International Journal of Pharmaceutics 535 (2018) 228–236

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Research Paper

Improving the direct penetration into tissues underneath the skin with iontophoresis delivery of a ketoprofen cationic prodrug

Shabbir Lobo[a], Guang Yan[b,*]

[a] Department of Biomedical and Pharmaceutical Sciences, Idaho State University, Pocatello, ID, 83209, USA
[b] Department of Pharmaceutical Sciences, MCPHS University, Worcester, MA, 01608, USA

ARTICLE INFO

Keywords:
Ketoprofen
Iontophoresis
Penetration
Cationic
Topical
Prodrug

ABSTRACT

Current topical nonsteroidal anti-inflammatory drugs (NSAIDs) showed marginal efficacy in treatment of musculoskeletal disorders due to their fast clearance by skin blood flow and thus little direct penetration into the underlying muscle and joint tissues. Using ketoprofen (Kt) as a model NSAID and converting it to a cationic ester prodrug ketoprofen choline chloride (KCC), this study was to investigate the iontophoresis delivery of the prodrug KCC for improving the drug retention in the skin and the direct penetration into underlying tissues. From in vitro flux study, anodal iontophoresis of KCC showed 5 times higher flux than cathodal iontophoresis of Kt across human epidermis skin, and also 1.5 times higher across full thickness rat skin. From in situ dual agar gel model rat study, anodal iontophoresis of KCC showed 35 times more drug penetrating across the live skin into underlying agar gel and 22 times more drug retained in the skin than those from cathodal iontophoresis of Kt. Co-iontophoresis of a vasoconstrictor phenylephrine with KCC did not show better result than the iontophoresis of KCC alone. Overall, iontophoresis delivery of the cationic prodrug KCC showed great potential for direct penetration into local tissues underneath the skin.

1. Introduction

Oral nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely used for pain relief in musculoskeletal disorders (MSD) such as arthritic joint pain, ankle sprain, and muscle aches. However, oral NSAIDs are associated with high risks of gastrointestinal bleeding/ulceration and cardiovascular adverse effects. On the other hand, topical NSAIDs showed much lower systemic blood drug concentration, and thus would have lower systemic adverse effect (Roth and Fuller, 2011). Therefore, topical treatment for MSD is appealing. However, topical NSAIDs only showed moderate musculoskeletal pain relief than placebo vehicle with around 60% of the patients responding to topical NSAIDs versus 50% of the patients responding to placebo (Derry et al., 2016). This big placebo effect could come from the physical rubbing effect. Currently, topical NSAIDs are recommended for early stage management of localized chronic pain such as osteoarthritis, and oral NSAIDs can be used if topical NSAID is not effective (Shah and Mehta, 2011). Studies also showed that lower muscle and joint tissue drug concentrations in patients treated with topical NSAIDs than those treated with oral NSAIDs (Miyatake et al., 2009; Rolf et al., 1999). It is reasonable to expect that enhancing topical NSAIDs direct penetration into the deep tissues (muscle and joint tissues) may improve their treatment efficacy.

For process of topical drug direct penetration into deep tissue, the drug molecules first need to penetrate across the epidermis, and then enter into the dermis, where a portion of the drug will be washed away by the capillary dermal blood flow in the dermis, and some other portion will diffuse into tissues underneath the dermis such as subcutaneous, fascia, muscle or joint tissues. Singh and Roberts reported that a series of NSAIDs (salicylic acid, naproxen, diclofenac, piroxicam, indomethacin) solutions applied topically on exposed rat dermis could only penetrate to a depth of 3 mm (dermis and some subcutaneous tissues) and beyond that the drug concentrations in deeper tissues were mainly from systemic circulation redistribution (Singh and Roberts, 1994c). Using the same procedure, they also observed that lidocaine could penetrate to a depth of 1 cm (Singh and Roberts, 1994b) and some bases and steroid compounds could penetrate 6–8 mm deep (Singh and Roberts, 1994a). Sugibayashi's group developed an in-situ method to investigate topical drug penetration across live rat abdominal skin and being collected by agar gel dishes (Hasegawa et al., 2008; Hasegawa et al., 2007; Yanagimoto et al., 1999). They observed that for NSAIDs (piroxicam and flurbiprofen), above 99% of the drug delivered into the skin was cleared away by blood circulation and less than 1% of the drug was collected by the agar gel; on the other hand 14% of

