

INVESTIGATIVE REPORT

In vitro and *In vivo* Characterization of a Novel Liposomal Butorphanol Formulation for Treatment of Pruritus

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As yet no transdermal topical formulations have been developed for the treatment of chronic itch. We developed a formulation containing 2 mg butorphanol tartrate in 100 µl purified water encapsulated into multilamellar phospholipid vesicles. Drug permeation experiments were studied with Franz diffusion chambers using human skin *in vitro* and on rat skin *in vivo*. Histological analysis of rat skins was performed to evaluate skin irritation of the formulation *in vivo*. Physical properties showed stable formulation with desirable viscosity. *In vitro* dermal penetration rate data suggest that there was significant permeation at time-points 2 h and 4 h, and a steady state was achieved afterwards to 24 h. Maximal plasma butorphanol concentration was noted at 2 h and steady state was achieved at 8 h. Visual skin assessment as well as histological analysis of excised rat skin did not demonstrate any evidence of inflammation and irritation. *In vitro* and *in vivo* analysis demonstrated release of a consistent amount of butorphanol in a sustained manner for 24 h. This liposomal transdermal delivery formulation could serve as a method to deliver butorphanol for patients with chronic pruritus. Key words: transdermal delivery; butorphanol; pruritus; liposome; kappa-opioid.

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It has been suggested recently that generalized pruritus is induced by an imbalance between the µ- and κ-opioid systems¹. κ-opioid receptor stimulation inhibits µ-receptor effects both centrally and peripherally (1–3). µ-opioid receptor agonists, such as morphine and endogenous opioid, are known to cause generalized pruritus (1, 2, 4–6). This has led to the use of µ-opioid antagonists, such

as naloxone and naltrexone, for the treatment of pruritus associated with cholestasis, uremia and dermatologic diseases. However, such µ-antagonists are associated with a number of significant side-effects and can be inconvenient to administer.

We have found that intranasal butorphanol, a commercially available κ-opioid agonist and µ-opioid antagonist, is highly effective in patients with intractable pruritus (7). While the rapid onset of action of intranasal butorphanol is more suitable for acute states of pain and itch, a transdermal delivery system would offer significant advantages by releasing butorphanol into the systemic circulation providing a steady blood level over a predictable time period. However, the skin acts as a principal barrier to efficient transdermal drug delivery. The stratum corneum plays a crucial role in barrier function for topical drug delivery by limiting the skin permeability. To overcome this, vesicular systems, such as liposomes, have been investigated to increase permeation (8).

The effectiveness of liposomal delivery on permeation depends on the liposome's physicochemical properties. Liposomal functions depend on multiple factors such as their composition and method of preparation, size, lamellar structure, charge, membrane fluidity or elasticity, and drug entrapment (9).

Given the need for medications that successfully treat severe, chronic pruritus and the profound impact that itch can have on patients' lives, we sought to develop a new transdermal formulation of butorphanol in order to improve ease of administration, provide a controlled and sustained effect, limit the side-effects, and provide an effective topical therapy for patients suffering from pruritus.

MATERIALS AND METHODS

Preparation of liposomes

Various factors were optimized to formulate the butorphanol liposome preparation, including composition and method of preparation, size, lamellar structure, charge, membrane fluidity or elasticity, and drug entrapment. For this study, 2 mg butorphanol tartrate in 100 µl purified water was encapsulated into multilamellar phospholipid vesicles. The lipid phase, consisting of a weight ratio of 2:3:5 egg yolk phosphatidylcholine, cholesterol and polyoxyethylene (POE, Brij 76) was dissolved in chloroform in a pear-shaped flask, then the solvent was evaporated to dryness in a rotary evaporator (Buchi, Buchi Co., Essen

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Germany) for 3 h until a smooth, thin lipid film was obtained on the surface of the flask. Subsequently, the dried thin film lipid was hydrated in 10.7 ml of phosphate buffered saline (PBS, pH 7.4, 154 mM NaCl, 5.6 mM Na₂HPO₄, 1mM KH₂PO₄) in a vortex mixer, then sonicated for 30 min in an ultra-sonicator (Branson 2210, Danbury, CT, USA) at 25°C, followed by 3 cycles of freezing at -70°C and thawing at room temperature according to the method of Lim et al. (10). All chemicals were from Sigma Chemical Co. (St Louis, MO, USA) and used as received. The morphology of the liposomes was observed by optical microscope (Axiovert 200M, Zeiss, Germany).

