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ORIGINAL ARTICLE

Local sustained delivery of bupivacaine HCl from a new castor oil-based nanoemulsion system

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Abstract
Bupivacaine HCl (1-butyl-2′,6′-pipecoloxylidide hydrochloride), an amide local anesthetic compound, is a local anesthetic drug utilized for intraoperative local anesthesia, post-operative analgesia and in the treatment of chronic pain. However, its utility is limited by the relative short duration of analgesia after local administration (approximately 9 h after direct injection) and risk for side effects. This work is aimed to develop a nanoemulsion of bupivacaine HCl with sustained local anesthetics release kinetics for improved pain management, by exhibiting extended analgesic action and providing reduced peak levels in the circulation to minimize side effects. Herein, biodegradable oils were evaluated for use in nanoemulsions to enable sustained release kinetics of bupivacaine HCl. Only with castor oil, a clear and stable nanoemulsion was obtained without the occurrence of phase separation over a period of 3 months. High loading of bupivacaine HCl into the castor oil-based nanoemulsion system was achieved with about 98% entrapment efficiency and the resulting formulation showed high stability under stress conditions (accelerated stability test) regarding changes in visual appearance, drug content, and droplet size. We show herein that the in vitro release and in vivo pharmacokinetic profiles as well as pharmacodynamic outcome (pain relief test) after subcutaneous administration in rats correlate well and clearly demonstrate the prolonged release and extended duration of activity of our novel nanoformulation. In addition, the lower C_{max} value achieved in the blood compartment suggests the possibility that the risk for systemic side effects is reduced. We conclude that castor oil-based nanomulsion represents an attractive pain treatment possibility to achieve prolonged local action of bupivacaine HCl.

Keywords Bupivacaine HCl · Amide local anesthetic · Nanoemulsion · Castor oil · Local analgesic · Cardiotoxic · Prolonged release

Introduction

Post-operative pain is a distressing feature and a challenge. Most patients suffer from pain, varying from mild to severe shortly after surgery, especially after complicated surgery. In addition to the direct narcotic benefits, pain relief has been shown to improve cardiac, respiratory, and gastrointestinal functions, thus improving patient survival [1–4]. Classically, opioids have been used to treat moderate to severe pain. However, due to the occurrence of adverse effects such as respiratory depression, drowsiness, nausea, vomiting, pruritus, urinary retention, and constipation, opioids are slowly left unused.

More recently, the application of local anesthetic drugs to provide post-surgical analgesia has become popular and implemented widely in the clinic [5, 6]. Bupivacaine HCl (1-butyl-2′,6′-pipecoloxylidide hydrochloride), an amide local anesthetic compound, is one of the most interesting local anesthetic drugs utilized for intraoperative local anesthesia, post-operative analgesia and in the treatment of chronic pain [5, 6]. The effectiveness of bupivacaine has been demonstrated by its favorable toxicity to potency ratio, latency of onset, degree of

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sympathetic, sensory and motor block, duration of analgesia, and regression time [6, 7]. Despite its potential promise for achieving pain relief, its anesthetic action after parenteral administration is not sustained enough in cases where the pain has become chronic and its use is then associated with an unsatisfactory clinical outcome. In addition, like the common local anesthetics, bupivacaine HCl demonstrates toxic effects on the heart and peripheral blood vessels due to unfavorable blood pharmacokinetics after local injection. Amide local anesthetics such as bupivacaine block the fast sodium channels in the fast-conducting tissue of Purkinje fibers and ventricles leading to a decrease of the rate of depolarization, the effective refractory period, and the duration of the action potential [8]. Therefore, the development of controlled release and sustained delivery formulations to overcome these problems associated with the current protocols for the local administration of anesthetics is of great interest.

This work is aimed to develop a nanoemulsion of bupivacaine HCl with sustained local anesthetics release kinetics for improved pain management, by exhibiting extended analgesic action and providing reduced peak level in the circulation to minimize side effects. Herein, biodegradable oils were evaluated for use in lipid-based nanoemulsion to enable sustained release kinetic of bupivacaine HCl. Castor oil is found to be the most effective oil to control the diffusion rate of the drug. We hypothesized that the surfactant properties of such oil may allow manipulation of the water-oil distribution coefficient of bupivacaine HCl and therefore of its local release kinetic. Castor oil (also known as Ricinus oil) is a vegetable, long-chain triglyceride oil extracted by mechanical cold pressing of the castor seed and slight refining/adulteration. Because of its surfactant properties, castor oil is also used in certain oral (e.g., vitamins) and parenteral formulations of drugs (e.g., cyclosporin A, phytonadione, tacrolimus, carbamazepine and steroids) [9–12].

To assess the prolonged duration of action of our novel bupivacaine nanoemulsion system, the animal response to electrical shock was observed repeatedly over a period of 24 h.

Materials and methods

Materials

Bupivacaine HCl was kindly provided by Dexa Medica Pharma Company (Cikarang, Indonesia), bupivacaine HCl US reference standard was purchased from USP (Roskville, USA), castor oil and PEG 400 were commercially obtained from Bratachem (Bandung, Indonesia), Tween 20 was purchased from Croda Indonesia (Jababeka, Indonesia). Other chemicals and ingredients used in the formulation and analysis were of pro analysis and pharmaceutical grade, respectively.

