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ROSUVASTATIN CALCIUM PERMEABILITY EVALUATION USING IN-VITRO PERMEATION TEST (IVPT) THROUGH HUMAN CADAVER SKIN

Padmanabha Reddy RV[1], Dipak Sahana[1], Sagir Ahmed Syed[1], and Craig Newby[2]

[1]Abbott Healthcare Pvt. Ltd. Plot no. 113, Marol Industrial Area, Plot no. 15, MIDC, Andheri East, Mumbai-400093, Maharashtra, India

[2]Abbott Products Operations AG, Hegenheimermattweg 127; 4123 Allschwil; Switzerland.

***Corresponding Author: Email:** preddyrv75@gmail.com

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ABSTRACT

Objective: *The goal of the study was to evaluate the permeability of Rosuvastatin Calcium through human cadaver skin using the in-vitro permeation test (IVPT) method and to correlate the data with simulated (GastroPlus 9.7 transdermal module) transdermal pharmacokinetic data.* ***Methods:*** *Rosuvastatin solubility study was performed in various aqueous and organic solvents. IVPT study conducted using human cadaver skin and franz diffusion cell. The IVPT data was correlated using simulation software GastroPlus 9.7 transdermal module for prediction of PK parameter through transdermal route.* ***Results:*** *Rosuvastatin was soluble in both water and 20 % dimethyl sulfoxide (DMSO); which were used as a solvent for the IVPT study. IVPT study data indicate permeation of rosuvastatin using water and 20% DMSO solution was 0.85% and 1.7% respectively. Simulation transdermal pharmacokinetic model revealed that the relative bioavailability was around 2% compared to the oral route.* ***Conclusions:*** *Rosuvastatin has a permeation potential through human cadaver skin. However, the simulated PK data revealed, that it was unable to achieve therapeutically effective concentration through transdermal route in comparison with a peroral route (PO). Further, a simulation study was conducted by increasing the patch size up to 50 cm^2, however, the permeation was not increased significantly. Hence, the development of transdermal delivery of Rosuvastatin is challenging using conventional transdermal patch.*

Keywords – *Rosuvastatin calcium; IVPT; Transdermal delivery; Diffusion; GastroPlus*

1. INTRODUCTION

Rosuvastatin is a synthetic HMG-CoA reductase inhibitor, belongs to a new generation of methane-sulphonamide pyrimidine and N-methane sulfonyl pyrrole-substituted 3, 5- dihydroxy-heptenoates. Inhibition of HMG-CoA resulting in reduction of cholesterol synthesis by preventing the mevalonate synthesis which is a precursor of Cholesterol. The drug is used in the treatment of hyperlipidemia and for the treatment of long-term hypertension, angina pectoris, and congestive heart failure [1]. Rosuvastatin is a member of 'superstatin' group. It is a BCS class II drug having low solubility and due to first-pass effect the oral bioavailability is low (~20%). It exhibits a crystalline structure which reduces its aqueous solubility [2]. Due to poor solubility in water, gastric fluid, and intestinal fluid, considerable first-pass metabolism, and poor bioavailability, an alternate route of administration like transdermal delivery is required to avoid first pass effect and improving the bioavailability for better patients' compliance [3].

The stratum corneum is a defensive and outer layer of skin, selectively permeable to certain drugs to reach in systemic circulation. The Lipinski's rule of 5 provides a framework of selecting drugs that are suitable for transdermal administration. The physicochemical properties of a drug could foresee the permeability of drug through the skin and determine the probability for transdermal drug delivery design [4-6]. The favorable physicochemical properties of Rosuvastatin as per Lipinski's rule (low molecular weight, 481.54; high lipophilicity, log P = 2.4, hydrogen acceptor count 8 and hydrogen donor count 3, dose 5 mg and 10 mg, oral bioavailability (20%), it seems to be an ideal candidate for exploration of transdermal route of delivery [7]. There have been several *in-vitro* permeation study data generated for the various molecules to determine the permeation potential through the skin. GastroPlus modeling is a tool, which can predict the PK performance of any drug molecules based on the *in-vitro* permeation data [8-9]. The main aim of this current study was to determine the permeation potential of Rosuvastatin through the skin using IVPT study and predict the PK parameters using GastroPlus modeling with respect to the oral route.

