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In vitro and in vivo iontophoretic transdermal delivery of an anti-parkinsonian agent

V. Vemulapalli [a], Y. Yang [a], S. Siddoju [a], R. Conjeevaram [b], H. Teunissen [c], T. Myers [b], A.K. Banga [a,*]

[a] College of Pharmacy and Health Sciences, Mercer University, 3001 Mercer University Drive, Atlanta, GA 30341, USA
[b] Solvay Pharmaceuticals, Marietta, GA, USA
[c] Solvay Pharmaceuticals, Weesp, Netherlands

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ABSTRACT

To objective of this work was to study the feasibility of iontophoretic delivery of SLV 318 (7-(4-benzyl-1-piperazinyl)-2(3H)-benzoxazolone methanesulfonate) across hairless rat skin in vitro and in vivo. The effect of counter-ions and temperature were investigated for optimizing SLV 318 solubility. The effect of electrode efficiency and total current applied on the delivery of SLV 318 were studied using Franz diffusion cells and samples were analyzed using HPLC. Delivery increased with increasing concentration. For current–time combinations, electrode had to be replaced every 9 h. Passive, iontophoretic (0.1 mA/cm^2 for 1 h) and intravenous studies were performed in vivo. Blood samples collected were analyzed using LC–MS/MS. SLV 318 had higher solubility with NaCl (75 mM) as a counter-ion at 25 °C than with other counter-ions tested. In vivo iontophoresis significantly enhanced the permeation and also reduced its lag time ($P < 0.05$). The C_{max} of SLV 318 during 1 h iontophoresis was 6.56 ± 0.68 ng/mL at 1.31 ± 0.29 h (T_{max}) as compared to 2.96 ± 0.29 ng/mL at 25.32 ± 0.67 h (T_{max}) by 24 h passive permeation. The in vitro and in vivo data has shown the feasibility to enhance delivery of SLV 318 by iontophoresis.

1. Introduction

Parkinson's disease is a chronic and progressive movement disorder caused by decreased dopamine levels in the brain. Depression occurs approximately in 40% of the patients with Parkinson's disease (Cummings, 1992). SLV 318 mesylate is a new synthetic compound and the chemical nomenclature of SLV 318 mesylate is (7-(4-benzyl-1-piperazinyl)-2(3H)-benzoxazolone methanesulfonate, mesylate) (Fig. 1). SLV 318 is a mesylate salt with two pK_a's (pK_a1: 7.0, pK_a2: 8.6) and the molecular weight is 405.48 Da. SLV 318 is freely soluble in water (solubility in water: 38 mg/mL) and with log P (octanol/water) of 3.2. SLV 318 is a potent agonist of the dopamine D_2 receptor and is predicted to show clear and potent anti-parkinsonian activity in humans combined with potential anti-depressant efficacy (Olanow et al., 2006; Hesselink et al., 2003; Nutt, 2007). SLV 318 has the potential to induce orthostatic hypotension in humans via a dopamine D_2 receptor mediated mechanism. Following intraperitoneal administration, SLV 318 showed an especially large dose range activity, i.e. 300-fold with no adverse activity displayed across vast dose (Lieberman, 2004). SLV 318 was delivered through skin to avoid first pass hepatic metabolism and poor oral bioavailability because of its mesylate salt form (Li et al., 2005b). To overcome these side effects and to deliver SLV 318 non-invasively over large dose range, transdermal route is preferred.

The main rate limiting barrier for transdermal delivery of hydrophilic molecules is the stratum corneum. To overcome the stratum cornuem barrier, various enhancements techniques such as iontophoresis, electroporation, laser ablation, ultrasound and microneedles are used (Banga, 2011). Iontophoresis is a non-invasive enhancement technique, which utilizes small electric current to deliver ionized drug molecules into skin (Kalia et al., 2004). It provides the advantages of improved patient compliance, avoids first pass metabolism, controlled release of drug from the patch and the possibility of programmed delivery (Nair et al., 1999; Naik et al., 2000).