Animals

Pathogen-free male Wistar rats (6–8 weeks, 150–200 g) and Webster mice (8 weeks, 30–40 g) (School of Pharmacy, Bandung Institute of Technology, Indonesia) were group-housed at the animal house of School of Pharmacy ITB, with sufficient daily food intake and unrestricted access to tap water. The animals were allowed to acclimate for 7 days before the experiments. Care of and experimentation with animals were performed in accordance with institutional guidelines under protocols approved by the Local Institutional Animal Care and Use Committee, number 1179/SK/I1.B03/KP/2015.

Preparation of bupivacaine HCl-loaded nanoemulsion (B-loaded NE)

Previously, we have developed an aqueous-based nanoemulsion formulation to encapsulate the lipophilic drug curcumin [13]. Using the same surfactant and lipid composition, we modified the lipid-based nanoemulsion system with different types of biodegradable oil. To select the most appropriate oil, formulations with and without co-surfactant PEG 400 were investigated. Briefly, a mixture of surfactant and distilled water with or without PEG 400 was prepared. The mixture was stirred gently until a clear dispersion was formed. Then, oil was added dropwise under constant stirring to produce a homogeneous preparation. The formulation was inspected visually. Further modification was performed at different temperatures using the most suitable oil (Table 1). The indicated temperature was kept constant during preparation followed by cooling down at room temperature after a homogeneous clear dispersion system was formed. Bupivacaine HCl loaded in the lipid-based nanoemulsion was performed using the same procedure to form a clear nanoemulsion dispersion. To determine the maximum loading capacity, various amounts of bupivacaine HCl were incorporated in the optimized nanoemulsion formulation (Table 2) via the aqueous phase prior to oil titration.

Physical characterization of nanoemulsion system

Droplet size and surface charge determination

As photon correlation spectroscopy cannot be applied to lipid-based nanoemulsions, the droplet size was determined manually from cryo-transmission electron microscopic digital images. A minimum of 50 droplets were selected randomly and the mean diameter was measured using computer software (Microsoft® PowerPoint® for Mac 2011). Further, the exact droplet size was calculated using the scale bar on the images. The droplet size was expressed as the average size of at least 50 droplets. The surface charge reflected by the zeta potential value was determined using the electrophoretic light scattering

Table 1 The influence of type of oil, co-surfactant, and temperature on the physical appearance of lipid-based nanoemulsions

Formula	Oil	Tween 20	PEG 400	Aquadest	Temperature (°C)	Physical appearance
F-1	Olive	8	–	2	25	Turbid
F-2	Soya	8	–	2	25	Turbid
F-3	Sunflower	8	–	2	25	Turbid
F-4	Castor	8	–	2	25	Clear
F-5	Olive	8	1	1	25	Turbid
F-6	Soya	8	1	1	25	Turbid
F-7	Sunflower	8	1	1	25	Turbid
F-8	Castor	8	1	1	25	Clear
F-9	Castor	8	1	1	40	Clear
F-10	Castor	8	1	1	60	Clear
F-11	Castor	8	1	1	80	Clear

A mixture of surfactant and distilled water was prepared in the absence or presence of PEG 400. The mixture was stirred gently at constant temperature indicated in the table until a clear dispersion system was formed. Then, oil was added dropwise while stirring constantly and subsequently a homogeneous preparation was produced. Visual inspection of physical appearance was used to assess turbidity

method (Delsa™Nano C Zeta Potential Analyzer, Beckman Coulter, USA). Measurements were performed in triplicate at 25 °C.

Microscopic analysis

The morphology of blank NE (F-9) and B-loaded NE (F-9-3) was studied using cryo-transmission electron microscope (Cryo-TEM, JEM 1400, JEOL, Tokyo, Japan). About 10 mL of sample was dropped on the specimen place and covered with a 400 mesh grid. After 1 min, 10 mL of uranyl acetate was dropped on top of the grid, and the sample was allowed to dry for 30 min before observation under the electron microscope.

Viscosity and rheological analysis

To determine the flow characteristics of the formulation (important for parenteral injection), both viscosity and rheology of the preparation were analyzed. The viscosity and rheology of blank NE (F-9) were measured using a Brookfield Rotational Digital Rheometer model DV-II+ (Middleboro, MA, USA). The viscosity of a 250-mL sample was measured at constant temperature of 22 °C, using spindle number 1 at a rotation speed of 10 rpm. The rheology property was investigated using similar volume and spindle number as used in the viscosity test. The shear stress (viscosity) of the sample was measured as a function of shear rate (rotational speed) at a constant temperature (± 22 °C). The measurements were carried out with increasing (forward measurement) and decreasing (backward measurement) shear rates. A shear rate at a range of 0.5–10 rpm was used. Both viscosity and rheology measurements were performed in triplicate.

pH determination

The pH value of B-loaded NE was determined using a HI9321 pH meter (Hanna Instruments Inc., Michigan, USA). The measurement was performed at 25 °C by direct immersion of a glass electrode into the sample.