* Corresponding author at: MCPHS University, 19 Foster Street, Worcester, MA, 01608, USA.
E-mail address: guang.yan@mcphs.edu (G. Yan).

https://doi.org/10.1016/j.ijpharm.2017.10.061
Received 22 August 2017; Received in revised form 6 October 2017; Accepted 31 October 2017
Available online 15 November 2017
0378-5173/

antipyrine and 23% of lidocaine delivered into the skin were collected by agar gel. Most NSAIDs show very high drug plasma protein binding (above 99%), while the protein binding of the other two drugs are much less: 70% for lidocaine and 2% for antipyrine (Yanagimoto et al., 1999). These results indicated that drug with higher plasma protein binding might have been cleared away from the skin by its blood flow more quickly, and thus little drug left in the skin for direct penetration into deeper tissues. This phenomenon was also demonstrated from a perfusion study by Roberts and Cross that drug with high plasma protein binding was quickly cleared away by blood circulation in the tissue (Roberts and Cross, 1999).

Many NSAIDs are negatively charged at physiological pH and cathodal iontophoresis delivery of NSAIDs for treating musculoskeletal diseases was also attempted, but the treatment result was not promising, as iontophoresis of ketorolac was no more effective than placebo iontophoresis (Saggini et al., 1996). It was shown that the penetration depth from the iontophoresis of sodium salicylate was only 3–4 mm (Singh and Roberts, 1993) and iontophoresis of diclofenac on rabbits showed a penetration depth only to subcutaneous tissue at 0.2 mA/cm^2 current density (Hui et al., 2001). These were probably also due to their high plasma protein binding and thus a rapid clearance from the skin and underlying tissue by the blood flow. On the other hand, the penetration depth from iontophoresis of lidocaine (a cationic drug) could reach more than 10 mm to deep muscles (Singh and Roberts, 1993), which indicated that topical delivery of drug to deep tissues is possible if the drug molecules have suitable physicochemical properties.

Many NSAIDs have a carboxyl group, which could be utilized to form an ester prodrug, and thus offers different physicochemical properties from their parent drug. In our previous study we synthesized some diclofenac ester prodrugs and those prodrugs showed lower plasma protein binding (around 90%) compared to diclofenac (> 99%). However, topically applying one of the diclofenac ester prodrugs did not show higher direct penetration across the skin from an agar gel rat model study compared to that from topically applying of the parent drug (Yan et al., 2014). It could be that the merely 10% lowering of the plasma protein binding from the neutral ester prodrug was not significant enough, and prodrugs with more drastic reduction in the plasma protein binding might be needed to show a significant impact on direct penetration.

Topical drug penetration into deep tissues has to pass through at least the dermis and subcutaneous tissues. However, the systemic blood flow in those tissues will clear away the drug molecules and lead to very limited drug molecules available for penetration into deeper tissues. Lowering the blood flow into the tissue will lower the drug clearance from the tissue. One way of lowering the blood flow in the local tissue is topically co-delivering a vasoconstrictor with the drug. Vasoconstrictors such as epinephrine, norepinephrine, and phenylephrine (PE) have been shown to enhance drug retention in local tissue or penetration into deeper tissues. Passive delivery of those vasoconstrictors across intact skin was not effective, and thus active delivery method has to be employed. These vasoconstrictors are positively charged at physiological pH. Co-iontophoresis of lidocaine and norepinephrine showed higher lidocaine retention in the skin (Riviere et al., 1992). Since most NSAIDs are negatively charged at physiological pH, they are not suitable for co-iontophoresis with a vasoconstrictor.

