European Journal of Pharmaceutical Sciences 121 (2018) 200–209

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Dissolving polymeric microneedle arrays for enhanced site-specific acyclovir delivery

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ARTICLE INFO

Keywords:
Acyclovir
Intradermal
Dissolving microneedles
Herpes labialis
Cold sores
Herpes simplex virus

ABSTRACT

Acyclovir is widely indicated for the treatment of herpes labialis (cold sores), typically caused by the herpes simplex virus type 1 (HSV-1). However, topical acyclovir has poor efficacy, due to its low skin permeability. The purpose of this study was, therefore, to evaluate the ability of dissolving polymeric microneedle (MN) arrays to improve the local delivery of acyclovir. Acyclovir-loaded dissolving MN arrays (0.49 cm^2) were formulated from aqueous blends of Gantrez® S-97 with 361 needles per array (589 ± 9.29 μm height). MN penetrated excised neonatal porcine skin, showing sufficient mechanical strength to resist compression and maintained their appearance after application of a 0.089 N per needle force for 30 s. Dissolution of the needles was observed within 15 min after application to skin and the needles had completely dissolved at 2 h *in vitro*. *In vitro* skin permeation studies revealed that the percentage of total acyclovir loading which permeated the skin over a 24 h period using MNs was approximately 45 times higher than that of a commercial cream formulation (Lipsore®). The accumulation of acyclovir at the basal epidermis, the target site of the herpes simplex virus, using MNs was a total of 21.5 μg/cm^3 *in vitro*, which is approximately 5 times greater than the 99% inhibition of viral cytopathic effect (ID_{99}) required for HSV infections. This level was also 16 times higher than that obtained using the cream formulation. An *in vivo* study showed that the use of acyclovir-loaded dissolving MN arrays successfully provided intradermal delivery of acyclovir over a 48 h period and the drug levels in the skin delivered using MN arrays (45.09 ± 13.28 μg/cm^3) were superior to those generated by the cream formulation (4.55 ± 1.37 μg/cm^3). Accordingly, acyclovir-loaded dissolving MN arrays could be a promising approach for effective local delivery of acyclovir.

1. Introduction

Acyclovir is a synthetic 2′-deoxiguanosine analogue with an inhibitory activity against herpes simplex virus type 1 (HSV-1), HSV-2 and varicella-zoster virus (Al-Dhubiab et al., 2015; Brunton et al., 2011). Acyclovir is also indicated for prevention and treatment of recurrent herpes labialis (cold sores), usually caused by HSV-1 (Arvin et al., 2007; Rahimi et al., 2012). The mechanism of action of acyclovir is inhibition of viral DNA replication. The replication site of HSV-1 is the basal epidermis (*stratum basale*), the deepest layer of the epidermis (Peira et al., 2007).

Acyclovir is widely prescribed internationally. It is administrated either orally or topically, or by both routes. For oral administration, 200–400 mg five times a day is suggested for treatment purposes in adults (Opstelten et al., 2008). Although oral administration is the most convenient route, it is problematic, because it can cause side effects, including nausea, vomiting, headaches, diarrhoea, abdominal pain, tiredness and malaise (Goldberg et al., 1986; Meng et al., 2011; Yildiz et al., 2013). Topical administration is also a convenient route, with very few or no side effects, as demonstrated in a clinical trial (Arndt, 1988). Accumulation of the drug in the desired target tissues is important for the efficacy of topical drug delivery. Simultaneously, low plasma levels observed following topical administration ensure low systemic burden and minimize systemic side effects (Marks and Dykes, 1994; Rannou et al., 2016; Siddoju et al., 2011).

However, topical acyclovir products have been reported to have only moderate efficacy and their efficiency is, in turn, dependent upon the severity of the infection. More frequent applications are required to improve its therapeutic efficacy, which may affect patient compliance (Hassan et al., 2016). The relatively poor efficacy of acyclovir after

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https://doi.org/10.1016/j.ejps.2018.05.009
Received 3 April 2018; Received in revised form 12 May 2018; Accepted 13 May 2018
Available online 16 May 2018
0928-0987/

topical application is mainly due to its low permeation through the skin, which can be attributed to its physicochemical properties (log P of acyclovir = −1.5) (Friedrichsen et al., 2002; Spruance and Crumpacker, 1982).

To improve the therapeutic efficacy of topically applied acyclovir, new drug delivery systems which can overcome the skin's formidable barrier and deliver the required dose in a targeted and sustained manner are required. Dissolving microneedles (MN) are a drug delivery system that is currently used to deliver a disparate range of drugs and vaccines. This platform is increasingly interesting for transdermal and intradermal drug delivery, as it has proven to be useful for a wide range of drugs, including insulin (Ito et al., 2006; Ling and Chen, 2013), polymyxin B (Dillon et al., 2017), meloxicam (Amodwala et al., 2017), lidocaine (Caffarel-Salvador et al., 2015) and doxorubicin (Amodwala et al., 2017; Sriraman et al., 2016). Dissolving MN have also been used extensively to target the rich population of professional antigen-presenting cells that reside within the viable skin layers (Quinn et al., 2014). Dissolving MN arrays comprise needles < 1 mm in length that can pierce the outer layer of skin, the *stratum corneum*, with little or no pain, and deliver their drug payload intradermally when in contact with skin interstitial fluid, with the drug then diffusing to its target tissue within skin or being systemically absorbed (Lutton et al., 2015; van der Maaden et al., 2012). Dissolving MN arrays are interesting, since they are self-dissolving and, thereby, leave no biohazardous sharp waste. Dissolving MN arrays can be made of many different materials, such as maltose (Lee et al., 2011), poly(vinylpyrrolidone) (Ronnander et al., 2017), starch and gelatin (Ling and Chen, 2013), chondroitin sulfate (Ito et al., 2014), dextran and hyaluronic acid (Ito et al., 2011). In addition, they provide advantages, such as accurate dosing, as only small amounts of the drug are lost during the production process (van der Maaden et al., 2012).

