In vitro Evaluation of the HIV Therapeutic IQP-0410 Through Transdermal Film Delivery

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ABSTRACT

Purpose: The pyrimidinedione IQP-0410 is a highly potent non-toxic, dual-acting HIV nonnucleoside reverse transcriptase inhibitor (NNRTI) that targets both virus entry and reverse transcription. With *in vitro* subnanomolar concentration activity as an NNRTI and nanomolar activity as inhibitors of virus entry, IQP-0410 is proposed to provide therapeutic opportunities not available with many other NNRTIs. Through conventional administrations, such as oral or injections, NNRTIs would be subjected to extensive first pass metabolisms. Therefore, transdermal patches are being investigated and developed for antiviral drug delivery that may improve patient compliance, reduce first pass metabolism, and provide long-term therapeutic delivery.

Methods: *In vitro* evaluations of antiviral activity, mechanism of action, and toxicity against primary and established cells evaluate the efficacy and toxicity of IQP-0410. IQP-0410 was formulated into an ethyl cellulose / HPMC based film patches via solvent cast manufacturing. *In vitro* patch characterizations evaluating drug content uniformity, film strength and physical characteristics, drug release and permeability, and packaged product stability were conducted.

Results: Flexible, strong transdermal patches loaded with 2% (w/w) of the HIV therapeutic IQP-0410 were produced that have no irritation to *ex vivo* skin tissue and no *in vitro* toxicity to HIV target cells CEM-SS and PBMC's. When applied to the skin model, IQP-0410 was delivered at a rate of 0.937 ± 0.060 µg/cm²/hr. Over a 3 day application to the skin model, the *in vitro* EC_{50} of the permeated IQP-0410 was 2.557 ± 0.401 nM (CEM-SS) and 0.0199 ± 0.001 nM (PBMC). In sealed light-protected packages, the transdermal patches demonstration no significant degradation at regular (35°C / 65%RH) and accelerated stability conditions (40°C / 75%RH) for over 3 months.

Conclusions: IQP-0410 is a promising HIV therapeutic with high level potency current being prepared for IND submission. Transdermal patches represent a drug delivery system that would allow the non-toxic delivery of IQP-0410 at therapeutic levels over extended periods of with a single application. Such controlled delivery system may be necessary and advantageous over traditional therapeutic delivery to improve the success of HIV therapeutic products.

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METHODS AND MATERIALS

IQP-0410. The pyrimidinedione IQP-0410 was synthesized and provided by Samjin Pharmaceutical Co. LTD (Seoul, Korea).

Virus and Cells. The HIV-1_{IIIB}, HIV-$1_{BaL}$and the CEM-SS cell line were obtained from the NIAID AIDS Research and Reference Reagent Program (Rockville, MD). Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood (Biospecialty Corporation, Colmar, PA) and were PHA-stimulated for 72-hours and then cultured in the presence of IL-2 prior to use.

Transdermal film formulation. The transdermal films were formulated through a solvent evaporation method. Based upon target dosing levels of other PYDs with similar activity against HIV-1 currently under development, a target dose of 2% (w/w dry polymer) was chosen. The homogenous polymer solvent + API mixture was poured through a film applicator to create a sheet of polymer film. The spread polymer was allowed to evaporate and the dried films were removed from the film applicator, cut, and packaged.

Physical characteristics. Polymeric transdermal films were evaluated for various aesthetic, physical, and mechanical parameters including visual inspection for appearance, color, and transparency.

Moisture Content and Film swelling. The moisture content of the films was determined immediately after manufacturing in an Arizona Instruments VaporPro system (Chandler, AZ). Swelling was evaluated by taking a weighed film from the desiccators and exposing the film to ambient, 75%, and 90% relative humidity in a stability chamber until a constant weight was obtained.

***In vitro* drug release of the transdermal films.** The initial *in vitro* release characteristics of the films were conducted in dissolution vessels as previously reported. In a Sotax CE7 Smart dissolution system (Westborough, MA), *in vitro* release of the drug from the films was evaluated under sink conditions (90:10 EtOH:DI Water) and in a USP 4 flow-through cell system until the dermal films displayed no additional release of API into the system. The rate of release was measured with a Thermo Scientific Evolution 300 UV-Vis spectrometer (Waltham, MA) at 267 nm.