Table 2 The loading of Bupivacaine HCl into the nanoemulsion based on F-9 formulation and resulting zeta potential and droplet size

Formula	Bupivacaine HCl (mg/mL)	Nanoemulsion characteristics			
		Entrapment efficiency (%, mean ± SD)	Loading capacity (mg/mL, mean ± SD)	Zeta potential (mV, mean ± SD)	Droplet size (nm, mean ± SD)
F-9	0	0	0	0.00	111.7 ± 3.11
F-9-1	5	97.3 ± 0.79	4.86 ± 0.04	+ 0.01	156.4 ± 3.41
F-9-2	10	97.4 ± 0.39	9.74 ± 0.14	+ 0.02	172.8 ± 14
F-9-3	15	97.9 ± 0.94	14.69 ± 0.04	+ 0.02	171.2 ± 18.28
F-9-4	20	89.5 ± 0.45	17.91 ± 0.09	+ 0.21	400.0 ± 22.70

Entrapment efficiency (EE) and loading capacity of bupivacaine HCl in nanoemulsion using formula F-9 were determined by a direct method. Bupivacaine HCl was determined using HPLC (Phenomenex® Luna C18 5 μ 100 Å (250 × 4.6 mm) column; mobile phase of phosphate buffer 0.05 M pH 6-acetonitrile (35:65); flow rate of 1.2 mL/min; detector UV at wavelength of 202 nm. Zeta potential value was determined using the electrophoretic light scattering method. Droplet size was an average of minimum 50 analyzed droplets measured from cryo-TEM images using computer software

Entrapment efficiency and loading capacity

The entrapment efficiency (EE) and loading capacity of bupivacaine HCl in nanoemulsion were measured by a direct method. The sample was centrifuged at 15,780×*g* for 30 min to precipitate free bupivacaine HCl. A 500-μL of supernatant was diluted with 10 mL distilled water. The content of bupivacaine HCl was measured using a HPLC (Knauer Smartline, Germany) method. Prior to HPLC analysis, the solution was filtered using a 0.45-μm disposable filter.

HPLC determination of bupivacaine HCl

A Phenomenex® Luna C18 5 μ 100 Å (250 × 4.6 mm) column was used as a static phase. A freshly prepared mobile phase of 0.05 M phosphate buffer pH:6-acetonitrile (35:65) was used at a flow rate of 1.2 mL/min. Bupivacaine HCl content was determined using an UV detector at a wavelength of 202 nm. A series of increasing concentrations bupivacaine HCl USP reference standard was used to obtain a calibration curve.

In vitro release study

The release of bupivacaine HCl from the NE samples (F-9-3) was determined against 250 mL 0.05 M phosphate buffer (pH 6) at 37 °C ± 0.5 °C, under constant gently stirring for 24 h. Two milliliters of sample was placed in a Slide-A-Lyzer® dialysis cassette 2000 MWCO (Thermo scientific, Rockford, IL, USA). A 3-mL sample was withdrawn from the receiver phosphate buffer at time intervals of 0, 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 h and an equivalent amount of fresh buffer added to keep the receiver compartment volume constant. For comparison, drug release from bupivacaine HCl without castor oil was also studied. The drug levels were determined using the HPLC method as described above. The tests were done in triplicate for each sample and the cumulative amount of bupivacaine HCl released was plotted as a function of time.

Accelerated stability test

Accelerated stability test of both B-loaded NE (F-9-3) and bupivacaine solution were conducted by investigating several parameters including phase separation, droplet size, polydispersity index, zeta potential, and bupivacaine HCl content. Samples were kept for 3 months in a climatic chamber at a humidity of 75% and temperature of 40 °C. Samples were taken monthly for analysis.

In vivo study

Pathogen-free male healthy Wistar rats were used to study the in vivo profile of bupivacaine HCl after administration of B-loaded NE. The animals were divided into two groups ($n = 6$): a group receiving 0.2 mL of 0.5% bupivacaine HCl solution and a group receiving 0.2 mL of 1.5% B-loaded NE (F-9-3). All preparations were given subcutaneously. A blood sample of 0.3 mL was collected from the tail vein at predetermined time intervals of 0.25, 0.50, 0.75, 1, 2, 3, 4, 6, 8, 12, 18, 24, and 48 h. Each blood sample was put in a 1.5-mL heparinized Eppendorf tube and immediately centrifuged at 13,610*g* for 3 min to obtain plasma. A 50-μL plasma sample was mixed with 100 μL of methanol, then vortexed for 10 s. Then, this mixture was centrifuged at 13,610*g* for 3 min to precipitate plasma proteins. Subsequently, 100 μL of supernatant was withdrawn and transferred to 1.5 mL tube and evaporated at 40 °C. Dried residue was subsequently re-dispersed in 125 μL distilled water and bupivacaine HCl content in plasma was determined by HPLC as described above. The main in vivo parameters of bupivacaine HCl (C_{max}, T_{max}, and AUC_{0-24}) were calculated using trapezoidal method plotted from 0 to 24 h.

Study the local response to electrical shock

To study the local effects of the preparations, an electrical shock was given to pathogen-free male Webster mice, as described by Gant et al [14]. The electrical device used in this experiment is presented in Fig. 1. The electrical current applied in the study was 3.161 ± 0.18 mA for 400–600 ms. The animals were divided into four groups ($n = 3$): group receiving a bupivacaine HCl solution 0.5% *W/V*, group receiving B-loaded NE 0.5% *W/V* (F-9-1), group receiving B-loaded NE 1.5% *W/V* (F-9-3), and group receiving only vehicle. All preparations (0.2 mL/animal) were given subcutaneously. The electrical shocks were given at predetermined times at 0, 0.25, 0.5, 1, 1.5, 2, 3, 6, 9, 12, 18, and 24 h.