2. MATERIALS

Rosuvastatin Calcium gifted by MSN Laboratories, reference standard was purchased from MSN Laboratories Ltd., Hyderabad. Different solvent system used for the solubility assessment like PEG 400, Propylene Glycol and Kollicream OA, was obtained as a gift sample from BASF and Transcutol P was obtained from Gattefosse. Other solvents like ethanol, acetonitrile, dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), and methanol were of analytical grade, purchased from Merck.

3. METHODS

3.1 HPLC method for determination of Rosuvastatin Calcium

The Rosuvastatin was weighed appropriately to prepare primary stock, followed by secondary stock in phosphate buffer saline (PBS), pH 7.4. The HPLC analysis was performed on Shimadzu, SPD 20A with a Symmetry® C18 column (5 µm particle size and 4.6 X 250 mm) with an UV detector. The calibration curve was constructed at 266 nm. The mobile phase was Acetonitrile: 20mM Phosphate Buffer (pH 4.5): Methanol (25:45:35). The flow rate was 1.5 mL/min. The standard curve ranges between 50 ng/mL to 100 µg/mL with R^2= 0.99. The solubility of samples was diluted with PBS as per the method developed.

3.2 Solubility study of Rosuvastatin Calcium in solvent system

Briefly, a weighed amount of the Rouvastatin was constantly added to the solvent system. The glass vials were placed in a water-bath shaker maintained at 25.0 ± 1.0°C. These glass vials were firmly closed to avoid loss of the solvent during the experiment. The addition of Rosuvastatin was continued until the saturation/equilibrium was achieved. After establishment of equilibrium, the content was centrifuged at 2795×g for 10.0 min to separate out the precipitated Rosuvastatin. The supernatant was used to determine the drug content at saturation by using the validated HPLC method at a λmax of 245 nm. The experiments were replicated for mean value ± SD (standard deviation) values (n = 3).

3.3 Preparation of skin for IVPT study

The human cadaver skin was stored at -20ºC until used. The skin was removed from the freezer and thawed at room temperature. After the skin attains room temperature, the skin is washed with deionized water. The excess water was blotted using soft tissue paper. Skin was cut into pieces of 3 cm diameter. The thickness of the skin was measured for the checking thickness uniformity and to avoid variation in permeation data. The thickness of the skin was measured using Mitutoyo micrometer by placing them in between the two-microscope slide. The differences in the skin thickness of the slides with and without skin gives the thickness of the skin. Human cadaver skin was evaluated for various physical properties like skin temperature, TEWL (Trans-epidermal water loss rate) and

resistivity for ascertain the skin integrity. The skin temperature was checked intermittently with IR thermometer (Microtek IT-1520). Once the skin attains 32ºC±1, the TEWL was measured using Delfin® Vapometer. The resistivity of the skin was checked using LCR Meter (BK Precision).

3.4 IVPT study details of Rosuvastatin in water and 20 % w/v DMSO

Human cadaver skin was sandwiched between the donor and the receiver with stratum corneum facing the donor compartment. The receiver compartments were filled with 8 ml of PBS pH 7.4. Hot water circulation was continued so that the skin temperature was attain 32ºC±1. The donor was filled with (0.5 ml) PBS pH 7.4. It was allowed to stand for a few minutes to attain the temperature of 32ºC±1.The buffer solution in the donor compartment was removed carefully and allowed to dry completely before adding the Rosuvastatin solution. 6mg/ ml Rosuvastatin solution was prepared using deionized water and placed in the donor compartment. The donor compartment was closed tightly with parafilm to mitigate any solvent evaporation. The details test parameters were represented in the (Table 1).

Table 1: IVPT test parameters of Rosuvastatin in water and 20 % w/v DMSO

Description of IVPT parameters	Parameters / Data	
	Water	**20 % w/v DMSO**
Number of Cells used	6 cells with 1cm^2 diffusional area	6 cells with 1cm^2 diffusional area
Barrier Used	Human Cadaver Skin	Human Cadaver Skin
Stirring speed	600 rpm	600 rpm
Water Bath Temperature	32ºC±1ºC	32ºC±1ºC
Donor Sample	6 mg/ ml of Rosuvastatin in water	6 mg/ ml of Rosuvastatin in 20 % DMSO
Donor sample volume	1ml	1ml
Receiver sample	Phosphate Buffer Saline (PBS) (pH 7.4)	Phosphate Buffer Saline (PBS) (pH 7.4)
Receiver sample volume	8 ml	8 ml
Volume of sample withdrawal	0.5 ml and replaced with 0.5 ml fresh PBS every time	0.5 ml and replaced with 0.5 ml fresh PBS every time

3.5 Dosing (infinite dose, 6mg per ml)

Immediately after placing the Rosuvastatin solution in the donor compartment, the zero (0 hr), 0.5 ml samples were withdrawn and replaced with 0.5 ml fresh PBS in the receiver medium. Further sampling was withdrawn at 1, 2, 4, 6, 8, 10, 12, 24 hr and the receiver medium was replaced with PBS each time. The samples were analyzed by HPLC (Shimadzu SPD 20A).

