International Journal of Pharmaceutics 531 (2017) 306–312

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

In vitro and *in vivo* release of dinalbuphine sebacate extended release formulation: Effect of the oil ratio on drug release

Chan-Jung Li[a,b], Mei-Yun Ku[a], Chia-Yin Lu[a], Yu-En Tien[a,b], Wendy H. Chern[a], Jin-ding Huang[b,*]

[a] *Lumosa Therapeutics Co., Ltd., Taipei, Taiwan*
[b] *Departments of Clinical Pharmacy and Pharmaceutical Sciences, Medical College, National Cheng Kung University, Tainan, Taiwan*

ARTICLE INFO

Keywords:
Pro-drug
Controlled release
Pharmacokinetics
Dissolution
Injectable

ABSTRACT

Nalbuphine is a semi-synthetic opioid indicated for the relief of moderate to severe pain. Dinalbuphine sebacate (DNS) is a prodrug of nalbuphine for which we have developed long-acting lipophilic formulations in a benzyl benzoate/sesame oil mixture for intramuscular (IM) injection. In this study, we found that the *in vitro* release profile of DNS could be affected by adjusting the weight ratio of benzyl benzoate to sesame oil (the solvent/oil ratio). A longer release period could be attained by increasing the solvent/oil ratio in the formulation. A pharmacokinetic study was conducted in beagle dogs to verify the relationship between the *in vitro* release and the drug release from the formulations *in vivo*. The pharmacokinetic study confirmed that the formulation with a higher benzyl benzoate to oil ratio exhibits a longer drug release profile with a lower maximum concentration (C_{max}) and a longer time to peak blood concentration level (T_{max}) than the formulation with a lower benzyl benzoate to oil ratio.

1. Introduction

Nalbuphine (Fig. 1A) is a semi-synthetic opioid and a kappa agonist/partial mu antagonist analgesic with an equivalent potency to that of morphine under intramuscular (IM) administration (Beaver and Feise, 1978). Nalbuphine HCl injection is indicated for the relief of moderate to severe pain, such as pain after surgery and myocardial infarction (Cai et al., 2011; Hardman et al., 2001; Jasinski and Mansky, 1972). Several advantages of nalbuphine are the limited ability to cause respiratory depression, low tolerance liability, and absence of significant withdrawal symptoms, which make it safer than morphine (Beaver and Feise, 1978; Cohen et al., 1992; Fournier et al., 2000; Hardman et al., 2001; Hoskin and Hanks, 1991; Lee et al., 1981; Miller, 1980; Minai and Khan, 2003; Romagnoli and Keats, 1978; Schmidt et al., 1985; Van den Berg et al., 1994). However, as the biological half-life of nalbuphine is approximately 5 h in humans, a "long-acting nalbuphine" for pain relief is highly desired.

Dinalbuphine sebacate (DNS, Fig. 1B) is a synthetic prodrug of nalbuphine with two active nalbuphine moieties connected by a sebacoyl ester. It can be efficiently converted back to the active component, nalbuphine, by various endogenous esterases (Leinweber, 1987; Williams, 1985). With a hydrophobic constant (Log P) of 3.15 and neutral aqueous solubility less than 250 ng/ml (Hu and Chang, 2012), DNS is relatively hydrophobic, and an oil-based extendedrelease formulation of DNS was thought to be able to prolong the duration of nalbuphine's analgesic effect.

Several sustained release formulations are available on the market: liposomes, microspheres, lipophilic solutions and aqueous suspensions. Liposomes and microspheres are the newer formulations with a high potential for development into numerous active compounds, but the complex and costly manufacture of these formulations is challenging (Freitas et al., 2005; Jiang et al., 2005). A long-acting oil base solution that has attributes such as uncomplicated manufacturing and good stability has been approved for steroid esters and antipsychotics for more than three decades (Chien, 1981; Davis et al., 1994). Despite the use of many lipophilic solutions in the clinic for a long time, it is difficult to establish a suitable *in vitro* release model due to a lack of information on the *in vitro* and *in vivo* correlations in lipophilic solutions (Larsen et al., 2002a). The basic *in vitro* characteristics that were thought to be key parameters of lipophilic solutions included (1) vehicle viscosity, (2) drug solubility in the vehicle, and (3) drug partition between the vehicle and aqueous buffer (Weng Larsen and Larsen, 2009). Three *in vitro* model systems have been developed, including lipophilic solution floating on the release medium method, dialysis method, and continuous flow method (Crommelin and De Blaey, 1980; Janicki et al., 2001; Larsen et al., 2002b; Soderberg et al., 2006).