Our study aims were to overcome the limitations of acyclovir permeation into skin. For the first time, dissolving MN arrays incorporating acyclovir were developed to deliver acyclovir to the basal epidermis of the skin, where herpes simplex viral lesions are usually located, in clinically-relevant amounts and in a controlled and sustained manner. For this purpose, dissolving MN arrays loaded with acyclovir were fabricated from aqueous blends of Gantrez® S-97 and characterized *in vitro* for their drug content, mechanical strength, insertion depth, dissolution kinetics and penetration into excised neonatal porcine skin and then evaluated *in vivo* in mice for effective intradermal delivery in comparison with a topically applied commercially available acyclovir cream (Lipsore cream).

2. Material and methods

2.1. Materials

Gantrez® S-97 (Mw = 1500), a copolymer of methylvinylether and maleic acid, was a gift from Ashland (Surrey, UK). Acyclovir was purchased from Activate Scientific (Hertfordshire, UK). Lipsore® 5% w/w acyclovir cream was obtained from Relonchem Limited (Cheshire, UK). Parafilm® M (~127 μm thickness), made of olefin-type materials, was purchased from Brand GMBH (Wertheim, Germany). Acetonitrile (ACN) was obtained from Sigma-Aldrich (Dorset, UK). All other chemicals used were of analytical reagent grade.

2.2. Fabrication of dissolving microneedle arrays incorporating acyclovir

MN arrays were fabricated by mixing aqueous blends containing 30% w/w Gantrez® S-97 with acyclovir powder to obtain a slurry of polymer gel: drug in a ratio of 90:10 w/w. The formulation was then dosed (0.5 g) onto laser-engineered silicone micromould templates (19 × 19 conical needles on a 0.49 cm^2 area with 600 μm needle height, 300 μm needle width, and 50 μm interspacing). The tips of the needle cavities were filled by centrifugation of the MN moulds at 3500 rpm for 20 min. MN arrays were left to dry at 25 °C for 48 h. Following this drying process, the MN arrays were removed from the moulds and their mechanical properties evaluated.

2.3. Evaluation of MN mechanical strength

The mechanical characteristics of the MN arrays were assessed using a TA-XT2 Texture Analyser (Stable Microsystems, Haslemere, UK) in compression mode, as previously described (Donnelly et al., 2011). MN arrays were imaged before and after application of a compression load using a Keyence VHX-700F digital microscope equipped with a VH-Z20R lens (Keyence, Osaka, Japan). Known axial compression forces (forces applied perpendicular to the baseplate of MN) were applied to the MN arrays to investigate the needle strength. The arrays were fixed to the moveable probe of the Texture Analyser using adhesive tape. The MN array was then pressed against a flat aluminium block at a rate of 0.5 mm/s until a maximum force was achieved. The maximum forces were varied from 0.028 N to 0.11 N per needle. The pre- and post-test speeds were both set at 1 mm/s and the trigger force was 0.049 N. Changes in the height of the needles were evaluated using the digital microscope. The mean percentage reduction in needle height *versus* applied force was then plotted.

2.4. Insertion force of MN arrays

Parafilm® M (PF) was used as a validated skin model for MN insertion experiments, as described previously (Larrañeta et al., 2014). MN arrays were applied perpendicularly into an eight-sheet PF laminate (approximately 1 mm in total thickness) using the Texture Analyser with a speed of 0.5 mm/s and an exerted force of 0.089 N per needle for 30 s. MN arrays were then carefully removed from the PF, the sheets unfolded and the number of holes created in each layer counted using the digital microscope. The percentage of holes created *versus* depth of PF was plotted. The thickness of each PF layer was previously shown to be 126 ± 7 μm (Larrañeta et al., 2014).

2.5. Insertion of MN arrays into excised neonatal porcine skin

In this study, full thickness neonatal porcine skin was used as a model of human skin because the structure of porcine skin is similar to that of human skin (Simon and Maibach, 2000; Touitou et al., 1998) and the percutaneous permeability of porcine skin is close to that of human skin (Neubert and Wohlrab, 1990). Full thickness skin was obtained from stillborn piglets and excised within 24 h of birth using an electric dermatome (Integra Life Sciences™, NJ, USA). The skin was then wrapped in aluminium foil and stored at −20 °C until use. The skin was washed with phosphate buffered saline (PBS, pH 7.4) before use. The skin surface was dried using tissue paper and placed dermis side down on a dental wax sheet. MN arrays were then applied perpendicularly onto the skin using the Texture Analyser at a speed of 0.5 mm/s, and an exerted force of 0.089 N per needle for 30 s. The probe with the MN arrays attached was removed upwards at the same speed. Methylene blue (5 mg/mL) was dropped onto the skin and left for 20 min, after which time holes appeared as dark blue dots. Excess methylene blue was then gently wiped off using tissue paper and then with normal saline solution. Afterwards, the skin was again visualized using the digital microscope. The number of holes created was counted and the percentage of holes created was investigated.

2.6. In vitro dissolution studies

As part of basic formulation screening efforts, preliminary dissolution study was performed, in which acyclovir-loaded dissolving MN arrays were immersed in 5 mL of PBS and stirred at 400 rpm. The temperature was maintained at 37 ± 1 °C. The dissolution time was then investigated. The dissolution rate of the MN array was also