Viscosity assessment

The viscosity of the prepared liposome was measured by a viscometer (DV-II+ Pro Viscometer, Brookfield, USA) at 25°C and 37°C and a speed of 0.5 rpm, using Cannon® certified viscosity standard solution as a reference and the primary standard, water at 20°C with a viscosity of 1.0016 cP.

Skin preparations

Excised full thickness human skin from 10 subjects undergoing excisions for cosmetic purposes or excisions of free margins around tumors was used. Excised healthy skin samples were approximately 1 cm in diameter. All subjects provided written consent and the study was approved by the Wake Forest University Health Sciences Institutional Review Board.

Skin conductivities

Skin conductivities for all the skin samples were measured using Ag/AgCl electrodes and a multimeter as an indicator of the skin barrier integrity. Only skin having an initial resistivity of 30 KΩ cm² or more were used to ensure that the skin was intact according to a well-accepted criterion for selecting suitable *in vitro* skin samples (11). Skin samples with an electrical conductance between 8 and 15 micro-ampere at applied voltage of 1.0 KHz were considered useful.

In vitro skin penetration of butorphanol

Using the full-thickness human skin sample, transdermal permeation measurement of the butorphanol liposome formulation was conducted *in vitro* with vertical diffusion Franz-cells. The donor compartment was filled with a known amount of butorphanol liposome. Samples were taken from the receiver at 2, 4, 8, 12 and 24 h after the start of the permeation experiment. As a control, full-thickness human skin without butorphanol liposome was mounted on the vertical diffusion Franz-cell and samples were collected at 2, 4, 8, 12 and 24 h after the start of the experiment. The ultraviolet absorption from these samples was used as a baseline for the sample group. The samples were analyzed by ultraviolet spectrophotometer (Bionate 3, Thermo Spectronic, USA). The kinetic profile of butorphanol penetration through full-thickness human skin was determined as a function on the measured butorphanol concentration over time, based on the following equation:

$$P = 1/A \times dQ/dt$$

where: P = penetrating rate; A = area of skin; and dQ/dt = cumulative amount of drug transported into the receiver compartment at time t.

In vivo pharmacokinetic study of butorphanol liposome

Plasma drug concentration when liposomal butorphanol was applied to the skin of Female Sprague Dawley (190~210 g)

was assessed (*n* = 20). Approximately 120 µg of butorphanol in liposome formulation was applied on the shaved back of rats and left for the following time periods: 2, 4, 8, 12 and 24 h. After the designated period, blood was withdrawn from the heart and the plasma was separated by centrifugation of whole blood at 1500 rpm, 4°C for 15 min. Plasma butorphanol was assayed using high performance liquid chromatography (HPLC) with electrochemical detector (ECD) (Waters, USA, mobile phase: 1.7 mM phosphoric acid (pH 2.3), 0.15 M carbonate buffer (pH 11) supplemented with 2.7 mM ethylenediaminetetraacetic acid (EDTA) and ethyl acetate, flow rate 0.3 ml/min).

Visual assessment of inflammation

Visual assessment was made on a 5-point Likert scale for edema and erythema, ranging from none, mild, moderate, moderate-to-severe to severe, independently by 2 investigators for the application sites from 2 h to 24 h after application (12).

Histological examination of rat skin

Skin at the sites of application of liposomal butorphanol and placebo control on the back of the rats was excised and fixed in 10% formalin. Histological assays of the skin samples by haematoxylin and eosin (H&E) staining were performed to examine the index of inflammation by assessing spongiosis in the epidermis and inflammatory infiltrate in the dermis and subcutaneous fat. Biopsies were taken at the following time-points: 2, 4, 8, 12 and 24 h after application of butorphanol. For each time-point 3 rats were tested with butorphanol and one with a control application of the liposome alone without butorphanol.

RESULTS

Physico-mechanical properties of formulation

A white, silky, smooth, homogenous, stable multilamellar, structured vesicle of less than 500 nm in diameter on the average was formulated (Fig. 1). This appearance was maintained for longer than 6 months on the shelf at room temperature, indicating the physical stability of the formulation.