3.6 Skin content

After the 24th hrs. sampling, the skin of each cell was unclamped, and the surface was wiped gently to completely remove the remaining Rosuvastatin solution. The skin was thoroughly washed and placed on an absorbent paper to remove the water. The diffusional area of each skin was cut with the help of biopsy punch and weighed. The skin was immersed in a centrifuged tube containing 1ml mobile phase and placed in refrigerator(4ºC) overnight. The tubes were centrifuged at 10,000 RPM for 20 minutes at 20ºC and the supernatant was analyzed by HPLC at 266 nm.

3.7 GatroPlus modelling for PK simulation through transdermal route using IVPT data

GastroPlus 9.7 (Transdermal module) was used for the PK simulation through transdermal route using IVPT data of Rosuvastatin. The model for rosuvastatin was build and the data was validated using published literature data through peroral route. Then the validated model was used for the prediction of PK parameters of Rosuvastatin using the IVPT data of the Rosuvastatin in both water and 20% DMSO solution. Then the predicted PK parameters were compared with the PK parameters of the oral route and the relative bioavailability was estimated.

4. RESULTS

4.1 HPLC method for determination of Rosuvastatin Calcium:

Rosuvastatin method was adopted from the pharmacopoeia and verified at laboratory. The HPLC analysis was performed on Shimadzu, SPD 20A with a Symmetry® C18 column (5 µm particle size and 4.6 X 250 mm) with an UV detector. The calibration curve was constructed at 266 nm (Figure 1). The mobile phase was Acetonitrile: 20mM Phosphate Buffer (pH 4.5) : Methanol (25:45:35). The flow rate was 1.5 mL/min. The standard curve ranges between 50 ng/mL to 100 µg/mL with R^2= 0.999.

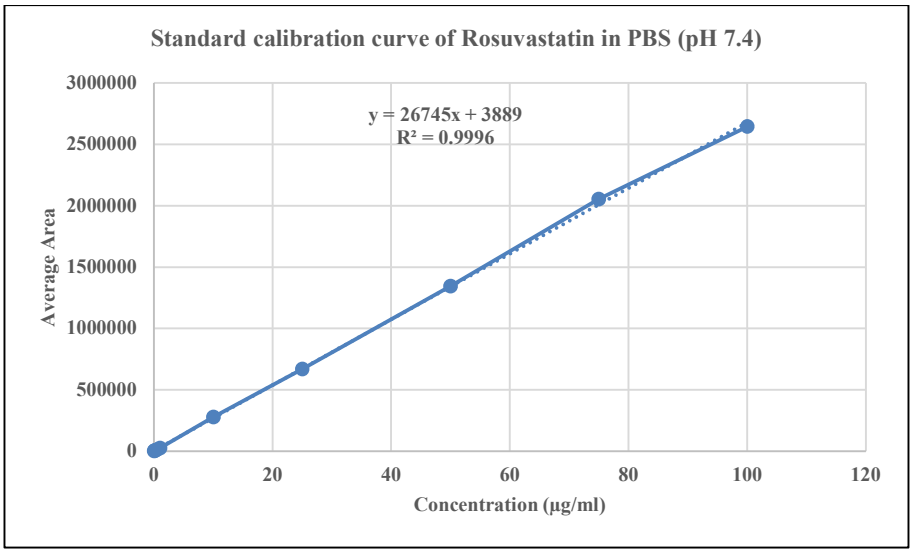

Figure 1: Standard calibration curve of Rosuvastatin

4.2 Solubility study

Solubility study was performed to determine the dose to be applied and to determine the donor and receptor media. Solubility data of Rosuvastatin in different media is presented in (Figure 2).