The objective of this study was to investigate the topical application of a NSAID prodrug with a low plasma protein binding for improving the direct penetration of the drug into deeper tissue. A ketoprofen cationic ester prodrug was synthesized in this study and showed low plasma protein binding (as demonstrated in the results), and then it was tested for topical delivery through anodal iontophoresis with a dual agar gel rat model. In addition, the ketoprofen cationic prodrug can be co-iontophoretically delivered with a vasoconstrictor, and thus another purpose of this study was to evaluate whether such combination would further enhance the direct penetration into deep tissue.

2. Material and methods

2.1. Material and animals

Ketoprofen (Kt) was obtained from TCI ltd (Tokyo, Japan), choline chloride was obtained from J.T. Baker (Phillipsburg, NJ), phenylephrine hydrochloride was obtained from Spectrum Chemicals (Gardena, CA), *N,N*'-dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine were obtained from Sigma (St. Louis, MO), agarose LF (PFGE grade) was obtained from Amresco (Solo, Ohio), silica gel ultra pure 40–60 μm was obtained from ACROS organics (Fair Lawn, NJ), dichloromethane was obtained from Fisher Scientific (Fair Lawn, NJ) and acetonitrile was obtained from EMD chemicals Inc (Gibbstown, NJ), methanol was obtained from BDH chemicals (Radnor, PA), trifluroacetic acid was obtained from Honeywell Riedel-de-Haen (Seelze, Germany) and fresh human plasma was obtained from Interstate Blood Bank Inc. (Memphis, TN). The direct current source used in the experiments was Activa Dose® II Controller Ionto Device purchased from ActivaTek Inc (Salt Lake City, Utah). The rats used in the study were Sprague Dawley rats bred and raised at the Animal Care Facility at Idaho State University and ranged between 280 and 350 g weight. All animal study protocols were approved by the IACUC of the Idaho State University. Human cadaver skin was purchased from New York firefighter skin bank and human epidermal membrane (HEM) was obtained with a heat stripping method.

2.2. Synthesis of cationic prodrug of ketoprofen

The esterification of the Ketoprofen cationic prodrug (Fig. 1) was carried out based on a procedure in our previous study (Lobo et al., 2014), with certain modifications. Specifically, choline chloride (0.00353 mol) was added to 50 ml of anhydrous acetonitrile, and dissolved with sonication bath at 50 °C. Kt (0.00118 mol) was then added and solubilized, followed by 0.000054 mol of 4-pyrrolidinopyridine. Reaction was initiated by addition of *N,N*'-dicyclohexylcarbodiimide (DCC) (0.00146 mol), and continuously stirring at room temperature for 18 h. A white precipitate was formed which was separated by filtering through non-absorbent cotton. The reaction vessel and the precipitate were further washed with 10 ml of anhydrous acetonitrile and filtered and combined with the initial filtrate. The obtained acetonitrile solution was evaporated under vacuum at room temperature, and a thick oil results in the flask which was the crude ester product. The oil was dissolved with 1 ml of dichloromethane and then was added to a flash chromatographic column packed with silica gel for purification. A combination of methylene chloride, acetonitrile and methanol

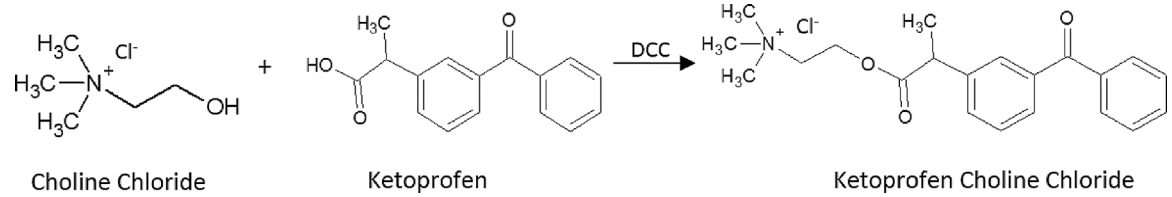

Fig. 1. Diagram of synthesis KCC from Kt.