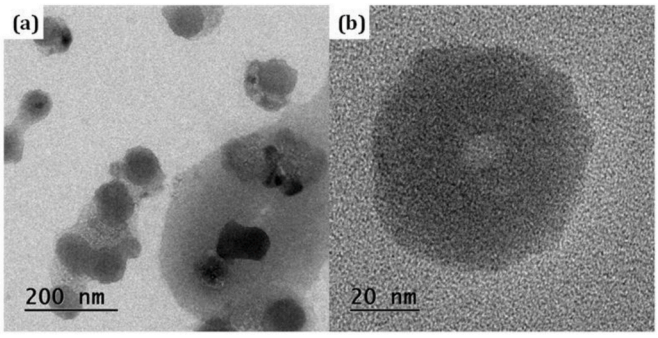

Fig: 2. a,b Transmission electron microscopy reveals uniform spherical shaped Liposomes with bilayer morphology, encapsulated with lidocaine.

solution and 1 ml of 10 % RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56 °C for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula

$$\text{Percentage Inhibition} = (\text{A of Control–A of Sample}) / \text{A of Control x100}$$

3. Results & discussion

3.1. Surface and functional characterization

Morphological assessment with SEM revealed particles were homogenous and uniform in size ranging 10–20 nm [Fig. 1 a,b]. Further TEM images also confirmed SEM findings of particle size. TEM also revealed a bilayer morphology similar to the findings reported by Franz-Montan et al. 2013.[10] [Fig. 2 a,b]. Their study characterized liposomal-lidocaine for application on oral mucosa as a topical anesthetic which aided in effective in vitro permeation and in vivo anesthetic efficacy than commercial lidocaine. Surface charge ζ-potential, of encapsulated lidocaine nanoparticles exhibited peak value at −20.0 mv and electrophoretic mobility at −0.000155 cm2/Vs. Negative charge, indicate enhanced interaction and activity. Values were similar to the untreated Lidocaine. [Fig. 3 a,b].

FTIR spectra of LA and L-LA showed strong peak at 1650 cm^{-1} representing the carbonyl group stretching of the amide group. The FTIR spectrum of the lidocaine hydrochloride illustrated two sharp bands materialized at the range 1450–1550 cm^{-1} attributable to C–N stretching where the one with superior energy as a result of the bond with elevated inductive outcome (O–C–N). Conversely, there was only one broad peak at the same range which is supporting the equivalency of the two C–N bonds in lidocaine base. [Fig. 4]. Shows the spectrum of the physical mixtures of PEG and either lidocaine base or lidocaine hydrochloride in a ratio of 1:1 correspondingly, where a broad peak emerges at the range 1650–1715 cm^{-1} because of abridgment of the two –COO groups stretching of the polymer and the LA drug. This signifies that there was no interface among the drugs and the polymer when they are physically mixed. Nevertheless, in the case of the evaporated powders having the same ratio of LA and the polymer, there was an upper field shift in the outline peak representing an interface took place among the drug and the polymer coating of LA. Difference in functional groups between LA and L-LA could also serve to mask bitterness which is an inherent characteristic of LA.

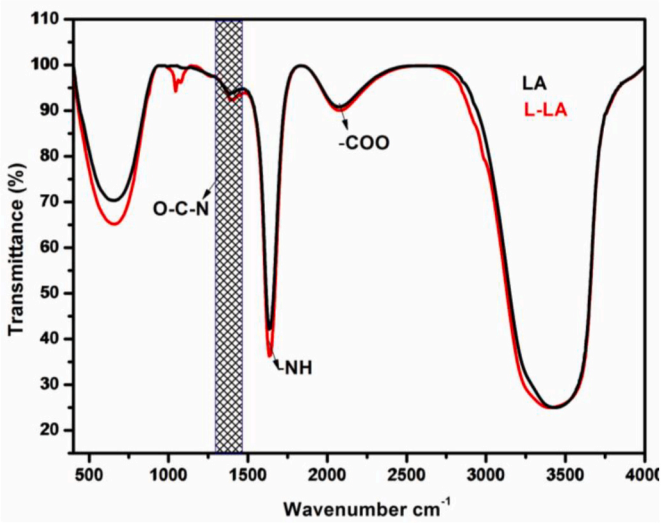

Fig: 4. FTIR spectra of LA and L-LA.