Solubility of Rosuvastatin was 5.0 to 8.65 mg/ml in aqueous media whereas in organic solvents the solubility was in the range of 158.21 to 334.22 mg/ml. The solubility of Rosuvastatin was 8.65 mg/ml in water whereas the solubility was 5.03 mg/ml in PBS (pH 7.4). The receiver solution volume was determined as 8 ml in IVPT study. Hence for maintaining the sink condition, 6mg of Rosuvastatin per ml of water was used in the IVPT study. DMSO is extensively used in transdermal formulation as a solvent and permeation enhancer. Also, the solubility data of Rosuvastatin was found very high in DMSO. Hence, as a comparative evaluation of IVPT data of Rosuvastatin both water and 20% DMSO solution were used.

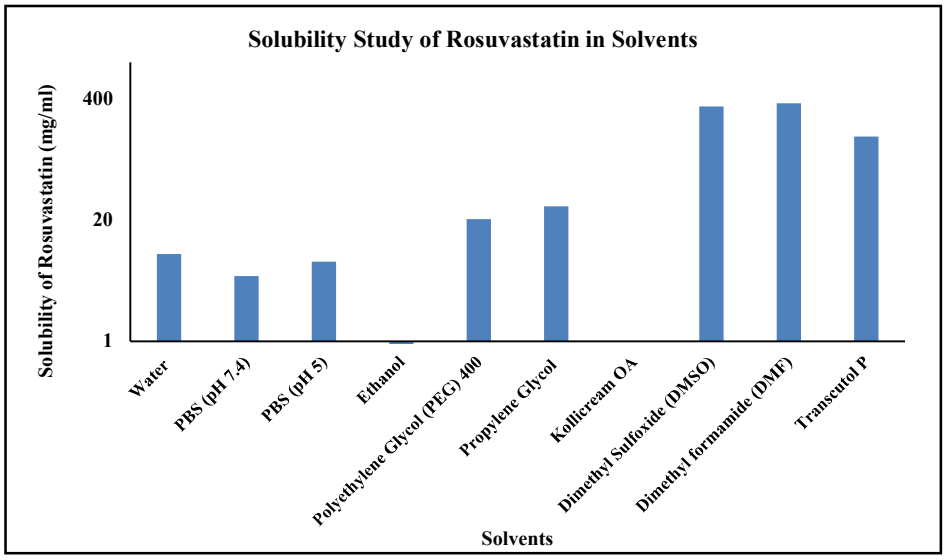

Figure 2: Rosuvastatin solubility data in different solvent system

4.3 Preparation of skin for IVPT study

The prepared skin was measured for thickness, temperature, TEWL, and resistivity. The data is tabulated in the (Table 2).

The thickness of the skin was found to be uniform for all 6 samples. The temperature of the skin was maintained in line with the body temperature throughout the study. TEWL value and resistivity were similar for all the six skin samples indicating that the skin was intact. For qualification of any skin for the IVPT, TEWL value should be less than 15 and resistivity is not over 10 kohm.cm^2. TEWL value was observed less than 15 for all the skin samples and the resistivity of all the skin is more than 10 kohm.cm^2, skins were intact. It was concluded that, they qualified for performing IVPT study.

4.4 IVPT studies results of Rosuvastatin across human cadaver skin

The cumulative amount of drug permeated in both the study across the human cadaver skin in 24 hours were 51.16 ±17.24 µg/cm^2 and 101.92 ± 20.10 µg /cm^2 respectively (Figure 3).

