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Research Paper

Dermal, Subdermal, and Systemic Concentrations of Granisetron by Iontophoretic Delivery

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Purpose. The purpose of this work was to demonstrate the iontophoretic delivery of granisetron hydrochloride by novel, self-contained iontophoretic patches and to determine the subcutaneous and dermal absorption kinetics using microdialysis.
Methods. *In vitro* iontophoretic delivery of granisetron hydrochloride was evaluated at 5, 10, or 20 mg/ml concentrations of donor using Franz diffusion cells and hairless rat skin as a membrane. *In vivo* studies were performed in hairless rats. Animals received either subcutaneous or dermal microdialysis probes and iontophoretic patches filled with drug formulation were applied on the abdominal area such that the probe lies below the anode chamber. Blood and microdialysate samples were collected at different time intervals. Intravenous administration of granisetron was also done to determine the basic pharmacokinetic parameters.
Results. Iontophoretic patches delivered current constantly throughout the patch application. The patches delivered granisetron hydrochloride at a rate of 14.91 ± 4.53 μg/min/kg. Similar concentrations of granisetron hydrochloride in dermal and subcutaneous tissue were observed. Depot formation was identified in the subcutaneous and dermal profiles, indicating that subcutaneous structures are also responsible for the depot formation of the drug in the dermis.
Conclusion. The patches successfully delivered granisetron hydrochloride by iontophoresis and depot formation was observed in the dermal and subcutaneous structures in the skin.

KEY WORDS: granisetron; iontophoresis; microdialysis; pharmacokinetics.

INTRODUCTION

Iontophoresis involves application of small amounts of current to push charged drug molecules through skin, resulting in higher fluxes of the drug molecules for which otherwise permeation through skin is negligible. It provides the advantages of improved patient compliance, avoidance of first pass metabolism, controlled rate of drug release from the patch and the possibility of programmed delivery (1,2). The present study utilizes a self-contained iontophoretic patch with built-in electrodes, which simplifies the bulky set-up of the commercially available iontophoretic devices.

Microdialysis is a sampling technique used to measure tissue concentrations of drugs in pharmacokinetic and metabolism studies. The simultaneous estimation of the pharmacokinetics in skin and plasma helps in understanding the kinetic relationship between absorption at the two sites (3) and gives an understanding of drug permeation before entering the systemic circulation. Dermal absorption caused by iontophoresis was previously investigated using microdialysis for the evaluation of dermal kinetics of various drugs including enoxacin, diclofenac, and acyclovir (4–6). Dermal microdialysis has also been tested in human volunteers to show the kinetics of propranolol after iontophoresis (7). In all these studies, microdialysis probes were inserted intradermally.

Subcutaneous microdialysis has been reported in various pharmacokinetic studies to measure drug kinetics in extracellular fluid (ECF). Subcutaneous tissue is homogenous in nature and the ECF is in constant equilibrium with the systemic circulation. The subcutaneous microdialysis technique is also considered to be easier than dermal microdialysis (8). With dermal microdialysis, probe insertion and control over insertion depth are difficult, and the probe itself can increase skin thickness (9,10).

Granisetron (MW 348.9, $K_{o/w}$ 0.38, pKa 9.4) is a selective 5-hydroxytryptamine-3 (5-HT3) receptor antagonist shown to be effective in treating nausea and vomiting

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ABBREVIATIONS: AUC, area under the curve; ECF, extracellular fluid; HPLC, high-performance liquid chromatography; NCA, noncompartmental analysis; RF, recovery factor.

induced by cancer chemotherapy. Intravenous infusion of 40 μg/kg of granisetron is effective to prevent nausea and vomiting for repetitive chemotherapeutic regimens. Granisetron is very potent, hydrophilic in nature and has high first-pass hepatic metabolism (11), thus making it a good candidate for transdermal iontophoretic delivery. Recently a report was published on transdermal delivery of granisetron by radiofrequency-driven skin microchanneling (12).