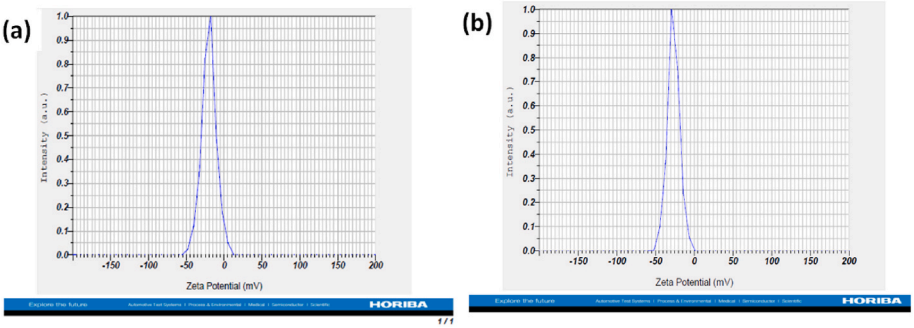

Fig: 3. a Surface charge ζ-potential, of encapsulated lidocaine nanoparticles b) Surface charge ζ-potential, of encapsulated lidocaine.

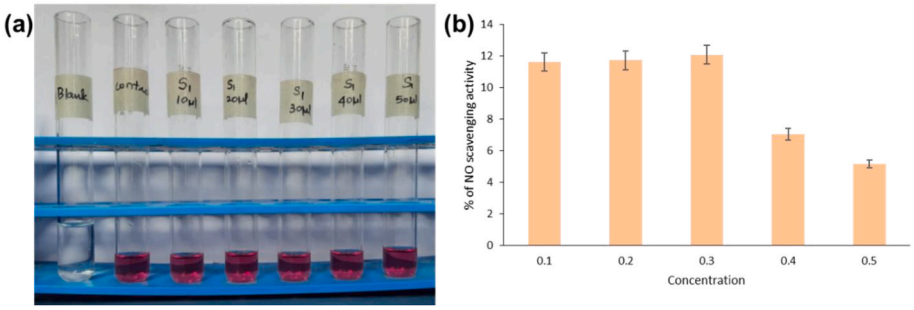

Fig: 5. a,b Nitric oxide scavenging assay.

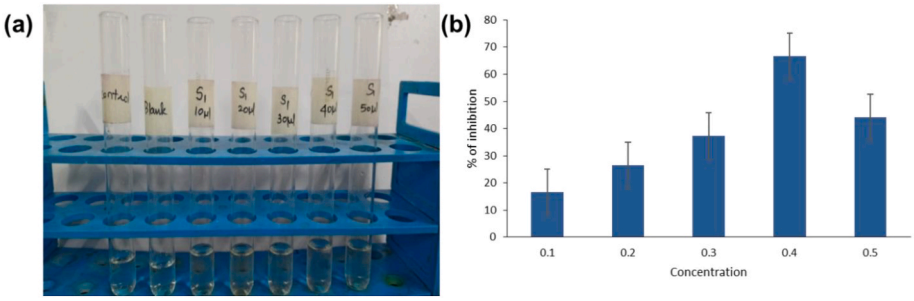

Fig: 6. a,b Protein denaturation.

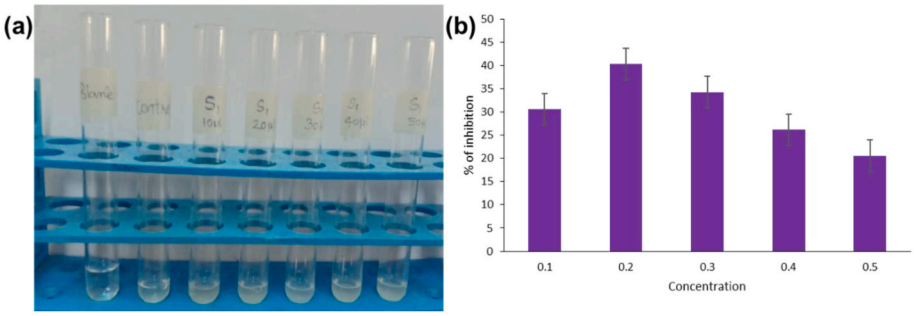

Fig: 7. a,b Protease inhibition.