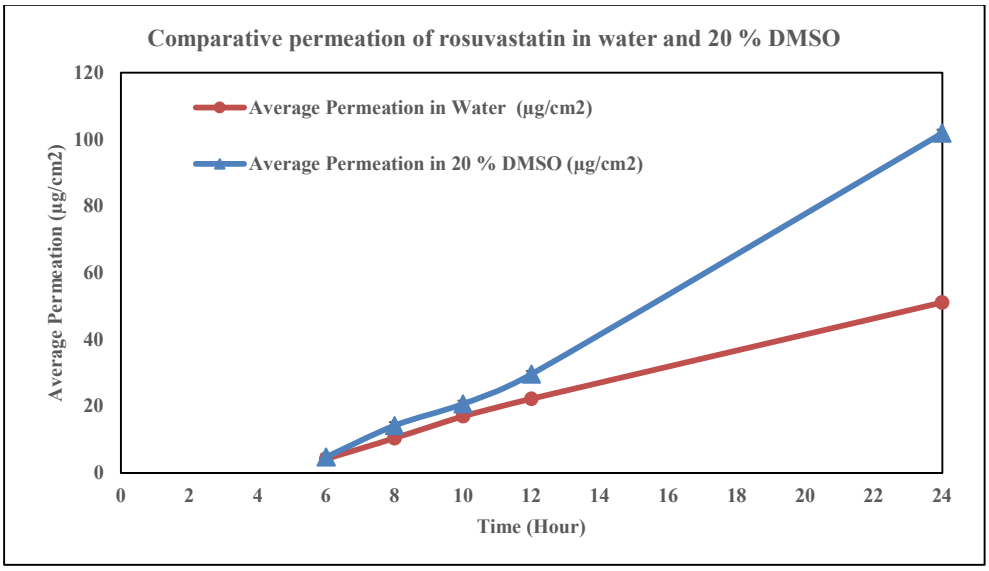

Figure 3: Comparative permeation data of Rosuvastatin in water and in 20% DMSO through cadaver skin. (Avg. ± SD, n=6)

The permeation profile (n=6 ± SD) of Rosuvastatin in water showed steady-state transdermal flux of 2.57 µg/cm^2 /h (Table 3). Also, the amount of drug permeated shown in (Figure 4). At the same time the transdermal flux was 5.48 µg /cm^2/h (Table 3) when used 20% DMSO solution (Figure 5). It was evident that the Rosuvastatin was permeated through the skin and when used 20% DMSO solution, the flux and the cumulative permeation was increased by around 2 folds than water was used as solvent. The reason might be the increase in solubility in DMSO. Also, DMSO acts as a permeation enhancer resulting in increased steady-state flux and total permeation of Rosuvastatin through the cadaver skin. There was a lag time of around 5 hours in both the tests which is a drawback of transdermal delivery. The amount of drug retained in the skin after 24 hours was negligible in the test i.e. less than 0.5 µg /mg reflecting complete drug was permeated through the skin (Table 4).