The aims of this study are to (1) evaluate the feasibility of delivering granisetron hydrochloride *in vitro* and *in vivo* by the self-contained and novel iontophoretic patch and (2) characterize iontophoretic permeation kinetics of granisetron using subcutaneous and dermal microdialysis sampling. The present report is the first known attempt to deliver granisetron by iontophoresis.

MATERIALS AND METHODS

Animals

Male CD-hairless rats (Charles River, Wilmington, MA) weighing 290–350 g were used. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication 85-23, revised in 1985). Food and water were provided *ad libitum.* The average number of replicates for each study was four.

Chemicals

Granisetron hydrochloride was purchased from Ultratech SPC PVT Ltd. (Mumbai, India). Sterile and pyrogen-free 0.9% sodium chloride USP was purchased from Baxter Healthcare Corporation (Deerfield, IL). Water, acetonitrile, glacial acetic acid, and sodium acetate were purchased from Fisher. All solvents used were of high-performance liquid chromatography (HPLC) grade.

Microdialysis System

A CMA 102 microdialysis pump with CMA 142 microfraction collector (CMA/Microdialysis AB, Stockholm, Sweden) was used. CMA 20 microdialysis probes (CMA/Microdialysis AB, Stockholm, Sweden) with 10-mm polycarbonate membrane, 20-kDa molecular weight cut off were used for subcutaneous insertion. Linear microdialysis probes (BASi, West Lafayette, IN) with 10-mm membrane window were used for the insertion into dermis.

Iontophoretic Patch—Principle of Operation

The Wearable Electronic Disposable Drug delivery (WEDD™) devices were obtained from Travanti Pharma, Inc., and were constructed of electrodes, supplemental power sources, internal resistor, absorbent pads, and medical adhesive tapes. For the electrodes, specific amounts of Zn and AgCl were coated on the anodes and cathodes respectively, and these electrodes were connected through an electrically conductive material.

The electrochemistry involved at the electrode interfaces was:

$$\mathrm{Zn} \rightarrow \mathrm{Zn}^{++} + 2\mathrm{e}^{-}(\text{At anode})$$
$$\mathrm{AgCl} + \mathrm{e}^{-} \rightarrow \mathrm{Ag} + \mathrm{Cl}^{-}(\text{At cathode})$$

The 1-volt potential provided by the electrode coatings was supplemented by an amplifier using a 3 V lithium (Li) battery, resulting in a total applied potential of 4 V. An internal series resistance of 10 KΩ was connected to reduce rate variability in the current due to the differences in skin resistances between individual rats. Alternatively, a series of Zn/AgCl electrodes can be utilized to completely substitute the Li battery.

In Vitro Iontophoretic Delivery of Granisetron Hydrochloride

In vitro permeation of granisetron hydrochloride by iontophoresis was evaluated using vertical Franz diffusion cells and freshly excised rat skin as membrane. The receptor chamber was filled with 0.9% NaCl solution and temperature was maintained at 38°C. Skin was mounted between the receptor and donor chambers of the Franz-diffusion cells. The donor chamber was filled with 500 μl of 5, 10, or 20 mg/ml granisetron in water. The pH of the formulation was observed to be between 4.0 and 4.5. For iontophoresis, proprietary Zn and AgCl electrodes were used as anode and cathode, respectively. The Zn electrode was placed in the donor chamber such that the electrode was immersed in the solution phase approximately 5 mm from the skin surface. Care was taken to avoid any contact between anode and the skin to avoid any high local voltage. The AgCl electrode was placed in the sampling port of the receptor chamber. Electrodes were connected in series and a constant current source (Keithley 2400 SourceMeter®, Keithley Instruments Inc., Cleveland, OH) was connected to the electrodes and 0.2 mA current was applied for a period of 2 h and samples (250 μl) were collected with replacement of receptor buffer at 0, 10, 30, 50, 70, 90, 110, and 130 min and stored at −20°C until analyzed by HPLC.