Non-invasive techniques such as transepidermal water loss (TEWL), chromamter, Laser Doppler Velocimetry (LDV) were used to evaluate skin integrity during iontophoresis. Measurement of transepidermal water loss (TEWL) across the stratum corneum yield important information regarding the barrier functions of the skin and its integrity (Anigbogu et al., 2000). Skin irritation can produce an erythema, changes in skin color were measured with the help of chromameter and measurements of subtle changes in dermal microcirculation at the treated sites were made using Laser

* Corresponding author. Tel.: +1 678 547 6243; fax: +1 678 547 6423.
E-mail address: banga_ak@mercer.edu (A.K. Banga).

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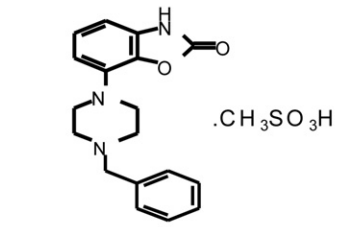

SLV 318: 7-(4-benzyl-1-piperazinyl)-2(3H)-benzoxazolone methanesulfonate

MW : 405.48	MP (DSC) – 270.80C
pKa1: 7.0	log P : 3.2 (Octanol/water)
pKa2: 8.6	Solubility in water - 38 mg/ml

Fig. 1. Structure and physico-chemical properties of SLV 318.

Doppler Velocimetry (LDV) (Hirvonen et al., 1993; Tanojo et al., 1999).

In this study, delivery of a new chemical molecule SLV 318, a potent dopamine agonist, was studied using iontophoresis. The first objective of this work was to study the influence of counter-ions and temperature on SLV 318 solubility in citrate-phosphate buffer, as SLV 318 is available as a mesylate form and then to study the effect of current density, altering electrode (electrode efficiency) and drug concentration on SLV 318 in vitro using freshly excised hairless rat skin. The second objective was to evaluate the feasibility to deliver SLV 318 in vivo and to characterize iontophoretic permeation kinetics of SLV 318 using a hairless rat model, since this has not been investigated previously. The skin irritation response and barrier function following iontophoretic application were also investigated.

This study investigates the feasibility to deliver a new chemical molecule starting with solubility studies, and then performing in vitro and in vivo work. The findings will also help in maximizing the therapeutic dose that can be delivered using iontophoresis.

2. Materials and methods

2.1. Materials

SLV 318 was obtained from Solvay Pharmaceuticals (Marietta, GA, USA). Dibasic sodium phosphate, citric acid and sodium chloride (HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Trans-Q® 1-GS iontophoretic drug delivery electrodes were obtained from IOMED, Inc. (Salt Lake City, UT, USA) and constant current source (Keithley®, Cleaveland, OH, USA).

2.2. Animals for in vitro and in vivo studies

Hairless rats (300–400 g) were obtained from Charles River (Wilmington, MA, USA), and were housed in the in-house animal facility at Mercer University. The studies were conducted as per the protocol approved by the Institutional Animal Care and Use Committee (IACUC) at Mercer University.

2.3. In vitro permeation studies

Franz diffusion cells were used for the in vitro permeation studies. The donor half was exposed to room temperature while the receptor half was maintained at 37 °C. Receptor compartment was continuously stirred to maintain sink conditions. Freshly excised hairless rat skin was mounted on the vertical diffusion cells for these studies. Drug solution was placed in the donor compartment and5 mL citrate phosphate buffer of pH 4.0 was added into the receptor compartment. SLV 318 has a positive charge at pH 4 and was hence delivered under anode. A silver wire was used as the anode in the donor and a silver/silver chloride wire was used as the cathode in the receptor compartment. Samples were taken at pre-determined time intervals from the receptor compartment and analyzed using HPLC method.

2.4. Effect of counter-ion and temperature on the solubility of SLV 318

The effect of various counter-ions (KBr, $MgCl_2$, and $CaCl_2$ at 37.5, 50 and 75 mM) and temperature (4 °C and 25 °C) on solubility of SLV 318 was investigated at pH 4 in citrate–phosphate buffer. Following this, the effect of NaCl as a counter-ion at 0, 50 and 75 mM at 4 °C and 25 °C for optimizing SLV 318 solubility was investigated. The samples were analyzed using HPLC.

2.5. Effect of electrodes

To determine the efficiency of the electrode for long periods of iontophoresis with a current density of 0.1 mA/cm^2, a study was performed with one electrode for 18 h. A drug concentration of 3 mg/mL was used for this study and sampling was performed at regular intervals until the end of 24 h.