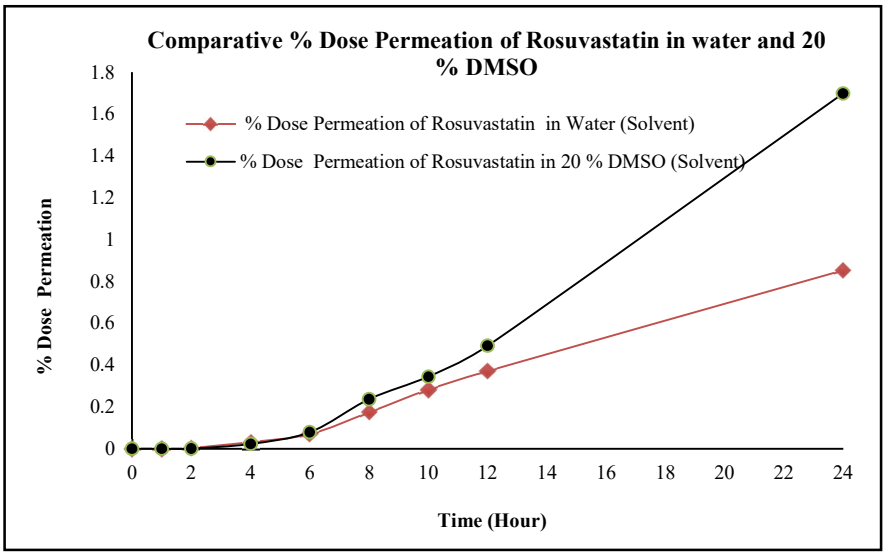

Figure 4: Percentage (%) Rosuvastatin permeate through the human cadaver skin

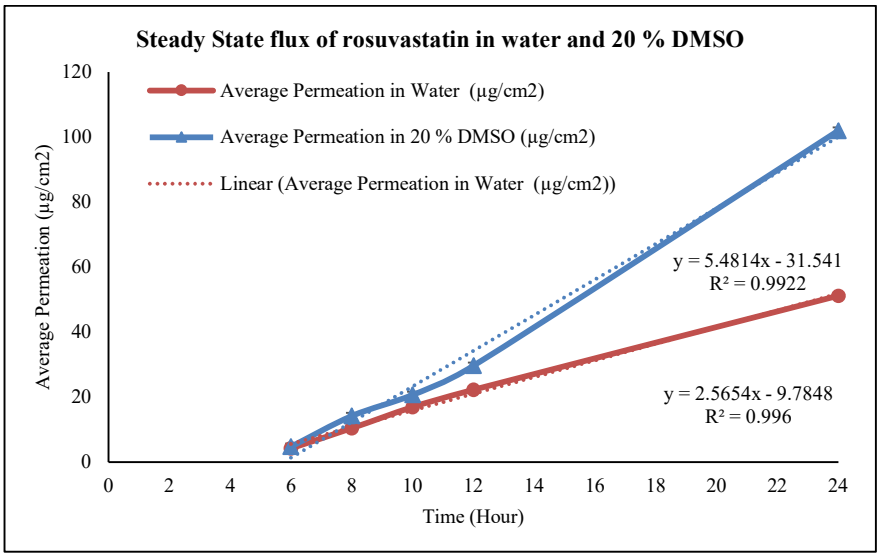

Figure 5: Calculated steady state flux value of Rosuvastatin.

Table 2: Thickness, Temperature, TEWL and Resistivity of human cadaver skin used in IVPT

Cell Number	Thickness of the skin (mm)	Temperature (°C)	TEWL (g/m^2h)	Resistivity ($Kohm/cm^2$)
1	0.17	31.7	13.8	46.63
2	0.29	32.1	13.3	55.29
3	0.19	31.8	10.8	63.10
4	0.14	32.2	11.6	40.25
5	0.12	31.7	10.9	44.67
6	0.18	31.9	9.5	66.13
7	0.15	31.1	7.0	92.04
8	0.17	31.4	10.5	46.80
9	0.10	31.6	9.3	70.00
10	0.19	31.5	8.3	73.52
11	0.18	31.6	6.2	42.91
12	0.19	31.2	9.6	54.92

Table 3: Summary of Rosuvastatin permeability data through cadaver skin in water and in 20% DMSO solution

Parameter	Water	20% DMSO Solution
Cumulative amount permeated in 24 hours (mcg/cm^2)	51.16 ± 17.24	101.92 ± 20.10
Steady state flux ($mcg/cm^2/h$)	2.57	5.48
Lag time (hr)	About 5	About 5

4.5 GatroPlus modelling for PK simulation through transdermal route using IVPT data

The present study was designed to evaluate the simulation PK data of Rosuvastatin in transdermal route using IVPT data and peroral (PO) PK data. It was observed in the IVPT study that 0.85% and 1.70% of Rosuvastatin was permeated through the skin when used water and 20% DMSO solution as solvent respectively (Figure 4).

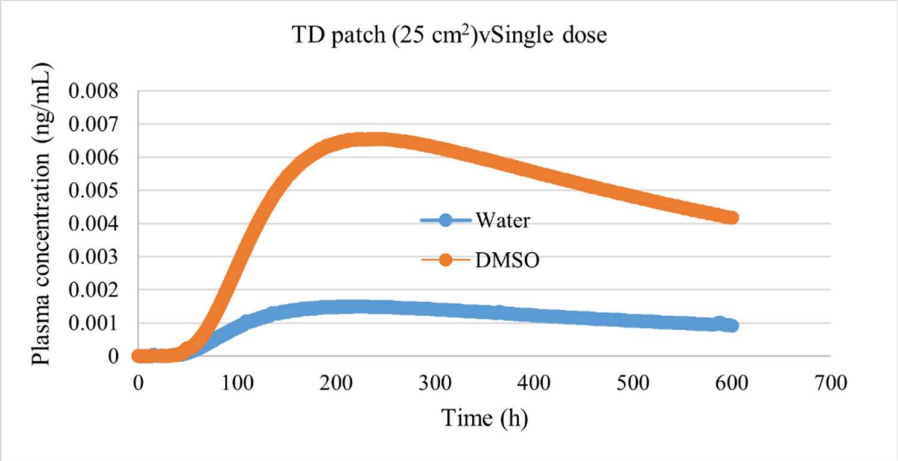

Figure 6: Comparative simulated PK data of Rosuvastatin based on the GastroPlus modeling at single dose

There was a substantial lag in the absorption of Rosuvastatin seen in the simulation model through the TD route. The rate of Rosuvastatin absorption was very slow indicated by the T_{max} of more than 200 h (Figure 6). The simulated plasma levels in transdermal route based on the IVPT data were observed in sub picomolar range at a steady-state level (Table 5).