The viscosity of the butorphanol liposome was 993.4 cP at 25°C and 459.5 cP at 37°C, compared with the viscosity of honey at 10,000 cP (13).

In vitro and in vivo drug permeation

The permeation of butorphanol in liposome formulation through human skin *in vitro* is shown in Fig. 2. The maximum permeation of 8.56 µg/cm²/h was achieved in 2 h, followed by 7.56 µg/cm²/h in 4 h. A steady state was achieved subsequently to 24 h. The permeation of butorphanol in liposome formulation through rat skin *in vivo* is shown in Fig. 3. These data demonstrate that a consistent amount of butorphanol was released and sustained in the blood for 24 h. This indicates effective therapeutic delivery of the formulation.

Irritation

Visual assessment of both placebo and butorphanol did not show any edema or erythema. Histology of

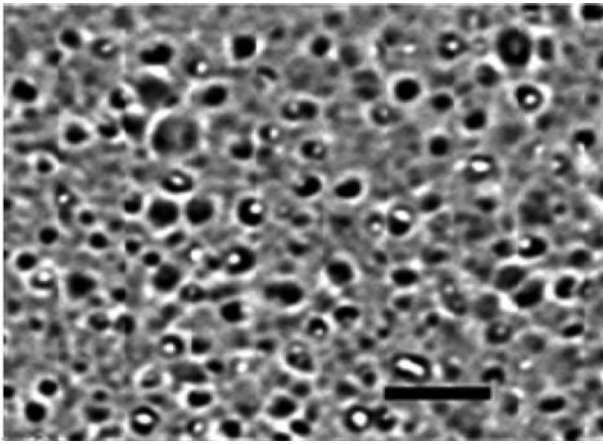


Fig. 1. Butorphanol liposomal preparation observed by optical microscope. The scale bar is 1 μm . (Axiovert 200M, Zeiss, Germany). A white, silky, smooth, homogenous, stable multilamellar, structured vesicle less than 500 nm in size was formulated. This appearance was maintained for longer than 6 months on the shelf at room temperature, indicating the physical stability of the formulation.

excised skin did not show any evidence of spongiosis and parakeratosis in the epidermis and no dermal or subcutaneous infiltrate.

DISCUSSION

These results demonstrate that we have developed a stable butorphanol liposome formulation that exhibits homogenous, multilamellar, structured vesicles. Pharmaceutical ointments are engineered to have rheological properties important to the physical performance of the product when used by the patient. Most ointments are intended to be thick when standing to prevent them from flowing away from the intended area of use. The

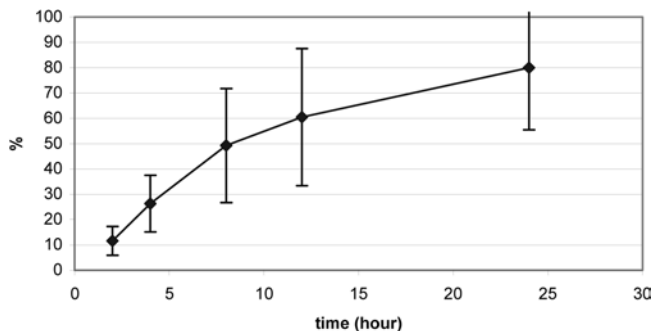


Fig. 2. Butorphanol release profile from liposome through full-thickness human skin by ultraviolet spectrophotometer (absorption at 280 nm, phosphate-buffered saline, 37°C). Using the full-thickness human skin sample, transdermal permeation measurement of the butorphanol liposome formulation was conducted *in vitro* with vertical diffusion Franz-cells. Samples were taken from the receiver at 2, 4, 8, 12 and 24 h after the start of the permeation experiment. As a control, full-thickness human skin without butorphanol liposome was used. The permeation of butorphanol through human skin shows that there was significant permeation at time-points 2, 4 and 8 h, and a steady state was achieved afterwards to 24 h (\blacklozenge mean \pm SD, $n=5$).