***In vitro* permeability of transdermal films.** *In vitro* permeability studies were carried out in Franz cell diffusion cells (PermeGear; Hellertown, PA) through a 0.45 micron hydrophilic PVDF membrane and through full thickness human epidermal tissue (Epiderm FT, MatTek; Ashland, MA). The entire assembly was magnetically stirred and maintained at a temperature of 37°C / 5% CO_2 for 72 hours. At regular intervals, samples from the receptor compartment of the Franz cell were taken to detect the presence of drug transport through the tissue. HPLC analysis was performed to determine permeation through the membrane.

***In vitro* efficacy of the transdermal films.** The IQP-0410 concentration released from the films with permeation through the skin tissue was determined using the described HPLC method. The concentration of IQP-0410 released from the film was then evaluated for anti-HIV efficacy and cellular toxicity performed in CEM-SS cells and human peripheral blood mononuclear cells (PBMCs). A highly standardized microtiter anti-HIV cytopathic effect (CPE) inhibition assay was performed as previously described. Anti-HIV efficacy and cellular toxicity was evaluated in a PBMC-based assay as previously described. Following the incubation, supernatants were collected for analysis of virus replication by supernatant RT activity and toxicity was assessed by analyzing cell viability utilizing XTT dye reduction. AZT was used as an internal assay control.

Irritation of the transdermal films to epidermal tissue. MTT studies were performed on the *ex vivo* epidermal tissues to evaluate toxicity of the transdermal film system following either a 24- or 72-hour application. The viability of the tissue was calculated as the optical density of the sample after exposure compared to the optical density of the negative control.

Stability of the transdermal films. A 3-month short term stability protocol of the film formulation was conducted under ICH recommended environmental conditions. Films were packaged in air tight foil pouches and stored under standard conditions (30°C / 65% Relative Humidity) and accelerated conditions (40°C / 75% R.H.). At regular time points, the films were removed from the chambers and assessed for their stability using the film evaluation assays described above. All tests were performed in triplicate (n = 3 ± SD).

RESULTS

PHYSICOCHEMICAL CHARACTERIZATION

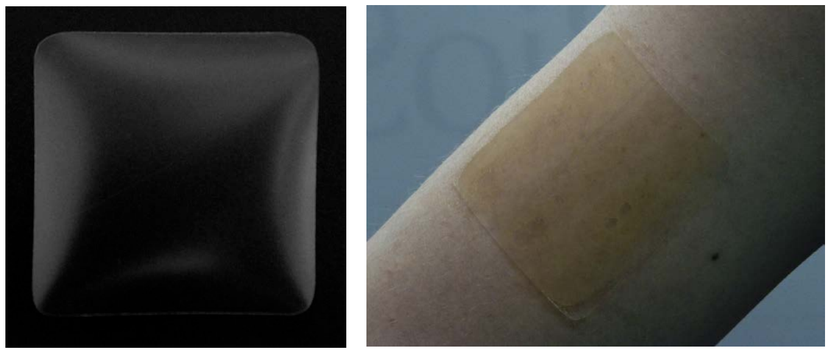


Image of transdermal films: IQP-0410 transdermal films (*left*), transdermal patch applied to arm (*right*). The patches were manufactured via solvent casting on a film applicator. The patches were cut from the drawn sheet and individually packaged in foil pouches.

IQP-0410 TRANSDERMAL FILM FORMULATION

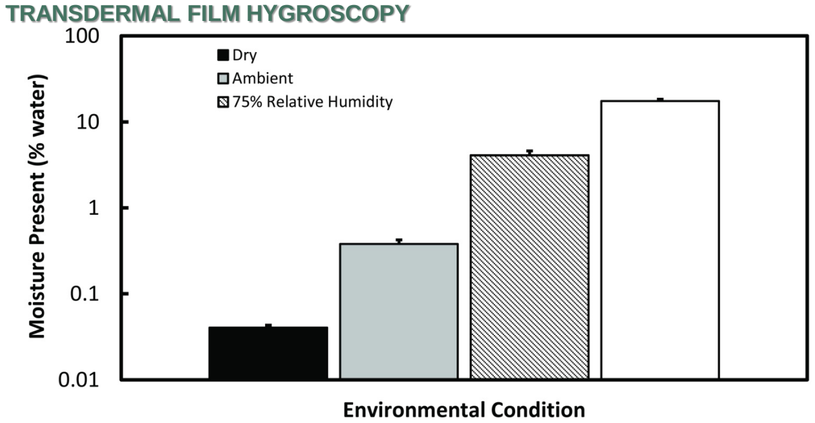
Formulation	EC (% w/w)	HPMC (%w/w)	PG (% w/w)	DnBP (% w/w)
D	60.0	20.0	8.0	12.0