As the Rosuvastatin permeation was comparatively better in DMSO solution than the water, hence, IVPT data in 20% DMSO solution was considered for further simulation in GastroPlus modeling. The simulated PK data of Rosuvastatin in transdermal route and PO route using a 6 mg dose were compared. It was observed that the C_{max} and AUC value were 0.00213-fold and 0.096-fold than the PO route (Table 6). When simulated TD data were compared with PO using 40 mg dose it was observed that the C_{max} and AUC were 0.005-fold and 0.02-fold respectively (Table 6). Based on the simulation PK data transdermal C_{max} and AUC were substantially lower even with 40 mg dose than plasma levels at 6 mg PO dose (Fig. 7).

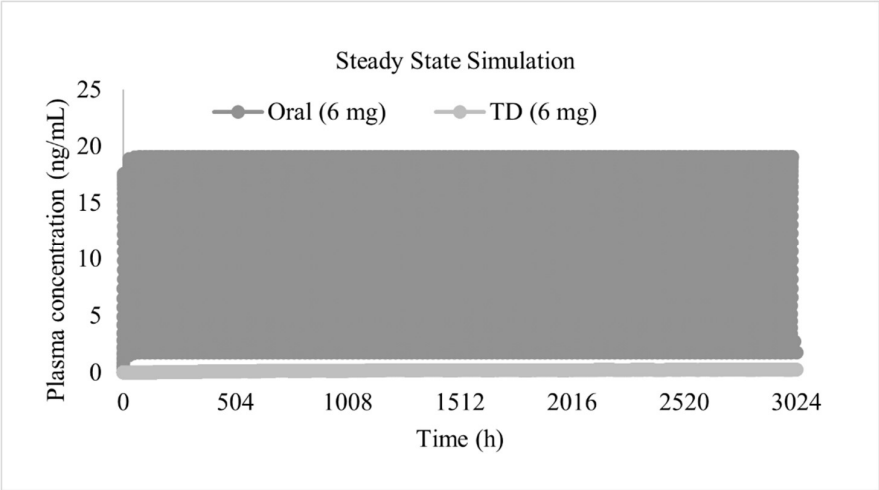

Figure 7: Comparative simulated PK data of Rosuvastatin based on the GastroPlus modeling at steady state

It was also evident on the model that the relative bioavailability was around 2% compared to the oral route. Further the simulation was conducted by increasing the patch size up to 50 cm^2, however, the permeation was not increased significantly (Table 7). The site of action of Rosuvastatin is liver and based on the literature the Ki value should be 3.5nM (1.7 ng/ml) in liver [10-11]. In PO route the simulated Ki value is 9.5 to 52.6-fold more than the target Ki value based on the dose, whereas the simulated Ki value in transdermal formulation were found 0.021 and 0.137 in the dose of 6mg and 40 mg respectively with respected to the target Ki value (Table no 8).

Table 4: Amount of drug retained in skin after IVPT study

Number of Skin	Amount of the drug retained (µg/mg) after IVPT study	
	Water	**In 20% DMSO solution**
Skin 1	0.014	0.71
Skin 2	0.010	0.64
Skin 3	0.016	0.03
Skin 4	0.012	0.03
Skin 5	0.027	0.58
Skin 6	0.021	0.02
Average	0.017	0.34
SD	0.006	0.34

Table 5: Comparative simulated pharmacokinetic data of PO and TD reservoir patch of 6 mg dose/day, area 25 cm^2, based on modelling.

Parameter	Single Dose		Steady State
Patch Size	25 cm^2		
IVPT Data	Water	20% DMSO	20% DMSO
Dose	6 mg		6 mg/day
C_{max} (ng/ml)	0.0015	0.0066	0.24
AUC_{0-t}(ng.h/ml)	0.65	2.78	41
T_{last} (h)	600	600	-
T_{max} (h)	217	237	3001

Table 6: Comparative simulated pharmacokinetic data of PO and TD reservoir patch of 6 mg and 40 mg dose, based on modelling

Parameter	Using DMSO IVPT data			
Route	**PO**	**TD reservoir patch**	**PO**	**TD reservoir patch**
Dose	6 mg	6 mg	40 mg	40 mg
C_{max} (ng/mL)	3.1	0.0066	17	0.008
AUC_{0-t}(ng.h/mL)	29	2.78	161	3.22
T_{max} (h)	4	237	4	445
Fold over PO exposures (C_{max})	-	0.00213	-	0.0005
Fold over PO exposures (AUC_t)	-	0.096	-	0.02
% Relative Bioavailability	-	-	-	2