Statistical analysis

All data are presented as mean ± SD. Statistical analysis using two-tailed distribution Student *t* test was conducted for in vitro and in vivo studies. A p value of < 0.05 was considered as statistically significant.

Results

A novel self-assembly lipid-based nanoemulsion system was established with low energy input using the changes in the spontaneous curvature of the surfactant (tween 20). The physical appearance and the characteristics of the lipid-based

Fig. 1 The device for electrical shock study. The device was set up to deliver an average electric current of 3.161 ± 0.18 mA, applied to each mouse for 400–600 ms

nanoemulsion are presented in Fig. 2 and Table 1. A number of factors related to the preparation conditions are critical in the spontaneous production of these lipid-based nanoemulsions: type of oil, the ratio of oil/tween 20/water, temperature and the presence of co-surfactant (PEG 400).

Influence of oil

The choice of oil will impact the formation and stability of lipid-based nanoemulsion by effects on several parameters such as viscosity, interfacial tension, interfacial flexibility,

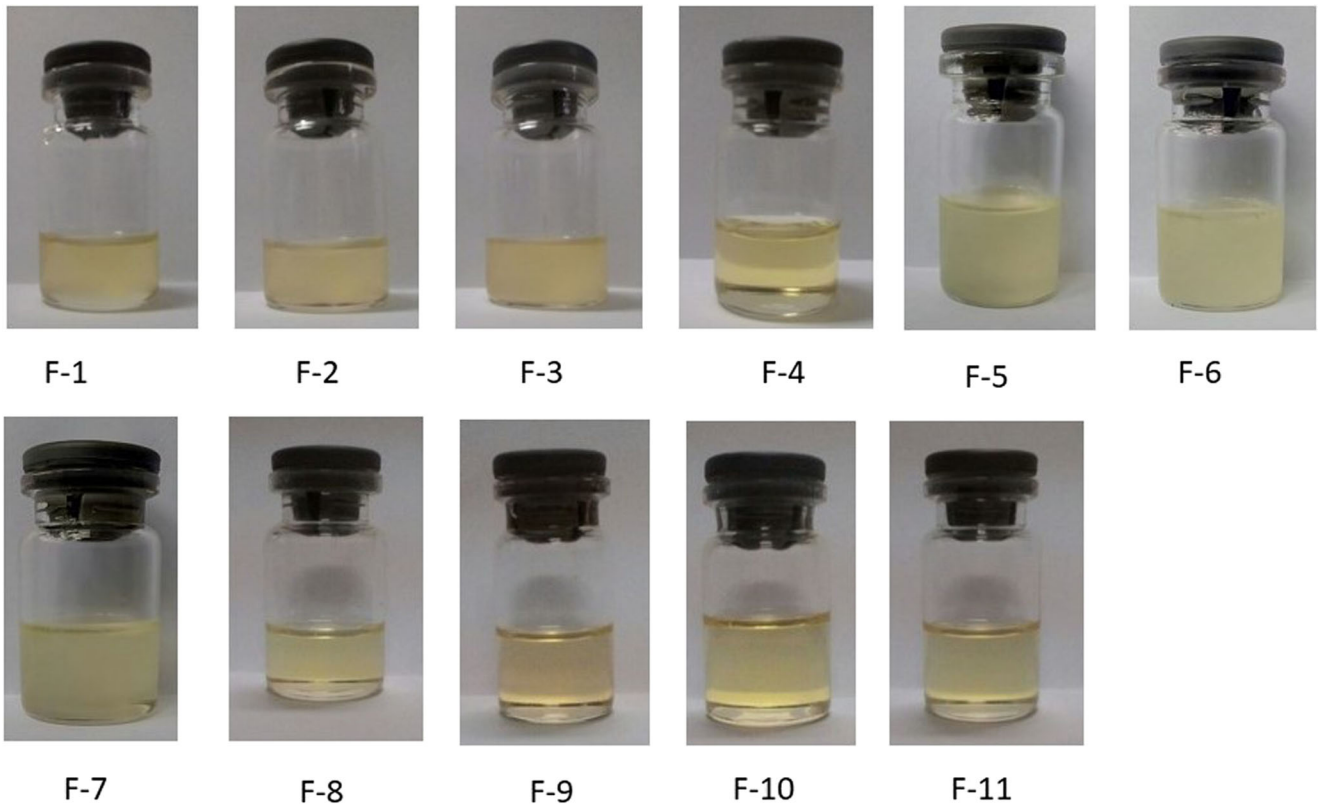

Fig. 2 Visual appearance of blank nanoemulsions using different formulas and temperature. The mixture of surfactant and distilled water with or without of PEG 400 was prepared (formulas are presented in Table 1). The mixture was stirred gently at constant temperature until a clear dispersion was formed. Then, oil was added dropwise under constant stirring until a homogenous preparation was produced