2.6. Effect of drug concentration

Effect of SLV 318 drug concentration on the delivery across hairless rat skin was studied at 1 mg/mL, 5 mg/mL and 10 mg/mL at 0.5 mA/cm^2 current density for 4.5 h. Donor compartment was filled with 1 mL of 1 mg, 5 mg and 10 mg drug during these studies. Samples were taken from the receptor at pre-determined time intervals until 24 h and analyzed using HPLC.

2.7. Effect of current density

The effect of current density on the delivery of SLV 318 was studied to see the effect of different current density–time combinations applied (0.1 mA/cm^2 for 18 h, 0.2 mA/cm^2 for 9 h, or 0.4 mA/cm^2 for 4.5 h) on the amount of drug delivered and also to compare to passive delivery of SLV 318. The donor SLV 318 concentration of 3 mg/mL was used for this study. For passive delivery, freshly excised hairless rat skin was mounted on the vertical diffusion cells with stratum corneum facing the donor compartment and drug solution was placed in the donor compartment and 5 mL citrate phosphate buffer (pH 4.0) was placed into the receptor compartment. Samples were taken from the receptor at pre-determined time intervals until 24 h and analyzed using HPLC.

2.8. HPLC method for in vitro receptor samples

SLV 318 was separated from its impurities by HPLC on an inertsil ODS column. SLV 318 and its known impurities were detected by UV-absorbance at 243 nm. The mobile phase was prepared by dissolving 1.54 g of ammonium acetate in 460 mL of water (pH was adjusted to 4.6 using acetic acid) and mixed with 540 mL of methanol. The mobile phase flow rate was set at 0.5 mL/min at a column temperature of 40 °C and equilibrated for 60 min before the start of analysis. Receptor samples were analyzed on Waters Alliance HPLC. Standard curve was the in the range of 0.15–25 μg/mL with an r^2 value of 0.9998.

2.9. In vivo studies

Hairless rats were anesthetized using intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). Trans-Q® patch (Iomed, UT) which utilizes Ag/AgCl electrode was filled with 1.5 mL of SLV 318 mesylate solution (3 mg/mL, base) in citrate-phosphate buffer (pH 4.0). The GelSponge® patch was then applied on the abdominal region of the rat. For iontophoretic delivery experiments, dispersive pad was applied next to the iontophoretic patch and a current density of 0.1 mA/cm^2 was applied for 1 h using constant current source. Each experiment was performed in triplicate. Blood samples were collected from the tail vein into the plasma microtainer tubes at predetermined time intervals during and after the iontophoresis period and the plasma containers were centrifuged at 7200 × *g* for 10 min. The supernatant plasma was analyzed by LC–MS/MS. For obtaining pharmacokinetic parameters, an intravenous dose of the SLV 318 mesylate in an isotonic solution (0.1 mg/kg base) was administered into the femoral vein of the rats. Blood samples were collected for 5 h, centrifuged at 7200 × *g* for 10 min. The supernatant plasma was analyzed by LC–MS/MS.

Skin irritation was measured using TEWL, chromameter and Laser Doppler Velocimetry (LDV) before and patch application. TEWL, chromameter and LDV measurements were taken after cleaning the patch area and at 30 min after removing the patch.

2.10. Chromameter

The chromameter (Minolta, Japan) uses a three-dimensional coordinate system with a brightness axis (L^*), a red-green axis (a^*), and a blue-yellow axis (b^*). All these values were used to determine the true color of the skin and a composite skin irritation index. In this study, however, only a^* observations were used because they provide a measure of erythema. The chromameter readings were measured before and after patch application.

2.11. Transepidermal water loss (TEWL)

TEWL values were recorded at three different areas before and after patch application using DermaLab® TEWL (cyberDERM, Inc., PA). TEWL measurement was based on the vapor gradient using the open chamber, which maintains the natural evaporation from the surface being measured without influencing the environment over the measurement area. The probe was held in position for 30 s to obtain a stable value before recording the TEWL at each site. The units of measurement for TEWL are g/m^2 h.