Surgical Procedures

Implantation of Subcutaneous Microdialysis Probe

The animals were anesthetized using intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). Each animal received two microdialysis probes, one at ventral, and another at dorsal subcutaneous region. For the placement of the probe, skin was pinched and an introducer needle (25G) with split tubing was inserted subcutaneously horizontal to the skin. The introducer was then removed, keeping the split tubing in the subcutaneous space. A microdialysis probe was inserted into the split tubing. By splitting the tubing, microdialysis probe was placed in the subcutaneous space and sutured with nonabsorbable sterile nylon sutures to the skin. A 1-h recovery period was provided after insertion of the probe and during this time probes were perfused with 0.9% NaCl.

Implantation of Dermal Microdialysis Probe

Animals were anesthetized as described previously and the abdominal area was washed and dried. Then, a 25G needle was inserted intradermally at the abdominal area and care was taken during insertion to ensure that the probe was inserted as superficially as possible. Then the microdialysis probe was passed through the needle and pulled with the needle through the skin such that probe lies in the skin. The openings in the skin created due to needle insertion were sealed using a Wetbond® (3M animal care products, MN) tissue adhesive which served to fix the microdialysis probe in skin.

Microdialysis Probe Recovery

For *in vitro* recovery estimation, probes were perfused with 0.9% NaCl for 30 min before starting the recovery experiment to remove the glycerol present in the probe. Then, the receptor chamber of Franz-diffusion cell was filled with 5 ml of 250 ng/ml of granisetron in 0.9% NaCl and temperature was maintained at 38°C (rat body temperature). Probes were placed over the receptor chamber and connected to tubing and then connected to the CMA 102 microdialysis pump. Probes were perfused at 2 μl/min and microdialysate samples were collected every 20 min. Samples were immediately analyzed by HPLC. Recovery factor (RF) was calculated by the following formula:

$$RF = \frac{C_m}{C_r} \quad (1)$$

where

C_m = concentration of microdialysate
C_r = concentration of receptor solution

After probe implantation, a 1-h recovery period was provided before the calibration was started. During this time, probes were perfused with 0.9% NaCl solution at 2 μl/min. *In vivo* probe recovery was determined by retrodialysis. In this method, probes were perfused at 2 μl/min with 250 ng/ml of granisetron in 0.9% NaCl solution and microdialysate samples were collected every 20 min and analyzed by HPLC. The recovery was performed until three constant values were obtained for recovery. RF was calculated by the following formula:

$$RF = \frac{C_p - C_d}{C_p} \quad (2)$$

where

C_p = concentration of perfusate
C_d = concentration of microdialysate

For dermal microdialysis probes, only *in vivo* recovery was calculated, while for subcutaneous microdialysis probes, both *in vitro* and *in vivo* recoveries were calculated.

***In Vivo* Iontophoretic Delivery of Granisetron Hydrochloride**

Animals implanted with subcutaneous or dermal microdialysis probes were used. Microdialysis probes were perfused for 30 min with perfusion fluid after determining the probe recovery. Then, the WEDD™ device, filled with 500 μl of granisetron hydrochloride in water (10 mg/ml) at the anode and 0.9% NaCl solution at the cathode, was applied onto the abdominal area such that the microdialysis probes lay below the anode chamber of the patch. The anode and cathode were connected to an amplifier in series and current flow was monitored using a multimeter during the patch application period. Patches were applied for 2 h. Microdialysate samples were collected every 20 min and analyzed by HPLC. Blood samples (300 μl) were also collected at different time intervals from the tail vein and serum was collected after clotting. Serum samples were stored at −20°C until analyzed by HPLC. At the end of each experiment, animals were killed using a CO_2 chamber. Skin sections from the probe insertion areas of the dermal microdialysis group were collected for microscopy. For the subcutaneous microdialysis group, the skin was cut and visually observed for the probe placement after the animal was killed. Control, passive experiments were also done with the same protocol, but the patches used were without the electrodes.