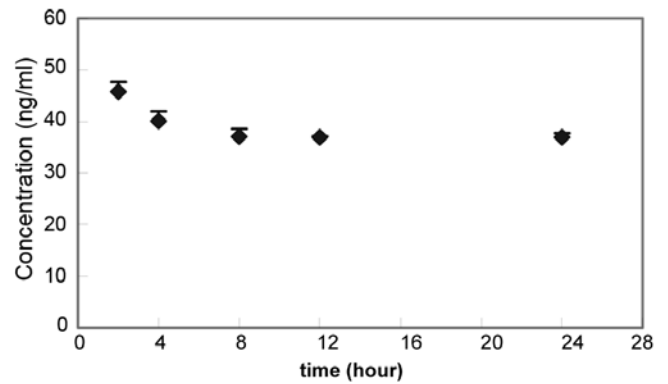


Fig. 3. Butorphanol plasma drug concentration assayed using high performance liquid chromatography (HPLC). Liposomal butorphanol was applied on the shaved back of rats and left for 2, 4, 8, 12 and 24 h. After the designated period, blood was withdrawn from the heart and the plasma was separated by centrifugation of whole blood at 1500 rpm, 4°C for 15 min. Plasma butorphanol was assayed using HPLC with electrochemical detector. Maximal plasma butorphanol concentration was noted at 2 h and steady state was achieved at 8 h (\blacklozenge mean \pm SD, $n=3$).

butorphanol liposome developed fulfills this criterion. The current butorphanol formulation was easy to apply and demonstrated desirable characteristics as a topical therapy. The transdermal delivery of butorphanol avoids gastrointestinal degradation and the hepatic first-pass effect of an oral administration, has potential for controlled and sustained delivery. Moreover, it is a user-friendly method and therefore could improve patient compliance.

The data demonstrate that there is no significant burst effect from the newly formulated liposomal butorphanol and that a consistent amount of butorphanol was released in a sustained manner for 24 h *in vitro*. This release pattern is appropriate for a once a day treatment regimen for patients with intractable pruritus. Pruritus has daily fluctuations and is typically intensified at night and can last in bouts of minutes to hours. Therefore, this type of preparation has an advantage over the present inhaler, which provides a transient effect for several hours and requires multiple administrations.

Pruritus can exert a profound impact on patients' lives, and there is a need for medications that successfully treat chronic, severe and intractable pruritus. There are currently no transdermal applications for anti-pruritic therapies in the US market. Recently, a study by Bigliardi et al. (14) reported a cream formulation of naltrexone, a μ -antagonist, to be effective for pruritus in atopic dermatitis. The need to develop a transdermal κ -agonist formulation for treatment of pruritus is supported by recent findings that there is significant downregulation of κ -opioids in the epidermis of patients with atopic dermatitis (15). κ -opioid receptor stimulation inhibits μ -receptor effects both centrally and peripherally (1–3). A recent double-blind controlled study demonstrated that nalfurafine, a κ agonist has anti-pruritic effect in patients with end-stage renal failure itch (16).

Butorphanol may be an ideal anti-pruritic medication since it targets both μ - and κ -opioid receptors without reversing the analgesic effects of opioid therapy. Recently, Lee et al. (17) demonstrated in monkeys that butorphanol is effective in blocking systemic or spinal morphine evoked itch via μ - and κ -opioid receptors. In contrast to μ -opioids butorphanol does not produce euphoria or dysphoria (18). An additional advantage of butorphanol is its favorable safety profile, it has minimal abuse potential compared with other opioid agonists. In clinical trials, less than 1% of patients developed physical dependence and tolerance (19). Furthermore, it is safe to administer in patients with hepatic and renal dysfunctions. The concentration used in the current study, 40 ng/ml, is in the effective dose range of 1–100 ng/ml mentioned in the formulations approved by the US Food and Drug Administration (FDA) (20–21).

Future studies to elucidate this mechanism are ongoing. The current investigation has developed a unique liposomal formulation of butorphanol. We believe that this preparation has significant potential for development of an efficient treatment for chronic itch.

CONCLUSION

This newly formulated liposomal delivery vehicle has the potential to deliver a pharmacologically effective amount of butorphanol in a controlled release fashion, enabling maintenance of its effective concentration in the treatment of chronic pruritus associated with imbalance of μ - and κ -opioids. In addition, this formulation has promising characteristics as a topical cream for skin application based on its appropriate color, viscosity, and skin compatibility.

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Conflict of interest: The authors G. Lim, Y. Ishiuj, A Dawn and G. Yosipovitch have filed a patent at Wake Forest University.

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