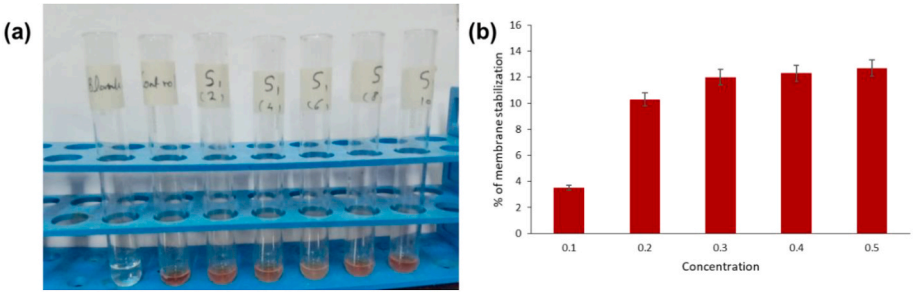

Fig: 8. a,b Membrane stabilization.

3.2. In Vitro anti-inflammatory studies

Nitric oxide scavenging assay revealed increase in concentration of NO activity suggestive of profound anti-inflammatory effect. [Fig. 5 a, b]. Protein denaturation was observed at lower concentration and is found to increase at 68ul indicating increase in ability to denature proteins. [Fig. 6 a,b]. Protease inhibition was evidenced at lower concentration of 20ul and is found to be varied with different concentration and decreases with increasing concentration. [Fig. 7 a,b]. Membrane stabilization of RBC's was observed only with higher concentration. [Fig. 8 a,b].

3.3. In Vitro drug release study of Liposomes loaded with anesthetic drug

The releasing profile of Liposomes loaded with anesthetic drug in PBS medium were estimated using UV–Vis spectrophotometer at 208 nm. For every 1 h time intervals the release profile of drug loaded Liposomes were taken up to 72 h and their cumulative drug release was

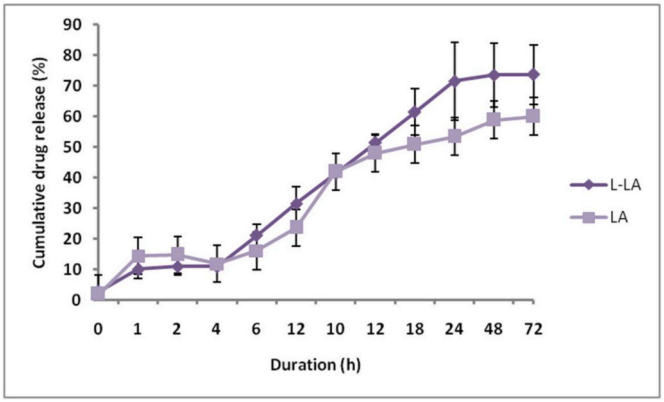

Fig. 9. Cumulative linear release of L-LA & LA.

calculated with the following equation. [Fig. 9 a,b].

$$\text{Drug releasing } (\%) = [\text{Initialconc(drug)-Finalconc(drug)} / \text{Initialconc(drug)}]\text{x } 100$$

After analyzing the cumulative drug release percentage from the drug kinetic test it shows that for 48–72 h, nearly more than 70 % of the drug was released. This suggests that the drug release is delayed by liposomal conjugation, which is advantageous in slow release applications.

4. Conclusion

Extension of duration of local anesthetics is best achieved by structural modifications. Gadus morhua derived lipoic acids exhibit promising results in terms of structural characteristics, anti-inflammatory property and sustained release. Most interesting and potential finding is the possibility to mask bitterness, which can make it an ideal agent for topical application. Novel modifications such as these offer a multitude of benefits which need to be validated further for translational utility.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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