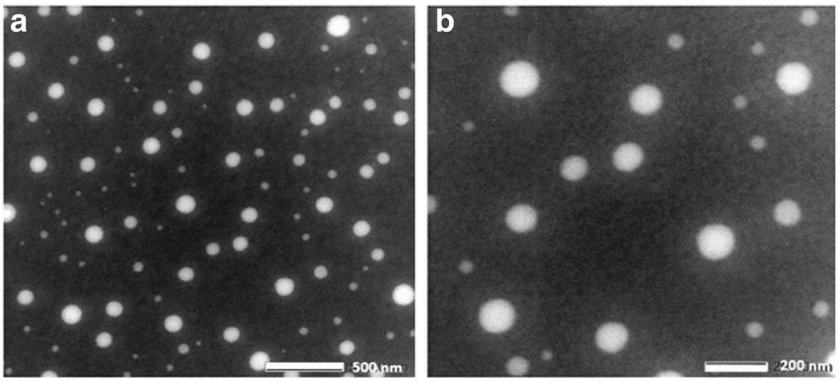

Fig. 3 Cryo-TEM presentation of blank nanoemulsion system (formula F-9) at magnification of × 12,000 (**a**) and × 20,000 (**b**)

and phase behavior, and will also influence the physical stability by differences in polarity and water-solubility of the oil molecules. In addition, the molecular structure of the oil also can play an important role in the spontaneous formation of nanoemulsions [15]. As shown in Table 1, only the use of castor oil was successful in forming a nanoemulsion system, as observed by visual inspection immediately after preparation, both in the absence (F-4) and presence of PEG 400 (F-8).

Influence of co-surfactant

The influence of the co-surfactant PEG 400 on the nanoemulsion formation is presented in Table 1. Co-surfactant was used to alter the bulk properties of the dispersion system (such as viscosity, density, refractive index, solubility, and interfacial tension) and/or change the structural properties of the surfactant solutions (such as optimum curvature, critical micelle concentration, and phase behavior) [16].

PEG 400 is likely to change the solubility and optimum curvature of Tween 20, hence lowering the interfacial tension at the water-oil interface [17]. In line with what we have reported previously [13], PEG 400, a short-chain alcohol, produced a clear dispersion when added to the formulation containing castor oil. When PEG 400 was not included in the formulation, a clear solution was also formed, but phase separation was observed during storage at room temperature.

Influence of temperature

Temperature changes commonly cause alterations in the physical stability of emulsions either inverting an emulsion or breaking it. Since the solubility of the surfactant normally shifts when the temperature increases, the physical stability of an emulsion may also change.

In low energy methods to produce nanorange droplets, the smaller droplets are formed when the system undergoes a phase inversion in response to changes in composition or temperature, and passes through a state of low interfacial tension. So, whether a turbid or clear nanoemulsion produced, it is related to droplet size. Differences in droplet size may be due to a decrease in viscosity (which facilitates the rapid movement of surfactant, oil and water molecules), a change in molecular geometry of the non-ionic surfactants used, an increase in oil-solubility of the non-ionic surfactant, and/or a decrease in interfacial tension when the phase inversion temperature (PIT) is reached. The effect of temperature on the formation of our self-assembly nanoemulsion was investigated with the formula using castor oil (F-4, Fig. 2, Table 1). PIT resulting in nanoemulsion systems was reached at 25 °C (F-8) and 40 °C (F-9). Above 40 °C, the system remained clear, but a minor color alteration was observed indicating heat-induced chemical degradation, likely of Tween-20 as this surfactant is known to be sensitive to higher temperatures [18].

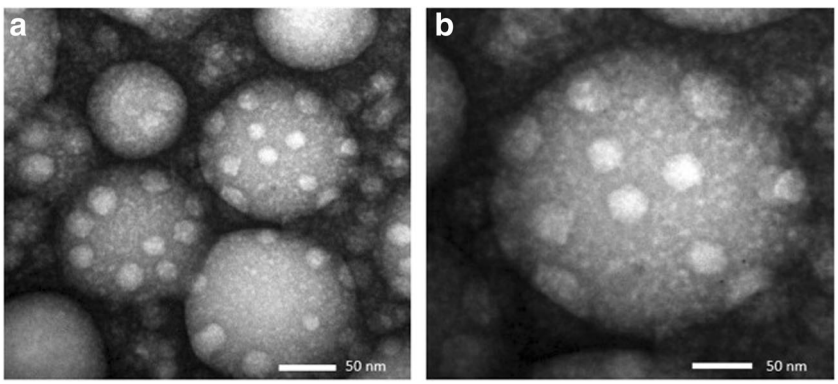

Fig. 4 Cryo-TEM presentation of B-loaded nanoemulsion system (formula F-9-3) at magnification of × 20,000 (**a**) and × 40,000 (**b**)

Loading of bupivacaine HCl

Based on the visual inspection, formulation of F-9 was selected as the preferred one. F-9 was therefore used for subsequent experiments on the loading of bupivacaine HCl in the nanoemulsion system. Table 2 shows that the drug can be efficiently entrapped in the carrier, with formulation of F-9-3 showing the highest entrapment efficiently (around 98%) with a droplet size of about 170 nm.

Cryo-TEM analysis

Cryo-TEM was applied to study the morphology of nanoemulsion formulation of F-9 and the B-loaded nanoemulsion based on F-9-3 (Figs. 3 and 4). As shown in both figures, the nanoemulsion systems were homogeneous with a narrow distribution size and nanodroplets of spherical shape. An interesting structure was formed in the B-loaded nanoemulsion, where the bupivacaine HCl were mostly located inside the droplets with a very low number in the external oil phase. This distribution pattern of bupivacaine HCl in the nanoemulsion system was suggested to play an important role in controlling the release of bupivacaine HCl: the localization of bupivacaine in the external phase facilitated the initial "burst" release followed by sustained release of the drug loaded in the droplet (Fig. 5). The size of droplets was calculated using a computer software analysis on the digital images of cryo-TEM (Table 2).