Abbreviations: DNS, dinalbuphine sebacate; IM, Intramuscular; ER, extended release; BB/oil ratio, benzyl benzoate/vegetable oil ratio; IV, intravascular

* Corresponding author at: National Cheng Kung University, No.1, University Road, Tainan City 701, Taiwan.
E-mail addresses: jdatncku@gmail.com, jinding@mail.ncku.edu.tw (J.-d. Huang).

http://dx.doi.org/10.1016/j.ijpharm.2017.08.083
Received 10 May 2017; Received in revised form 28 July 2017; Accepted 15 August 2017
Available online 25 August 2017
0378-5173/

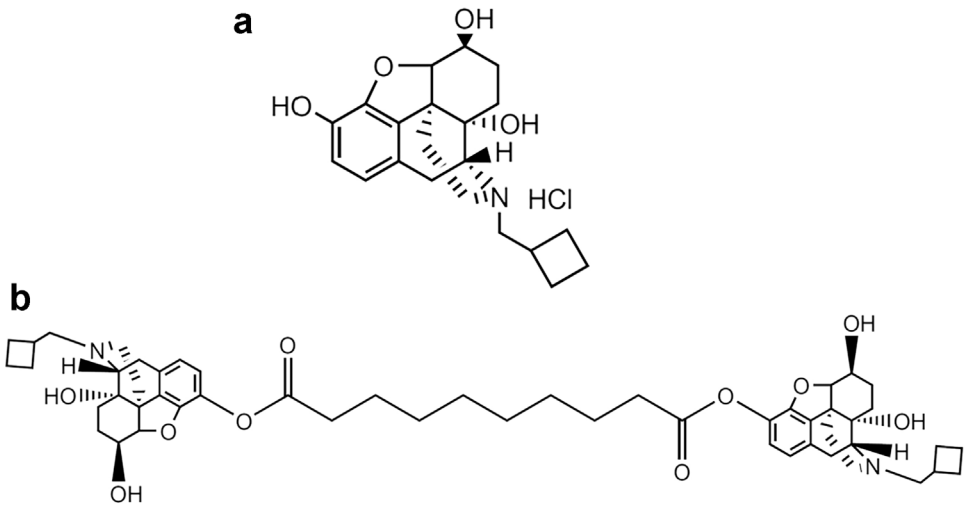

Fig. 1. Chemical structures of (A) nalbuphine and (B) dinalbuphine sebacate.

However, these systems were difficult to apply to a DNS lipophilic solution due to their very slow release. Therefore, we aimed to develop an *in vitro* release method for DNS lipophilic solution in this study. In the present study, we showed that adjusting the weight ratio of benzyl benzoate, a solubilizing agent, to vegetable oils had a direct impact on the release profile of DNS *in vitro*. A pharmacokinetic study was conducted in beagle dogs by IM injection to investigate the relationship between the *in vitro* and *in vivo* release. Establishing the *in vitro* and *in vivo* correlation is very helpful to accelerate development of lipophilic solutions and develop a useful control method for product quality.

Recently, a benzyl benzoate/sesame oil-based DNS formulation was studied in a randomized, placebo-controlled, single dose, parallel design clinical study for the treatment of moderate to severe post-operative pain in patients who underwent hemorrhoidectomy (Yeh et al., 2017). The oil-based injections demonstrated 7–10 days of analgesic effect, correlating to the pharmacokinetic profile of nalbuphine in patients treated with the extended release formulations. The clinical results from this study validated the formulation design and demonstrated that a simple oil-based formulation has great potential in drug delivery (Yeh et al., 2017).