Intravenous Administration of Granisetron Hydrochloride

Animals were anesthetized as described previously. Granisetron hydrochloride in water for injection was injected (3 mg/kg dose) into the femoral vein of the animal and blood samples were collected from tail vein at different time intervals. Serum was collected after clotting and stored at −20°C until analyzed by HPLC.

HPLC Analysis

A Waters® Alliance HPLC system with Empower® software and a Waters® 2475 fluorescence detector were used. A Spherisorb Cyano column (4.6 × 250 mm) was used. Samples were eluted in acetate buffer (25 mM)–acetonitrile (72:28) at a flow rate of 1 ml/min. The fluorescence detection was done at 305 nm and 350 nm as excitation and emission wavelengths, respectively and gain of the detector was set at 1. Serum samples were extracted with toluene and evaporated to dryness under nitrogen and reconstituted in mobile phase and injected into the column. Extraction efficiency was found to be more than 85%. *In vitro* samples were injected directly

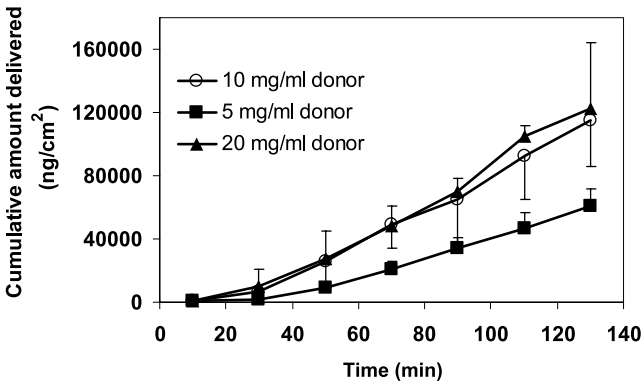

Fig. 1. *In vitro* iontophoretic delivery of granisetron hydrochloride (average ± SD).

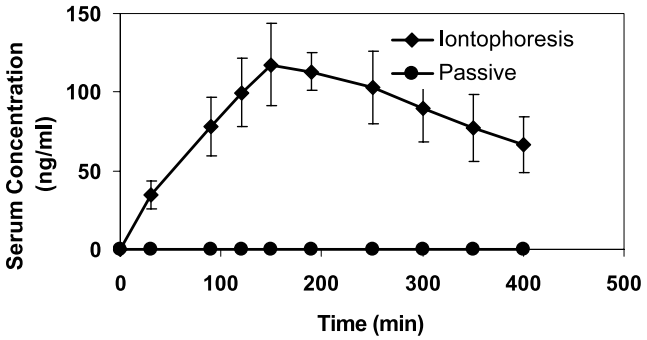

Fig. 2. Serum concentrations of granisetron hydrochloride in hairless rats (average ± SD).

onto the column. Calibration curves were prepared at a concentration range of 10 ng/ml–1000 ng/ml. Inter-day and intra-day precision of the assay was less than 6%.

Pharmacokinetic Data Analysis

Serum concentration *vs.* time profiles from i.v. injection and iontophoretic delivery of granisetron hydrochloride were analyzed using noncompartmental analysis (NCA) by WinNonlin (4.1). Pharmacokinetic parameters such as AUC_{0-inf}, terminal elimination rate constant (λz), clearance/F, and C_{max} were calculated. Clearance obtained from i.v. data was used to calculate the dose delivered during iontophoresis by the following equation, with the assumption that iontophoretic delivery provides a zero-order infusion:

$$F^*\text{Dose delivered} = AUC_{\text{iontophoretic}} \times \text{Clearance}_{IV} \quad (3)$$

Rate of infusion (R_0) at steady state was calculated by following equation:

$$R_0 = F^*\text{Dose delivered}/\text{Duration of patch application} \quad (4)$$

F represents the fraction of dose absorbed into systemic circulation. It represents the drug loss in the skin and subdermal layers. F*Dose delivered will be calculated as a single function from Eq. (3).