Viscosity and rheology

Parenteral administration requires comfort and easy injection via a syringe; hence, viscosity and rheology are important parameters. The nanoemulsion system with the preferred formulation (F-9-3) showed a low viscosity value of 68.74 ± 2.26 mPa•s. The nanoemulsion showed thixotropic property as presented in the rheogram curve (Fig. 6) in which the shear stress (viscosity) is expressed as function of the shear rate (rotational speed). The presence of the hysteresis loop is due to the decrease of the fluid's viscosity during shearing.

The thixotropic property plays an integral role in determining the therapeutic efficacy of pharmaceutical formulations through facilitating a prolonged retention time at the injection site. A non-Newtonian thixotropic system can become plastic under pressure, which is of strong interest for the clinic as applying pressure to a syringe for injection will liquefy the product and let it pass through a needle without losing its structure. An ideal thixotropic liquid should have high consistency during storage, yet enable removal easily.

Accelerated stability test

The stability of the B-loaded nanoemulsion (F-9-3) regarding changes in drug loading, visual appearance and zeta potential during 3 months of storage under the stress condition of a climatic chamber (75% humidity, 40 °C) are presented in Table 3. No phase separation, loss in drug encapsulation

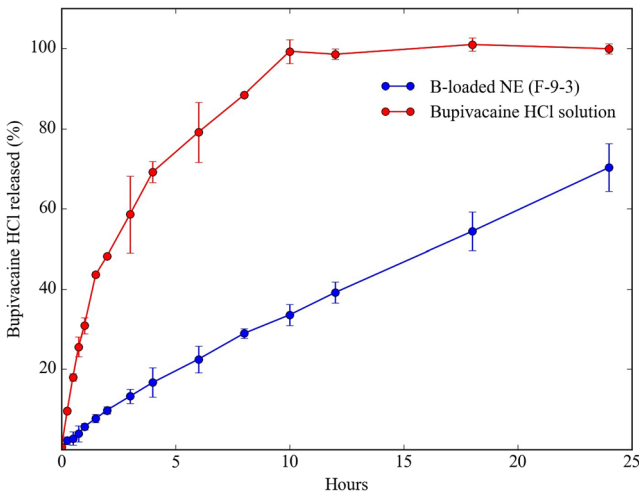

Fig. 5 In vitro release profile of bupivacaine HCl from a conventional formula (red line) and nanoemulsion system (formula F-9-3, blue line). A comparison release profile of bupivacaine HCl in the surfactant/co-surfactant solution and in nanoemulsion system was performed using a dialysis bag method with 2000 Da cut off. A 3-mL sample was withdrawn from the receiver medium at 0, 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 h. The release profile of both samples showed significant different ($p < 0.05$)

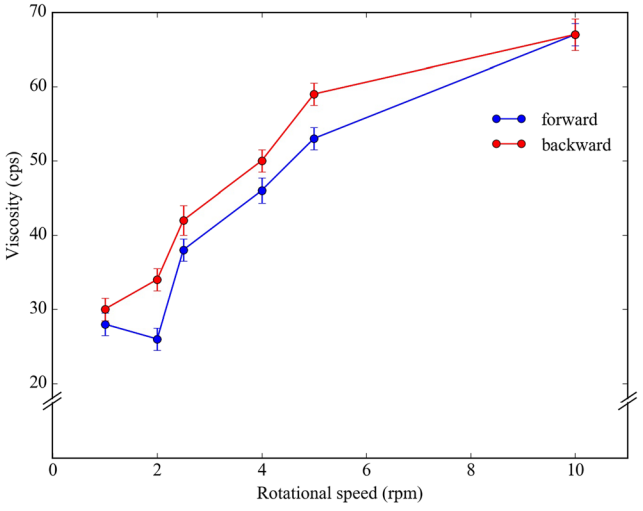

Fig. 6 The rheology property of B-loaded nanoemulsion system (formula F-9). The rheology of 250 mL sample was measured at constant temperature (22 °C) using spindle number 1. The shear stress (viscosity) of the sample was measured as a function of shear rate (rotational speed). The measurements ($n = 3$) were carried out with increasing (forward measurement, blue line) and decreasing (backward measurement, red line) shear rates. The used shear rate range was 0.5–10 rpm

Table 3 Stability of the B-loaded nanoemulsion based on F-9-3 formulation during storage under stress conditions of 75% humidity and 40 °C for 3 months

Sampling time (week)	Bupivacaine content (%, mean ± SD)	Visual appearance	Zeta potential (mV)
0	98.30 ± 0.74	Homogenous-clear	+ 0.02
2	97.70 ± 0.30	Homogenous-clear	+ 0.02
4	99.52 ± 0.14	Homogenous-clear	+ 0.04
6	95.69 ± 0.11	Homogenous-clear	+ 0.03
8	96.49 ± 0.25	Homogenous-clear	+ 0.03
12	96.10 ± 0.36	Homogenous-clear	+ 0.04

Bupivacaine HCl content was determined using HPLC (Phenomenex® Luna C18 5 μ 100 Å (250 × 4.6 mm) column; mobile phase of phosphate buffer 0.05 M pH 6-acetonitrile (35:65); flow rate of 1.2 mL/min; detector UV at wavelength of 202 nm. The zeta potential value was determined using the electrophoretic light scattering method. The experiment was done in triplicate

efficiency, drug precipitation, alterations in surface charge and color were detected after a 3-month observation period.

