Journal of Drug Delivery Science and Technology 29 (2015) 173–180

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Research paper

Lopinavir metered-dose transdermal spray through microporated skin: Permeation enhancement to achieve therapeutic needs

Dhruval Patel, Praveen Kumar, Hetal P. Thakkar*

TIFAC CORE in NDDS, Pharmacy Department, Faculty of Technology & Engineering, The M.S. University of Baroda, Vadodara 390 001, India

ARTICLE INFO

Article history:
Received 27 January 2015
Received in revised form
22 May 2015
Accepted 7 July 2015
Available online 11 July 2015

Keywords:
Metered-dose transdermal spray
Lopinavir
Microneedles
Skin microportion
Permeation enhancement
Kollidon® VA 64

ABSTRACT

A user friendly metered-dose transdermal spray (MDTS) of Lopinavir was developed and a combination of chemical and physical penetration enhancement techniques was utilized to enhance overall drug permeation across skin. Formulation was optimized varying Kollidon® VA 64 level while optimized batch was exhaustively characterized for *in vitro* drug release, *ex vivo* skin permeation and *in vivo* bioavailability. Safety and stability were also ascertained. Formulation containing 5 %w/v of Kollidon® had best sprayability and volatilization property. A significantly high permeation enhancement ratio (1.77) and steady state transdermal flux (52.5 μg/cm^2/h) through microporated pig ear skin *ex vivo* indicated the permeation enhancement potential of the techniques applied. A remarkable 3-fold rise in relative bioavailability via transdermal route ($F_{MDTS\ via\ microporated\ skin}$ = 291.15%) as compared to oral suspension of marketed tablet ($AUC_{0-\infty}$ = 45.94 h*μg/ml) further confirmed the validity of our hypothesis. The outcomes of *in vitro*, *ex vivo* and *in vivo* characterization of Lopinavir MDTS represented the system as safe, effective and stable which seems promising for their extended clinical evaluation.

Acquired immune deficiency syndrome (AIDS), caused by human immune deficiency virus (HIV) infection, is one of the most serious infectious diseases that challenges public health globally [1]. As per the UNAIDS report 2013, even with a 33% decrease since 2001, new HIV infections reported in 2012 being significantly high (about 2.3 million) with approximately 35.3 million people suffering from HIV worldwide [2]. Due to lack of efficacy of vaccines and macromolecular entry inhibitors, there is a growing consensus on the use of drugs with proven antiretroviral activity for prophylaxis of HIV. It is anticipated that the presence of sufficient concentrations of antiretroviral drugs at the site would help to prevent HIV infection. Hence, drugs which act on HIV entry, HIV fusion, HIV reverse transcriptase and HIV integrase are being explored for the HIV prophylaxis [3]. Current optimal highly active antiretroviral therapy (HAART) regimens consist of two nucleoside analogue reverse transcriptase inhibitors (NARTIs or NRTIs) along with either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (NNRTI). Use of antiretroviral drug combination is a commonly employed strategy for HIV therapy to increase efficacy and reduce resistance and side effects. One of the classes of anti-HIV drugs that inhibit growth of virus is retroviral protease enzyme inhibitor which has been highly beneficial to many HIV-infected individuals since its introduction in 1996, when the protease inhibitor-based highly active antiretroviral therapy (HAART) initially became available. Lopinavir is a potent protease inhibitor indicated for the treatment of the HIV infection [4]. However, the oral administration of Lopinavir is limited by its poor bioavailability (~20%) due to its low aqueous solubility (0.01 mg/ml), poor dissolution, extensive first pass metabolism primarily mediated by cytochrome P450 3A4 and cytochrome P450 3A5 isoenzymes and high efflux by P-glycoprotein efflux system. Currently, it is co-administered with subtherapeutic dose of Ritonavir, a pharmacokinetic enhancer of Lopinavir. Ritonavir is a potent inhibitor of cytochrome P450 3A4 in liver microsomes. However, even in presence of Ritonavir, oral bioavailability of lopinavir is only around 36% [5]. Moreover, this combination is reported to cause several adverse effects including Diarrhea; headache; mild stomach pain or upset; nausea; tiredness; vomiting and weakness.

Transdermal delivery, an attractive alternative to oral delivery is associated with a number of advantages, particularly, avoidance of hepatic first-pass metabolism that can prematurely metabolize

* Corresponding author. Pharmacy Department, Faculty of Technology & Engineering, The M.S. University of Baroda, Kalabhawan, Vadodara 390 001, Gujarat, India.
E-mail address: hetal_thakkar11@yahoo.com (H.P. Thakkar).