2. Materials and methods

2.1. Materials

Benzyl benzoate and cottonseed oil were purchased from ACROS Organics (Geel, Belgium). Benzyl alcohol and castor oil were acquired from PanReac AppliChem (Barcelona, Spain). Ethanol, 2-propanol and t-butanol were purchased from Merck (Darmstadt, Germany). 1-propanol was purchased from Sigma-Aldrich (St Louis, MO, USA). Sesame oil was purchased from Croda International Plc (Snaith, UK). 1-butanol was obtained from Fisher Chemical (Pittsburgh, USA). Nalbuphine HCl standard was purchased from MedChem Express (NJ, USA). Dinalbuphine sebacate was synthesized and qualified by Lumosa Therapeutics Co., Ltd. (Taipei, Taiwan).

2.2. Drug analysis

The quantification of DNS was performed using an Acquity UPLC® system (Waters Corp., Milford, MA, USA) with an Acquity UPLC® BEH C18 Column (1.7 μm, 2.1 mm ID*50 mm; Waters Corp., Milford, MA, USA) at 35 °C. The mobile phase consisted of 88 mM acetate buffer and methanol (40:60, v/v). The flow rate was set at 0.1 mL/min, and the detection wavelength was set at 280 nm.

2.3. Study of the solubility of DNS in solvents

The solubility of DNS in benzyl benzoate, benzyl alcohol, ethanol, 1-propanol, t-butanol and sesame oil was tested. Weighted appropriate amount of DNS in each 0.4 mL solvent and incubated on vortex mixer (Benchmark scientific, B3D1008, Sayreville, USA) at room temperature for overnight. Undissolved DNS was removed by centrifugation at 3000 rpm for 10 min, and the supernatant DNS solution was diluted with ACN for drug analysis. Based on the solubility of DNS in the above solvents, six solvent systems comprised of sesame oil, benzyl benzoate and different alkyl alcohols (*e.g.*, methanol, ethanol, 1-propanol, 2-propanol, and 1-butanol) were prepared in various solvent/oil ratios (w/w%), and the solubility of DNS in each solvent system was measured.

2.4. In vitro method of DNS dissolution

Formulations with various DNS concentrations ranging from 50 to 150 mg/mL were prepared in benzyl benzoate and sesame oil mixtures of various weight ratios (benzyl benzoate/sesame oil ratio ranged from 0.5–16). The *in vitro* release of each DNS formulation was assessed using a MS-MP8 magnetic stirrer (Witeg Labortechnik GmbH, Wertheim, Germany) and a pH 6.0 dissolution medium comprised of 11.8 mM phosphate buffer and 0.1% tween 80. For each experiment, 500 mL of medium was placed in a beaker on a magnetic stirrer. A formulation sample (50–150 μL) containing 7.5 mg of DNS was dropped into the medium, followed by stirring to disperse the sample solution into small oily droplets. The temperature of the medium was controlled at 25–27 °C, and the stir speed was set at 360 rpm. At each time point, 5 mL of the resulting medium was withdrawn for the analysis of DNS. At the end of the dissolution period (180 min), 200 μL of 6 N HCl was added into the medium, and stirring was continued for an additional 20 min to allow 100% release of DNS from the formulation under acidic conditions. Five milliliters (5 mL) of the medium at the termination point was collected, and the drug concentration was determined as the 100% release reference point to calculate the dissolution rate of DNS at each time point. All dissolution samples were filtered with 0.22-μm PVDF filters (Millipore, Millex-GV) immediately prior to analysis of DNS by UPLC.

2.5. Pharmacokinetic study in dogs

The animal pharmacokinetic study was performed in Sundia MediTech Company (Shanghai, China) under the IACUC approval. Beagle dogs (7.8–9.6 kg, 8–10 months) were housed in stainless cages and had free access to dry dog food. F2, F3, and F4 formulations were