In vitro drug release

Characterizing in vitro drug release profile from nanoemulsion system is a challenging task because of the submicron size of the droplets and the difficulty in separating the continuous and dispersed phases. Several experimental techniques such as the dialysis bag method, diffusion cell method, centrifugal ultrafiltration, ultrafiltration at low pressure, and continuous and in situ flow methods have been used to measure the release of drug from liquid preparations.

Comparative release profiles of bupivacaine HCl in case of the surfactant/co-surfactant mixture and B-loaded nanoemulsion system (F-9-3) were obtained using a dialysis bag method with 2000 Da cut off (Fig. 5). An obvious difference in release rate exists between the conventional and prolonged release formulations. A burst release was observed in case of bupivacaine HCl in the reference surfactant/co-surfactant mixture, 100% drug release was reached after 10 h of dialysis. In case of the castor oil enabled nanoemulsion, drug release was much slower and sustained, with linear release kinetics up to a level of 70% drug release at the 24 h of observation time point.

In vivo drug profile after subcutaneous administration

In line with the outcome of the in vitro release experiment, the castor oil-based nanoemulsion sustained the release of bupivacaine HCl in vivo up to 24 h after subcutaneous administration into rats (Fig. 7). The main pharmacokinetic parameters of both preparations were presented in Tables 4 and 5. As indicated in the table, rapid systemic absorption of bupivacaine HCl from the reference bupivacaine HCl formulation was observed, with a C_{max} value of about 250 ng/mL at 0.5 h after administration and complete disappearance from the circulation at 8 h after administration. In comparison, the nanoemulsion system showed a reduced rate of systemic absorption with a lower C_{max} value of 135 ng/mL at 1 h after administration and sustained blood levels up to 24 h.

Local pain relief response test

A simple electrical device (Fig. 1) was designed to evaluate the pharmacodynamic action of the castor oil enabled B-containing nanoemulsion in terms of pain relief after subcutaneous administration. As shown in Table 4, the onset and duration of action of the bupivacaine HCl was corresponding with the in vitro-in vivo data. The conventional formulation of bupivacaine HCl showed the fastest onset of action starting at 0.5 h and the pain relief effect lasted only up to 3 h after administration. The depot formulation containing the same concentration of 0.5% bupivacaine HCl exhibited no effect over the entire observation period, indicating that effective

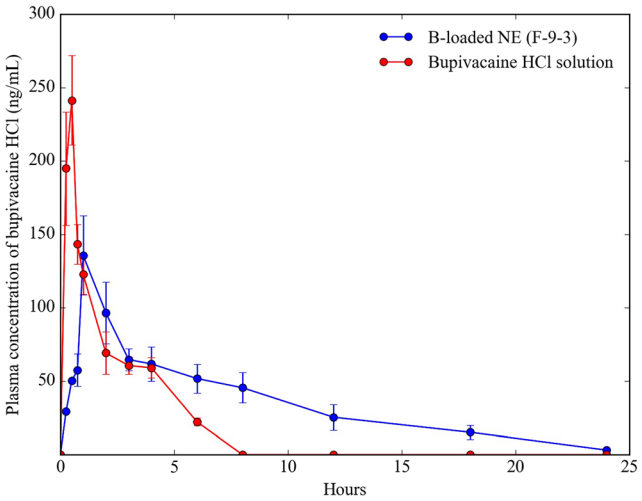

Fig. 7 The plasma concentration as a function of time of bupivacaine HCl preparations (bupivacaine HCl solution and bupivacaine HCl-loaded nanoemulsion, F-9-3) after subcutaneous injection to Wistar rat. Blood samples were collected at predetermined times: 0.25, 0.50, 0.75, 1, 2, 3, 4, 6, 8, 12, 18, 24, and 48 h. Bupivacaine HCl content was determined by HPLC. The in vivo profile of both samples showed significant different ($p < 0.05$)

Table 4 Scoring of pain relief response after subcutaneous administration of preparations containing bupivacaine HCl after an electrical shock given to pathogen-free male Webster mice

Time of observation (h)	Negative control			Bupivacaine solution (0.5%)			B-loaded NE (0.5%)			B-loaded NE (1.5%)		
	1	2	3	1	2	3	1	2	3	1	2	3
0	–	–	–	–	–	–	–	–	–	–	–	–
0.25	–	–	–	–	–	–	–	–	–	–	–	–
0.5	–	–	–	+	+	+	–	–	–	–	–	–
1	–	–	–	+	+	+	–	–	–	+	+	+
1.5	–	–	–	+	+	+	–	–	–	+	+	+
2	–	–	–	+	+	+	–	–	–	+	+	+
3	–	–	–	+	+	+	–	–	–	+	+	+
6	–	–	–	–	–	–	–	–	–	+	+	+
9	–	–	–	–	–	–	–	–	–	+	+	+
12	–	–	–	–	–	–	–	–	–	+	+	+
18	–	–	–	–	–	–	–	–	–	+	+	+
24	–	–	–	–	–	–	–	–	–	+	+	+