% w/w weight of excipient to total dry weight of film. EC = ethyl cellulose, HPMC = hydroxypropyl methylcellulose, PG = propylene glycol, DnBP = Di-*n*-butyl phthalate

TRANSDERMAL FILM PHYSICAL PROPERTIES

Formulation	Thickness (mm)	IQP-0410 (%, w/w)	IQP-0410 Amount (µg/cm²)	Texture
D1	50	2.0	315.31 ± 6.57	Smooth; Very low tensile strength; Very high pliability
D2	100	2.0	454.49 ± 14.04	Smooth; Low tensile strength; Very high pliability
D3	150	2.0	448.14 ± 22.19	Smooth; Moderate tensile strength; Very high pliability
D4	250	2.0	667.76 ± 54.90	Smooth; Moderate tensile strength; Moderate pliability

TRANSDERMAL FILM HYGROSCOPY



The patches were placed under various environmental conditions: dessicant (black), ambient humidity (grey), 75% Relative humidity (striped), and 95% relative humidity (white). The patches remained under these conditions until a stable mass was measured. n = 3 ± standard deviation (SD)

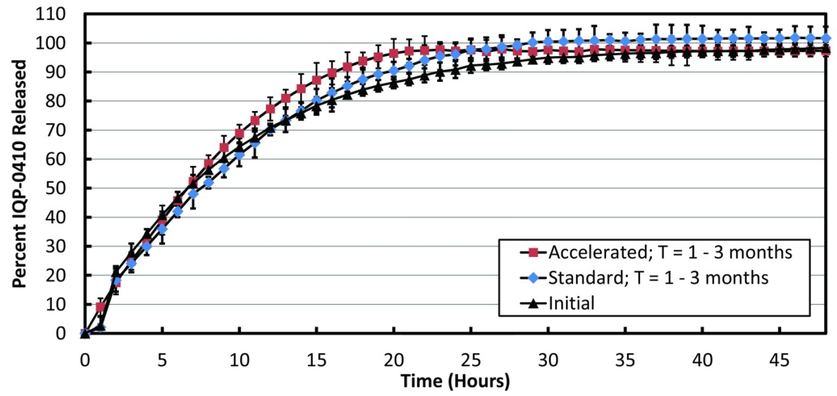
TRANSDERMAL FILM WATER CONTENT

Formulation D3	Water Content (%)	
	30°C / 65 %R.H.	40°C / 75 %R.H.
0 days	1.151 ± 0.260	
7 days	1.475 ± 0.604	1.284 ± 0.418
1 month	1.224 ± 0.234	0.561 ± 0.053
2 months	1.2979 ± 0.001	1.128 ± 0.476
3 months	0.7896 ± 0.177	0.936 ± 0.084

TRANSDERMAL FILM FORMULATION STABILITY

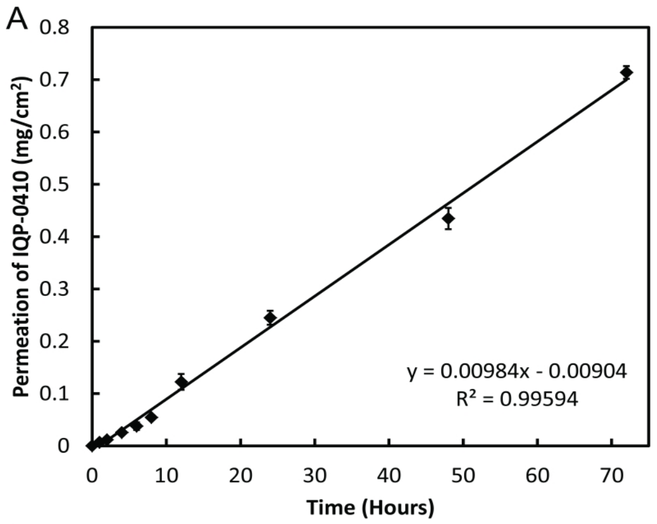
Formulation D3	30°C / 65 %R.H.		40°C / 75 %R.H.	
	IQP-0410 Recovery	Patch Uniformity (% RSD)	IQP-0410 Recovery	Patch Uniformity (% RSD)
0 days	100.15 ± 0.60%	1.022%	100.15 ± 0.60%	1.022%
7 days	93.61 ± 1.36%	1.457%	97.66 ± 3.34%	3.424%
1 month	99.54 ± 2.01%	2.894%	101.58 ± 4.78%	2.455%
2 months	122.89 ± 2.45%	1.991%	123.27 ± 9.29%	5.291%
3 months	96.53 ± 4.94%	5.118%	101.61 ± 7.27%	4.273%