http://dx.doi.org/10.1016/j.jddst.2015.07.004
1773-2247/

drugs. Metered dose transdermal spray (MDTS) formulations are gaining significant industrial attention because of their easy scalability and potential to overcome the limitations with other existing transdermal drug delivery systems. For example, problems with irritation and adhesion are often encountered with transdermal patches [6]; conventional gels must be applied over large surface areas and the possibility of passive transfer to clothing, or to another person, must be considered [7]. MDTS, on the other hand, is user friendly and permits a metered dose to be administered to a fixed skin area [8,9]. Being a solution based formulation, MDTS consists of drug and film forming polymer (with or without chemical penetration enhancer) dissolved in a volatile solvent. Once applied, the volatile components rapidly evaporate leaving behind a thin, uniform transdermal film with drug at high thermodynamic activity that is rapidly taken up into the skin [10]. However, overall transdermal flux remains limited by the extremely tough barrier property of stratum corneum. Achievement of desirable plasma concentration without the aid of a penetration enhancer is usually difficult. Isopropyl myristate (IPM) was selected as a chemical penetration enhancer that reversibly affect the bilayer architecture of the SC lipids by introducing phase separation and by direct perturbation of a proper lamellar arrangement [11]. Microneedle roller was also chosen as a physical way to facilitate the permeation that utilizes sub-millimeter sized microneedles designed to pierce the skin's stratum corneum barrier in a painless manner and create microchannels therein. Microneedle rollers have been used to treat large areas of skin for cosmetic purposes and to increase skin permeability for drug delivery [12,13].

Thus, the present investigation aimed at development of metered dose transdermal spray of Lopinavir and to study its permeation through microporated skin. The various formulation parameters were optimized to obtain a spray formulation with the desired drug loading, spray pattern etc. The optimum formulation was evaluated for the *in vitro* drug release, *ex vivo* permeation through pig ear skin. *In vivo* pharmacokinetic studies were also performed using Wistar rats and comparison of the pharmacokinetic parameters was done with the oral suspension of marketed Lopinavir tablet.

1. Experimental

1.1. Materials

Lopinavir was obtained as a gift sample from Aurobindo Pharma Ltd., Hyderabad. Kollidon® VA 64 was procured from BASF, Mumbai. Isopropyl myristate and cellophane membrane (12kD) were purchased from HiMedia Labs, Mumbai. Metered dose spray containers were purchased from Valois India Pvt. Ltd., Mumbai. Microneedle rollers (CTS-150, Clinicares) were procured from Coherent Medical Systems, Mumbai. All other reagents used were of analytical grade.

1.2. Preparation of metered dose transdermal spray

Accurately measured quantities of Kollidon® VA 64 (Table 1) and isopropyl myristate (0.15 ml) were dissolved in 30 ml of acetone:ethanol (1:1). 600 mg of Lopinavir was uniformly mixed into it using bath sonication for 3 min followed by mechanical stirring for 10 min. Resulting formulations were then filled in metered dose spray containers and characterized for different parameters as discussed in subsequent sections.

1.3. Characterization of metered dose transdermal spray

1.3.1. Viscosity of spray solutions

The viscosity of the spray solutions was measured at 25 ± 1 °C using Ostwald viscometer.

1.3.2. Film drying time and its coverage area

Formulations were sprayed through the MDTS onto a glass plate from a distance of 3.0 cm. The time required for complete evaporation of solvent and the average diameter of the fully dried film formed (d) was observed. The area covered by the film was then calculated from its diameter using Equation (1).

$$\text{Area covered by film} = \frac{\pi d^2}{4} \tag{1}$$

1.3.3. Drug content per spray and content uniformity

The drug content per metered dose was determined by actuating the pump five times in a beaker containing measured volume of Acetonitrile. The sprayed content was allowed to dissolve under mild agitation and the drug content per spray was quantified using first derivative UV spectroscopy as described elsewhere [14]. The content uniformity was also evaluated by quantifying the drug emitted in 1st, 5th, 10th, 15th and 30th actuation.

1.3.4. Percent solid content per spray

The initial weights (W_1) of the MDTS containers were recorded. The containers were then actuated for five times (n) and their final weights (W_2) were recorded. The average solid content per spray ($SC_{avg/spray}$) was calculated using Equation (2) and was divided by theoretically calculated solid content per spray ($SC_{T/spray}$) as given in Equation (3) to get percent solid content per spray ($SC_{\%/spray}$).

$$SC_{avg/spray} = \frac{W_1 - W_2}{n} \tag{2}$$

$$SC_{\%/spray} = \frac{SC_{avg/spray}}{SC_{T/spray}} \tag{3}$$

1.3.5. Spray pattern

The spray pattern was assessed by delivering the spray through the MDTS onto a paper held at a distance (L) of 3.0 cm. The resulting wet region on paper was outlined and the average radius (r) was measured. Spray angle was then calculated using Equation (4).

$$\text{Spray angle}(\theta;\ \text{radian}) = 2 \times \tan^{-1}\frac{(r)}{(L)} \tag{4}$$

1.3.6. Pump seal efficiency test

The initial weights (W_1) of the MDTS containers under test were recorded and then placed in the upright position at 30° for 3 days. The final weight (W_2) of containers were measured and the leakage rate was calculated using equation (5).

$$\text{Leakage rate} = \frac{W_1 - W_2}{W_1} \tag{5}$$

1.3.7. pH and clarity of spray solution

The pH of the spray solution was determined by spraying them on pH paper while the clarity was observed visually.

1.4. In vitro drug release study

The *in vitro* release study was done using specially designed