Four groups of animals were used ($n = 3$): negative control (group receiving only blank nanoemulsion); group receiving bupivacaine HCl solution 0.5% *W/V*; group receiving B-loaded NE 0.5% *W/V* (formula F-9-1), group receiving B-loaded NE 1.5% *W/V* (formula F-9-3). All preparations were given subcutaneously at a dose of 0.2 mL/animal. An electrical shock was applied with electrical current of 3.161 ± 0.18 mA for 400–600 ms. The electrical shock was given at predetermined time intervals after subcutaneous administration of the formulations: 0, 0.25, 0.5, 1, 1.5, 2, 3, 6, 9, 12, 18, and 24 h. The signs (−) or (+) indicate pain or no pain response, respectively

blood concentrations of the drug were not achieved. By increasing the concentration in the nanoemulsion to 1.5%, the pain relief effect started 0.5 h later when compared to the reference drug formulation but the activity was maintained much longer, up to 24 h after administration.

Discussion

Only a few reports describe the sustained release of bupivacaine HCl after injection. The only commercially available product is in the form of liposome [19]. A phospholipid bilayer with nanorange vesicle encapsulating bupivacaine HCl demonstrated depot characteristic and was claimed to maintain its effect for 72 h. In addition, administration can be directed to the site of action by irrigation or injection near the target. By this means, possible systemic toxicity can be minimized as systemic absorption is avoided and/or limited. Our report presents similar benefits, using a simple method and materials, and more importantly it is easy to do pilot scale or even production scale transfer without complicated adjustments both in the composition and process, because of simple self-formation of the nanoemulsion system with low energy input.

Bupivacaine is a potent amide local anesthetic possessing an extended effect. It targets sensory nerves more than motor nerves to produce analgesia without motor blockade [5, 6]. When prolonged blocks are used, either by continuous infusion or by repeated bolus administration, the risks of reaching a toxic plasma concentration or inducing a local neural injury must be considered. Like all local anesthetic drugs, bupivacaine may cause acute toxicity on the central nervous and cardiovascular systems if utilized for local anesthetic procedures, resulting in high blood concentrations of the drug. This is especially the case after unintentional intravascular administration. Major peripheral nerve blocks may

Table 5 The main pharmacokinetic parameters of bupivacaine HCl solution and B-loaded nanoemulsion (F-9-3) after subcutaneous administration of the preparations containing 0.5 and 1.5% of bupivacaine HCl, respectively

Preparation	Main pharmacokinetic parameters		
	T_{max} (hours)	C_{max} (ng/mL)	$AUC_{0–24}$ (ng h/mL)
Bupivacaine HCl solution (0.5%)	0.5 ± 0.0	259.3 ± 36.2	420.0 ± 16.2
B-loaded NE (F-9-3, 1.5%)	1.0 ± 0.0*	135.7 ± 26.7*	840.0 ± 94.3*

*Significantly different at $p < 0.05$ as compared to bupivacaine HCl solution 0.5%

require the administration of a large volume of local anesthetic in areas of high vascularity, often close to large vessels where there is an increased risk of intravascular injection and/or systemic absorption. This may lead to high plasma concentrations. Injection of repeated doses of bupivacaine HCl may cause significant increases in blood levels with each repeated dose due to a slow accumulation of the drug. Epidural anesthesia with any local anesthetic can cause hypotension and bradycardia which should be anticipated and appropriate precautions were taken [8].

Our report offers an attractive approach to improve the conventional mode of bupivacaine HCl release by applying a castor oil-based nanoemulsion system. The presence of small amounts of castor oil showed a significant impact to delay the initial release of bupivacaine HCl both in vitro and in vivo. The formula also reduced the systemic absorption as indicated by the plasma concentration versus time curve. The construct as depicted in Fig. 4 and also confirmed by the percentage of encapsulation explained the release pattern then the onset and duration of action. As compared to conventional immediate release formula of bupivacaine we prepared, the depot release of castor oil-based nanoemulsion droplet system reduced the systemic absorption of bupivacaine HCl significantly (Fig. 7), hence decreasing the toxicity. In this depot formulation, the rate limiting step of drug absorption is the dissolution of drug particles in the formulation or in the tissue fluid surrounding the drug formulation. Thus, drug absorption can be controlled by slow dissolution of the drug particles. The rate of drug dissolution (Q/t)d under sink conditions is defined by (Q/t)d = Sa Ds Cs/hd (I), where Sa is the surface area of the drug particles in contact with the medium; Ds the diffusion coefficient of drug molecules in the medium; Cs the saturation solubility of drug in the medium; and hd the thickness of the hydrodynamic diffusion layer surrounding each drug particle.

Conclusions

Castor oil-based nanoemulsion represents a new pain treatment possibility to achieve prolonged local action of bupivacaine HCl. At present, only few studies addressed the need for sustained release formulations of bupivacaine HCl for local application, with liposomal bupivacaine being commercially available. Though comparative release studies are still lacking, our nanoemulsion system approach promises similar patients benefits, with potential production superiority as the self-assembly of the nanoemulsion system requires only simple production technology, readily available inexpensive materials and low energy input.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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