2.12. Laser Doppler Velocimetry (LDV)

Laser Doppler Velocimetry (PeriFlux System 5000, Perimed AB, Sweden) was used to measure skin blood flow before and after patch application at the site of iontophoresis. The laser light is transmitted through a fiber optic probe and undergoes a shift when it hits the moving blood cells, and this shift correlates to the change in blood perfusion. The perfusion measurement was made at one spot on the patch application site.

2.13. Sequence of measurements

Before patch application, visual scoring was done first followed by Chromameter, TEWL and LDV measurements. Following patch removal, the same sequence of measurement was repeated.

2.14. Sample extraction and LC–MS/MS analysis of plasma samples

A $^{13}C_4$-labeled form of SLV 318 was used as the internal standard. Plasma samples, 50 μL, were transferred to a 96-well plate and spiked with 25 μL of the appropriate calibration spiking standard and/or internal standard solution. Quality control samples were also prepared at 3 levels (4, 25, and 75 ng/mL). The well plate was transferred to a Quadra 3 (TomTec) platform for processing, where subsequent additions of reagents took place. Acetic acid (100 μL, 0.01 M) was added to all wells on the sample plate. An MP1 SPE 96-well plate (Varian) was preconditioned with 250 μL MeOH followed by 250 μL 0.01 M acetic acid. Samples were transferred from the sample plate to the extraction plate and allowed to elute completely. The extraction plate was then washed with 250 μL 75:25 0.01 M acetic acid: MeOH and eluted with 250 μL 49:49:2 H_2O:MeOH:NH_4OH, with 750 μL 0.01 M acetic acid added to the eluted volumes.

The HPLC system consisted of an Agilent HP1100 binary pump/vacuum degasser and an HTS PAL autosampler (CTC Analytics). The analytical column was a Phenomenex Luna C-18, 5 μm, 50 mm × 2.0 mm, with a flow rate of 100 μL/min. The mobile phase consisted of 75:25 10 mM ammonium acetate (pH adjusted to 4):acetonitrile. The mass spectrometer was a Waters Quattro Ultima triple quadrupole mass spectrometer, operated in positive electrospray MS/MS mode. The calibration range was 1–200 ng/mL. A quadratic calibration model was applied with $1/x^2$ weighting. The mean coefficient of determination (R^2) was 0.9995. Accuracy of the quality control samples and calibration standards was within ±15% in all cases except one (one low QC at −19%).

2.15. Pharmacokinetic data analysis

Plasma concentration vs. time profile from intravenous injection and transdermal delivery of SLV 318 were analyzed using non-compartmental analysis (NCA Model 201 Winonlin® version 4.0, Pharsight, CA). Pharmacokinetic parameters such as AUC_{0-inf}, AUC_{all}, T_{max}, and C_{max} were calculated. Clearance obtained from intravenous (i.v.) data was used to calculate the dose delivered by iontophoretic and passive delivery by the following equation.

$$\text{dose delivered} = AUC_{0-\text{inf}} \times Cl_{IV}$$

2.16. Statistical analysis

All values were presented as mean ± SE. Statistical analysis of the data obtained pre and post-treatment were done using Student's paired *t*-test, and the data obtained from different groups were done using ANOVA. The level of significance was taken as $P \leq 0.05$.

Table 1
Solubility of SLV 318 with different counter-ions at 75 mM and 37.5 mM.

Counter-ions	Solubility of SLV 318 (μg/mL)	
	75 mM, 25 °C	37.5 mM, 25 °C
$MgCl_2$	155.24	296.80
$CaCl_2$	248.94	297.69
KBr	138.79	297.91

3. Results and discussion

3.1. Solubility studies

The counter-ions at anode (Ag wire) are necessary to initiate the electrochemistry and sodium chloride is the most commonly used counter-ion. Sodium chloride ionizes to produce Na^+ which competes with drugs to carry positive charge, whereas Cl^- reacts with Ag wire to form AgCl (Phipps et al., 1989; Sage and Riviere, 1992). The electrochemistry at anode and cathode is as follows:

$$\text{Anode}: \quad Ag + Cl^- \rightarrow AgCl + e^-$$

$$\text{Cathode}: \quad AgCl + e^- \rightarrow Ag + Cl^-$$

When NaCl was added to the drug solution, precipitate formation was observed and eventually the concentration of SLV 318 decreased. To increase the solubility of SLV 318, counter-ions like $MgCl_2$, $CaCl_2$ and KBr at 75 mM and 37.5 mM were investigated (Table 1). Although different counter-ions were used to prevent precipitation, adding these counter-ions to SLV 318 solution resulted in precipitation as also reported for some other drugs (Mudry et al., 2006; Murdock, 1999). This can be possible due to conversion of mesylate salt to hydrochloride salt, and hydrochloride salt in turn has low solubility, which in turn can result in the formation of precipitate (Li et al., 2005a,b). Decreasing the counter-ion concentration from 75 mM to 37.5 mM decreased the precipitation by increasing the solubility of SLV 318 in citrate-phosphate buffer and this type of observation has been reported in the literature (Nugroho et al., 2004).

The samples with NaCl 50 mM and 75 mM stored at 4 °C showed precipitation and the samples at room temperature did not show any precipitation (Table 2). Thus all the in vitro samples were stored at room temperature to avoid any precipitation. In all the counter-ions the samples with sodium chloride had less precipitation when compared to with other counter-ions. The samples of SLV 318 with NaCl 50 mM at room temperature had the highest solubility among others. In the following experiments, 50 mM NaCl was used as counter-ion along with SLV 318 in the donor compartment.

3.2. In vitro permeation studies

The advantage of using Ag wire as anode and Ag/AgCl as cathode is that Ag/AgCl electrodes avoid change in pH. The drug is positively charged at pH 4.0 and was delivered under anode using electrorepulsion. The current density of 0.1 mA/cm^2 was applied for 18 h to study the effect of current density on the delivery of SLV 318 (Fig. 2). The flux decreased after 9 h and the decrease in flux was found to be due to the saturation of electrode material (AgCl) at the electrode surface. Therefore, in subsequent studies, the electrode was changed every 9 h. When the electrode was changed at 9 h, the average flux continued to increase after 9 h (data not shown).

Table 2
Solubility of SLV 318 with different concentrations of NaCl at 25 °C and 4 °C.

	Solubility of SLV 318 (μg/mL)		
NaCl	0 mM	50 mM	75 mM
25 °C	294.08	298.84	290.03
4 °C	282.52	284.41	204.53

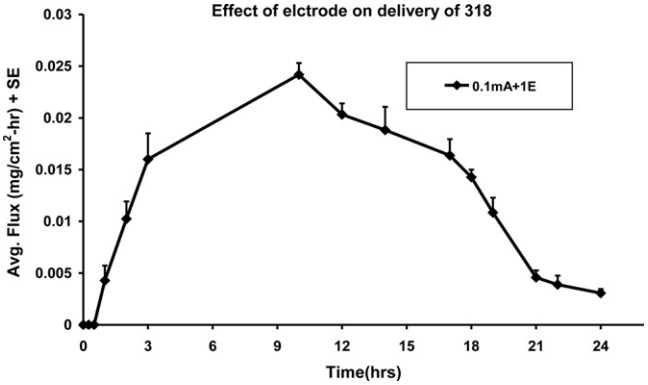

Fig. 2. Linear increase in flux during iontophoresis till 9 h when the flux starts to drop due to depletion of electrode.

In order to maintain higher flux with a current density at 0.5 mA/cm^2 for 3 h the drug concentration was increased from 1 mg/mL to 5 mg/mL (Fig. 3). However, when the drug concentration was further increased to 10 mg/mL, there was no significant increase in cumulative amount of the SLV 318 delivered from 5 and 10 mg/mL concentration during the first 3 h, but the delivery from 10 mg/mL was slightly higher ($P > 0.05$) than 5 mg/mL at 24 h (Bouwstra et al., 2009). The possible reason could be that the conversion of SLV 318 mesylate salt to hydrochloride salt in the presence of sodium chloride was higher at 10 mg/mL than 5 mg/mL (Li et al., 2005a,b).