Table 7: Predicted simulated pharmacokinetic data of TD reservoir patch by change in dose and patch size

Parameter	TD reservoir patch			
Patch Size	10 cm^2	25 cm^2	25 cm^2	50 cm^2
Dose	40 mg	6 mg	40 mg	40 mg
C_{max} (ng/mL)	0.006	0.001	0.008	0.0095
AUC_{0-t}(ng.h/mL)	2.89	0.50	3.22	3.77
T_{last} (h)	600	600	600	600
T_{max} (h)	446	439	445	401

Table 8: Comparative simulated liver Ki value of Rosuvastatin in PO and transdermal route in different dose

Fold over targeted Ki Value (3.5 nM) In liver		
Dose	PO route	TD route
6 mg	9.5	0.021
40 mg	52.6	0.137

5. DISCUSSION

The present study was designed to evaluate the permeation of Rosuvastatin calcium using IVPT study through human cadaver skin. To evaluate the same solubility of Rosuvastatin calcium was evaluated first in different polar and non-polar solvents. The solvents were selected based on the solubility study water and DMSO. 20 % DMSO was selected as solvent for Rosuvastatin IVPT study. It was evident that the Rosuvastatin permeated through the skin and when use 20% DMSO solution, the flux and the cumulative permeation were increased by around 2 folds than water was used as solvent. The reason might be the increase in solubility in DMSO and DMSO acts as a permeation enhancer. Enhanced permeation of DMSO is probably due to the formation of hydrogen-bonded complexes with stratum corneum lipids resulting in changes in the structure of stratum coronium which leads to increased permeability [11-12].

The simulated PK data of Rosuvastatin was compared in the transdermal route and PO route using a 6 mg dose. In PK data of transdermal simulation, it was observed C_{max} and AUC were substantially lower even with 40 mg dose than plasma levels at 6 mg PO dose. It was also revealed, the model that the relative bioavailability was around 2% compared to the oral route. Further, the simulation was conducted by increasing the patch size up to 50 cm^2, however the permeation was not increased significantly. Also, there was a lag time of around 5 hours in both the tests which is a drawback of transdermal delivery.

Hence, the transdermal development of rosuvastatin using conventional transdermal technology using chemical permeation enhancers seems to be difficult. Other techniques like microneedle patch, sonophoresis and iontophoresis can be explored to improve the permeation of rosuvastatin through the transdermal route [13-15].

6. CONCLUSION

The present study was designed to evaluate the permeation and the IVIVC relationship between the IVPT data. The IVPT data indicate permeation of Rosuvastatin using water and 20% DMSO solution as the solvent was 0.85% and 1.7% respectively, proved that the Rosuvastatin can permeate through the skin. However, the simulated plasma levels (GastroPlus 9.7 transdermal module) through transdermal route (TD) were substantially lower as compared to oral formulations. The rate of absorption was very slow with (T_{max} >200h) and more lag time (time of ~4-5h) observed in simulated TD. The unbound plasma and liver levels of Rosuvastatin achieved through the TD route using simulated 6 mg to 40 mg dose were much below the reported K(i) values of rosuvastatin for HMGCoA reductase. Even change in the size (50 cm^2) in patch in simulation modeling was unable to provide significant permeation. Further increase in size, would compromise patient compliance. Hence, the transdermal development of Rosuvastatin using conventional transdermal technology using chemical permeation enhancer seems to be difficult. Other techniques like microneedle patch, sonophoresis, iontophoresis can be explored to improve the permeation of Rosuvastatin through the transdermal route.

It was also evaluated based on the model that the relative bioavailability was around 2% compared to the oral route. Further, the simulation was conducted by increasing the patch size up to 50 cm^2, however, the permeation was not increased significantly.

7. ACKNOWLEDGEMENT

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8. FUNDING

Abbott Healthcare Pvt. Ltd. had provided the funding for conducting this research work.

9. CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest, funding, or financial relationships.

10. AUTHOR CONTRIBUTIONS

All authors contributed to the study's conception and design. Formulation and development activity performed by Dipak Kumar Sahana, and analytical activity performed by Dr, Sagir Syed. Dipak kumar Sahana wrote the first draft of the manuscript, and the final corrections were made under the supervision of Dr Padmanabha RV Reddy and Dr, Craig Newby. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

11. AVAILABILITY OF DATA AND MATERIALS

The datasets generated during this study are available from the corresponding author upon request.

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