Pharmacokinetic Modeling

To validate the calculations involved in noncompartmental analysis, the serum profiles were fitted using WinNonlin (4.1) software, to the one-compartmental continuous infusion model with zero-order absorption and first-order elimination as follows:

$$C_p = (F^*\text{Dose delivered}/V_z{}^*K)^*\left(1 - e^{-kt}\right) \quad \text{If } t \le T_{abs} \quad (5)$$

$$C_p = (F^*\text{Dose delivered}/V_z{}^*K)^*\left(1 - e^{-kt}\right)^*\left(e^{-k(t-Tabs)}\right) \quad \text{If } t > T_{abs}, \quad (6)$$

$$F_{inf} = F^*\text{Dose delivered}/T_{abs} \quad (7)$$

where

C_p = serum concentration of granisetron
V_z = volume of distribution
F_{inf} = zero-order absorption rate
K = elimination rate constant
T_{abs} = time of absorption

The parameter estimation was done using a Gauss-Newton algorithm with Levenberg-Hartley modification. A number of other pharmacokinetic models were also evaluated and include the one-compartment model with first-order input and two-compartment models with constant and first-order inputs. Goodness of fit criteria included the Akaike information criteria (AIC) (13), lack of systemic deviations in the residuals, and a high correlation coefficient.

Statistical Analysis

All data are presented as means ± SD. Statistical analysis was performed using analysis of variance (ANOVA). A value of $p < 0.05$ was set *a priori* as significant.

RESULTS AND DISCUSSION

Average fluxes *in vitro* for 20 mg/ml, 10 mg/ml, and 5 mg/ml donor concentrations were 1171.3 ng/cm^2/min, 1082.4 ng/cm^2/min, and 603.3 ng/cm^2/min, respectively. The cumulative amounts delivered for 5 mg/ml and 10 mg/ml donors were significantly different ($p < 0.05$) but not for 10 mg/ml and 20 mg/ml donors (Fig. 1). In the absence of background electrolyte in the formulation, no dependence of iontophoretic flux on drug concentration was reported (14). An increase in the iontophoretic flux with increasing donor concentration was observed, however, but reached a plateau after the 10 mg/ml donor concentration. This could be due mainly to two reasons, counter-ion effect by Zn^{2+} ions generated as a result of the electrode chemistry and transport number reaching an asymptotic limiting value (15). The increase in efficiency with increasing donor concentrations

Table I. Pharmacokinetic Parameters After Iontophoretic Delivery, Calculated by NCA

Parameter	Units	Serum	Subdermal	Dermal
Cmax	ng/ml	123.9 ± 9.6	11,973.8 ± 978.5	8024.6 ± 4887.9
AUC_{0-inf}	μg*min/ml	53.1 ± 8.8	1641.1 ± 257.5	1533.5 ± 875.2
Lambda Z	min^{-1}	0.004	0.02	0.008
Half-life	min	204.9 ± 33.9	47.8 ± 11.8	94.6 ± 23.5
Cl/F	ml/min	98.7 ± 14.1	3.4 ± 0.5	5.9 ± 2.6
Dose delivered	mg/kg	2.5 ± 0.4	–	–

Table II. Pharmacokinetic Parameters Calculated by Model Regression

Parameter	Units	Estimate
Terminal elimination rate constant (λz)	min^{-1}	0.005 ± 0.001
Volume of distribution (Vz)	L	4.02 ± 0.59
F_{inf}	μg/min/kg	14.91 ± 4.53
T_{abs}	min	159.25 ± 28.62

is related to the competition for the delivery with Zn^{2+} ions. At low granisetron concentrations a high proportion of delivery was zinc and the system was inefficient. At higher granisetron concentrations, the proportion of zinc delivery was lower and the 10 mg/ml donor concentration was enough of a drug excess for the zinc counter-ion effect to be negligible.