The effect of current density and time of application on the delivery of SLV 318 was studied in vitro and the total amount of current delivered was kept constant (1.8 mA/cm^2 in 18 h), but the current density was varied with time of application (0.4 mA/cm^2 for 4.5 h, 0.2 mA/cm^2 for 9 h or 0.1 mA/cm^2 for 18 h) (Fig. 4). The initial delivery of drug with 0.4 mA/cm^2 was higher than both 0.2 and 0.1 mA/cm^2, but the cumulative amount delivered at 24 h had no significant difference between three different current densities and time of application. Therefore, the initial amount of SLV 318 being delivered is influenced by the current density, but the total amount of SLV 318 delivered is determined by total current applied. For future studies, current density and duration can be adjusted to reach the programmed drug delivery from the TranQ® patch.

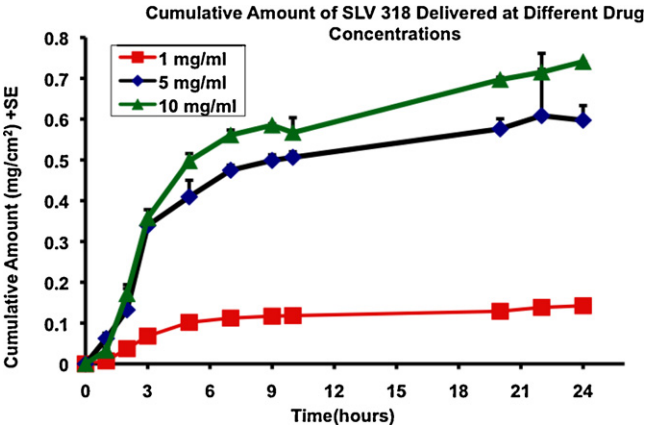

Fig. 3. Effect of varying concentration of SLV 318 on cumulative amount of drug delivered at 0.5 mA/cm^2.

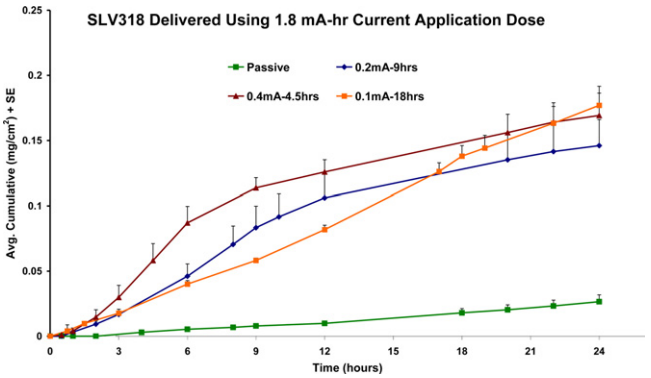

Fig. 4. Effect of total constant current applied on the delivery of SLV 318.

Table 3
Pharmacokinetic parameters of SLV 318 after i.v. administration. Pharmacokinetic parameters of SLV 318 after i.v. administration.

Parameters	Units	Estimate (mean ± SE)
$T_{1/2}$	h	1.47 ± 1.12
AUC_{0-t}	ng h/mL	35.79 ± 3.17
AUC_{0-inf}	ng h/mL	38.36 ± 3.43
Vz	mL	1888.98 ± 520.16
CL	mL/h	891.64 ± 82.04

Table 4
Pharmacokinetic parameters of SLV 318 after passive and iontophoretic delivery.

Parameter	Units	Passive	Iontophoresis
Cmax	ng/mL	2.96 ± 0.29	6.56 ± 0.68
T_{max}	h	25.32 ± 0.67	1.31 ± 0.29
AUC_{all}	ng h/mL	16.90 ± 3.02	25.07 ± 3.64
AUC_{0-inf}	ng h/mL	32.67 ± 8.72	32.78 ± 8.73
Dose delivered	ng	29,130.88 ± 7776.5	29,224.20 ± 7787.06

3.3. *In vivo studies*

SLV 318 was administered intravenously in hairless rats and the plasma concentration–time profile (Fig. 5) was analyzed using non-compartmental approach Winonlin® (Chaturvedula et al., 2005; Stagni and Shukla, 2003), and the primary pharmacokinetic parameters were obtained as shown in Table 3. After an initial distribution phase, SLV 318 concentration in the plasma declined with a half-life of 1.47 h. Fig. 6 shows the plasma concentration of SLV 318 after iontophoretic and passive delivery. The patch was applied for 24 h in passive permeation and for 1 h with 0.1 mA/cm^2 current density during iontophoresis. As shown in Table 4, the C_{max} of SLV 318 delivered by 1 h iontophoresis