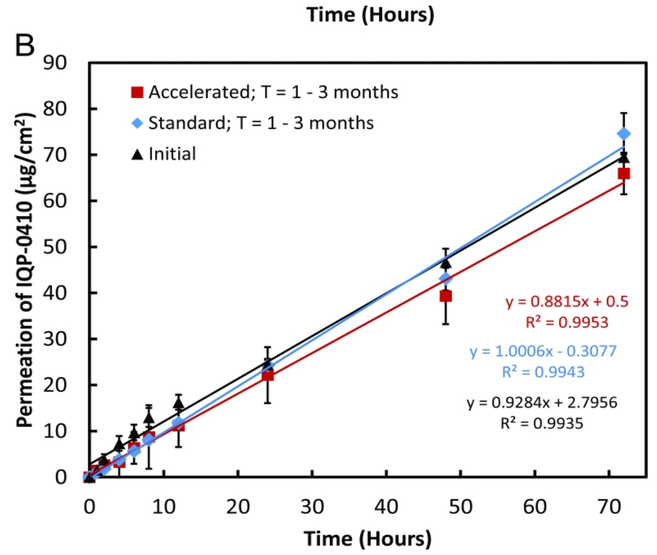
IN VITRO RELEASE AND DRUG DELIVERY



In vitro release for IQP-0410 from transdermal films. Under 90:10 EtOH-Water sink conditions, the *in vitro* release of IQP-0410 from the transdermal films were evaluated in a USP-4 apparatus for 48 hours upon initial manufacturing (black triangles) (n = 3 ± SD). The transdermal films were packaged into air-tight foil pouches and stored under standard (30˚C / 65% R.H.) and accelerated (40˚C / 75% R.H.) environmental conditions for 3 months. At 1 month, 2 months, and 3 months' time points, the transdermal films were removed from storage and tested for drug release. The combined average (n = 9 ± SD) of the transdermal films stored under standard environmental conditions for 3 months (blue diamonds). The combined average (n = 9 ± SD) of the transdermal films stored under accelerated environmental conditions for 3 months (red squares).

IN VITRO / EX VIVO PERMEABILITY OF IQP-0410 FROM TRANSDERMAL FILM FORMULATIONS





the *in vitro* release and subsequent transport of IQP-0410 from the transdermal film through a PVDF membrane over 72 hours (A) (n = 3 ± SD). The *ex vivo* release and permeability of IQP-0410 from the transdermal films through full thickness epidermal tissue over 72 hours (B – *black triangles*) (n = 3 ± SD). The combined average (n = 6 ± SD) of the transdermal films stored under standard environmental conditions for 3 months (B - *blue diamonds*). The combined average (n = 6 ± SD) of the transdermal films stored under accelerated environmental conditions for 3 months (B - *red squares*).

IN VITRO ANTI-HIV EFFICACY OF IQP-0410 FROM TRANSDERMAL FILMS

Time (days)	Antiviral Efficacy CEM-SS/HIV-1_{IIIB} EC_{50} (nM)	Antiviral Efficacy PBMC/HIV-1_{BaL} EC_{50} (nM)
Unformulated IQP-0410	2.84	1.42
1	1.705 ± 0.002	0.0568 ± 0.019
2	1.989 ± 0.164	0.0284 ± 0.009
3	2.557 ± 0.401	0.0199 ± 0.001