The iontophoretic patches delivered an average current of 0.18 mA during the patch application period in hairless rats. The serum concentration *vs*. time profile (Fig. 2) shows enhanced delivery of granisetron hydrochloride by iontophoresis over passive diffusion. Passive experiments did not give any measurable serum levels. In iontophoretic experiments, serum levels continued to increase, even after the patch removal at 120 min until 150 min. This could be due to a depot formation of the drug in the skin. The limitation of diffusion cell studies is the absence of cutaneous microvasculature, which is important to the absorption process *in situ* (16). In case of *in vitro* studies, the lack of active microvasculature results in inefficient removal of the depot present in the skin. The calculated rate of input *in vivo* was higher than the rate *in vitro,* which could be attributable to the faster drug clearance from the skin depot by cutaneous microcirculation. The presence of a larger competing ion reservoir in the skin *in vitro* when compared to *in vivo* has been reported for other drugs such as tacrine and ropinirole, and the correlation between *in vitro* and *in vivo* fluxes is dependent on the nature of the molecule, experimental conditions and individual skin source (17,18). Because granisetron hydrochloride is a highly hydrophilic small molecule, the contribution of transcellular pathways in iontophoretic delivery is expected to be negligible and evident in this study by the lack of passive diffusion. Data from dermal and subcutaneous microdialysis groups were analyzed by NCA and pharmacokinetic parameters are presented in Table I.

In the pharmacokinetic modeling of serum data, a zero order absorption rate (F_{inf}) and the time of absorption (T_{abs}) were estimated as model parameters. The AIC criterion was improved with zero-order absorption compared to first-order absorption for all the individual animals. Pharmacokinetic parameters estimated by model regression are given in Table II. The model described the delivery of granisetron well (Fig. 3) and no systemic deviations were observed in the residual plots. The zero-order absorption rate can be related to the transport number of the drug by the following equation (19,20):

$$F_{inf} = \frac{t_n \cdot I_t}{Z_i \cdot F} \quad (8)$$

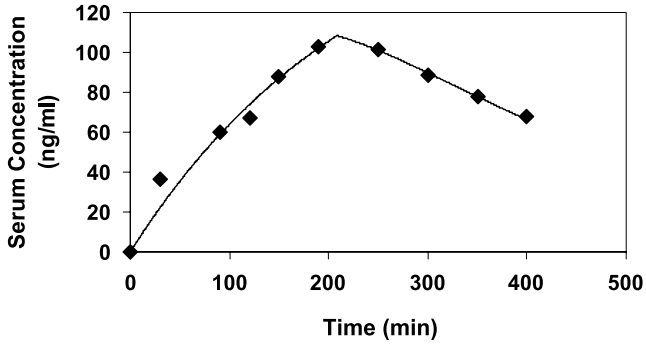

Fig. 3. Model fitting of the serum concentration *vs*. time profile. Closed circles represent experimental data and solid line is the prediction from the model.

where F_{inf} is zero-order absorption rate, t_n is transport number of solute, I_t is applied current, Z_i is the solute charge, and F is Faraday's constant.

Probe recoveries calculated *in vitro* and *in vivo* were different for subcutaneous microdialysis probes (Table III). We therefore used the *in vivo* RF for our calculations. This difference can be attributed to the tortuosity or increased path length through the tortuous tissue matrix that molecules must travel to reach the microdialysis probe (21). For dermal microdialysis probes we calculated only *in vivo* recovery, because these probes are hard to handle once they are wet and the probe insertion would also become more difficult.

In the subcutaneous microdialysis experiments, after cutting open the skin it was observed that the probe was placed in the thin muscle layer below the hypodermis, which is in accordance with the reports in the literature. Mathy *et al.* showed that subcutaneous microdialysis probe insertion in hairless rats resulted in a probe depth of about 1.8 mm below the skin surface (22). The microdialysis probe at the dorsal subcutaneous region served to quantify the extent of granisetron hydrochloride reaching subcutaneous tissue from systemic circulation. Subcutaneous levels of granisetron at the contralateral site (dorsal subcutaneous region) were negligible compared to the patch application site and were closer to the systemic levels (Fig. 4). The lower systemic levels of granisetron hydrochloride compared to the patch application site shows the dilution of the drug from the venous blood flow after it enters into the systemic circulation and proves that the subcutaneous microdialysis is a relevant site of sampling for determining absorption kinetics after iontophoresis. This finding is in accordance with reports that