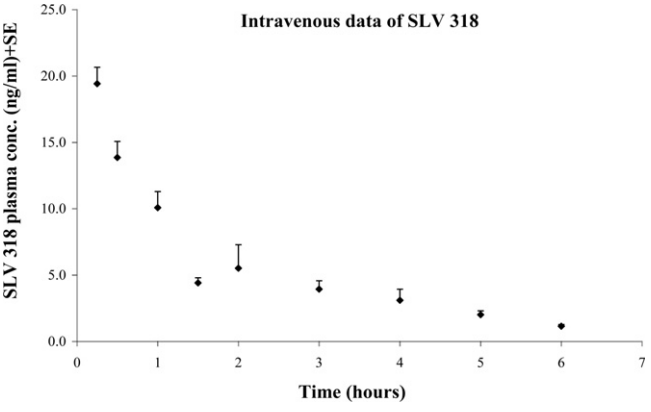

Fig. 5. Plasma concentration after intravenous administration of SLV 318.

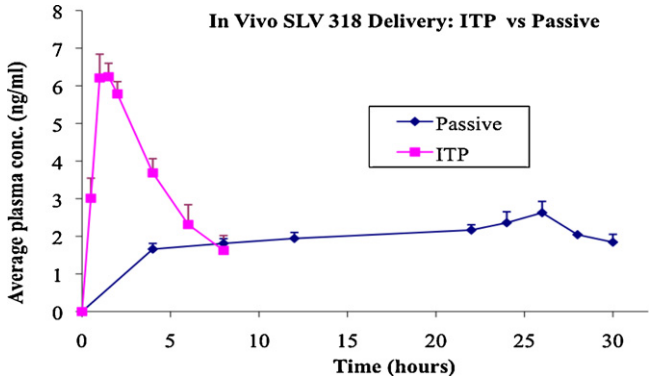

Fig. 6. Plasma concentration after passive and iontophoretic delivery of SLV 318 in vivo.

reached to 6.56 ± 0.68 ng/mL at 1.31 ± 0.29 h (T_{max}) as compared to 2.96 ± 0.29 ng/mL at 25.32 ± 0.67 h (T_{max}) delivered by 24 h passive permeation. The total dose delivered by passive and iontophoresis permeation showed approximately same amount in two groups (29,130.88 ± 7776.5 ng for passive, 29,224 ± 7787.06 ng for iontophoresis). The reason is due to the long patch application period for passive permeation studies (24 h) while the iontophoretic patch was removed after 1 h. Although the total delivered amount was similar between passive and iontophoresis groups, iontophoresis enhanced the efficiency of the delivery of SLV 318.

From a comparison of efficacy with pharmacokinetic data from the MPTP marmoset obtained in a separate study (data not shown), therapeutic efficacy of SLV 318 is expected at a plasma exposure of 2 ng/mL. As shown in Fig. 6, the plasma SLV 318 concentration delivered by iontophoresis reached 3 ng/mL at 0.5 h while the passive permeation needed 12 h to reach 2 ng/mL.

In comparison of plasma concentration of SLV 318 at 1 h delivery, iontophoresis (6.5 ng/mL) increased the concentration by approximately 10-fold as compared with 24 h passive permeation (0.5 ng/mL). All these results demonstrated the feasibility of iontophoresis in achieving faster and higher delivery of SLV 318 in vivo. The skin irritation and barrier function were measured using a chromameter, LDV and DermaLab® TEWL system. The differences in values taken before patch application and after patch removal were used to quantify the skin irritation. In this study neither passive nor iontophoretic delivery of SLV 318 produced any significant erythema nor any increased transepidermal water loss (data not shown).

4. Conclusions

Iontophoresis has shown the feasibility to deliver SLV 318 both in vitro and in vivo. Solubility of SLV 318 was affected by the strength and type of counter ions. 50 mM NaCl as counter-ions at room temperature provided the highest solubility of SLV 318 among the counter-ions. Changing the current density and time of application by keeping the applied current constant did not significantly change the amount of SLV 318 delivered at the end of 24 h. SLV 318 did not cause any irritation and erythema in vivo during ITP and passive study.

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