Table III. Probe Recovery in Microdialysis Experiments

Microdialysis probe	*In vitro*	*In vivo*
SP1	0.27 ± 0.01	0.21 ± 0.04
SP2	0.34 ± 0.01	0.29 ± 0.03
SP3	0.53 ± 0.07	0.24 ± 0.04
DP1	–	0.24 ± 0.01
DP2	–	0.33 ± 0.03
DP3	–	0.26 ± 0.05

DP, dermal microdialysis probe; SP, subcutaneous microdialysis probe.

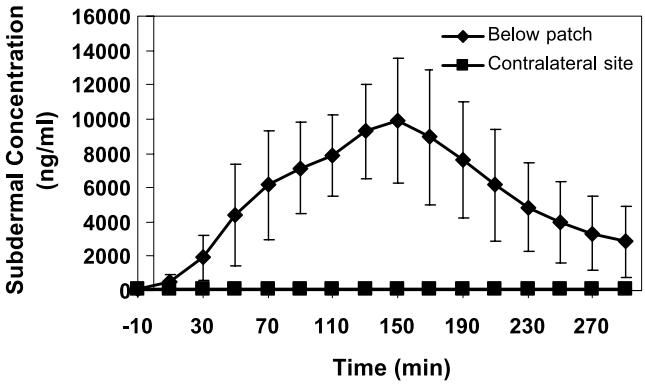

Fig. 4. Subcutaneous concentrations of granisetron hydrochloride after iontophoresis in hairless rats (average ± SD).

iontophoresis of salicylic acid resulted in high local concentration in the subcutaneous structures up to 3–4 mm below the application site and concentrations in the deeper underlying tissues tend to be similar to the plasma concentrations (23).

For insertion of dermal microdialysis probe, the method described by Stagni and Shukla was followed and the needle was inserted in the skin as superficially as possible in such a way that the needle was clearly visible through the superficial skin layer (3). Microscopic sections showed the probe insertion site as deeper dermis (data not shown). The dermal concentrations of granisetron hydrochloride are shown in Fig. 5. We have observed more variability in the dermal concentration measurements. No difference was observed between dermal and subdermal concentrations during the iontophoresis. However, at a 90% confidence interval, the concentrations at 10 min for dermal and subdermal concentrations were different ($p < 0.1$). Subdermal concentrations were higher than dermal concentrations. The reason for this is not clear but may be attributable to transport pathways. The predominant mechanism for iontophoretic delivery is via transappendageal pathways which extend all the way into the dermis. The drug will thus travel to the dermis and will be released into the subcutaneous tissue. In addition, the low current used will create a high current density at the epithelial lining at the base of the hair follicles. This will increase the permeability of the membrane to allow drug release as a result of electroporation (24). Turner and Guy demonstrated the dependence of iontophoretic pathways on the physicochemical properties of the penetrant (25).

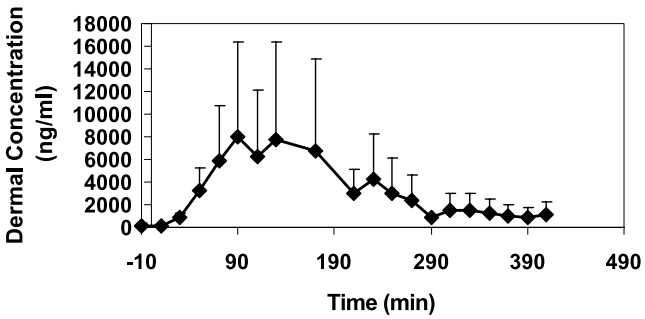

Fig. 5. Dermal concentrations of granisetron hydrochloride after iontophoresis in hairless rats (average ± SD).

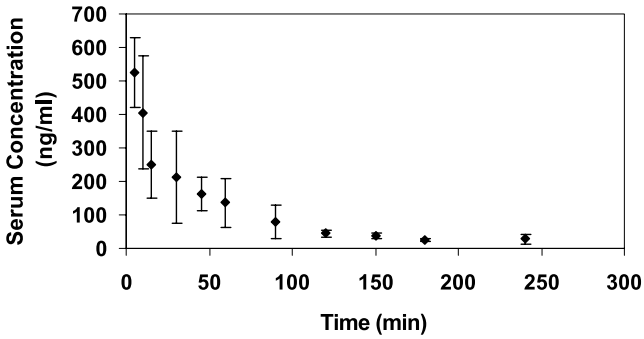

Fig. 6. Pharmacokinetic profile of granisetron hydrochloride after i.v. administration (average ± SD).

The relative contribution of transcellular and paracellular pathways of delivery will be dictated by the nature of the molecules. For hydrophilic drugs such as granisetron hydrochloride, the transcellular pathways may be minimal (24,26) and the drug reservoir is expected to be limited to the dermal region. However, in our observations, similar drug concentrations in both dermal and subdermal layers indicate that subdermal layers also contribute to a drug reservoir. This may be a positive implication for local therapies using iontophoresis targeted to subdermal structures. The rate that drug molecules passes through the dermis may also be faster than the rate taken up by the blood vessels. This may explain the results observed with iontophoretic delivery of methotrexate in rabbits by Stagni and Shukla. In these studies, increasing current density increased the blood levels of the methotrexate but not the dermal exposure, that is, area under the curve of dermal concentration *vs.* time profile (3), showing that the drug passed through the dermis and was absorbed from the subcutaneous region. Singh and Roberts determined the blood flow rates of skin and subcutaneous tissue as 5.18 and 3.59 ml/min per 100 g of tissue, respectively, showing almost equal clearance can be observed for the drugs delivered into deeper tissues by iontophoresis (27).

Serum concentrations of granisetron hydrochloride after i.v. administration are shown in Fig. 6. Pharmacokinetic parameters such as terminal elimination rate constant (λz), half-life, clearance, volume of distribution (Vz), and AUC_{inf} were calculated by NCA and are given in Table IV. Lower clearance and higher half-life were observed compared to Sprague-Dawley rats (21).

As discussed earlier, *in vitro* flux was less than *in vivo* flux in iontophoresis, which may be due to lack of microvasculature *in vitro*. A good correlation ($r^2 = 0.9918$) was observed between the *in vitro* cumulative amounts delivered, and the AUC_{0-n} calculated from *in vivo* subdermal microdialysis data. Thus, *in vitro* set up can still give infor-

Table IV. Pharmacokinetic Parameters After i.v. Administration

Parameter	Units	Estimate
Terminal elimination rate constant (λz)	min^{-1}	0.011 ± 0.006
Half life	min^{-1}	80.23 ± 43.08
Clearance	ml/min/kg	46.40 ± 10.41
Volume of distribution (Vz)	L/kg	5.81 ± 4.19
AUC_{inf}	min*μg/ml	26.21 ± 6.32

mation regarding the iontophoretic flux *in vivo*. A good correlation was also observed (r^2 = 0.9837) between the AUC_{0-n} of subdermal concentrations and the AUC_{0-n} of serum concentrations.

CONCLUSION

The iontophoretic patches delivered granisetron at a rate of 14.91 ± 4.53 μg/min/kg, at a current of approximately 0.18 mA. Drug depot in the skin was observed *in vivo* and was most likely responsible for prolonged absorption observed after the patch removal *in vivo*. Similar concentrations of granisetron hydrochloride in dermal and subdermal tissues suggest that subdermal structures may also responsible for the depot effect. The delivery profile can be described by a one- compartment model with zero-order absorption. A good correlation was observed between the *in vitro* cumulative amounts delivered and the AUC_{0-n} calculated from *in vivo* subdermal microdialysis data. Thus, subcutaneous microdialysis can be used to describe absorption kinetics of hydrophilic drugs delivered by iontophoresis.

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