TRANSDERMAL DELIVERY OF ISRADIPINE THROUGH EXCISED RABBIT SKIN: EFFECT OF VEHICLE AND DRUG CONCENTRATION

Saleh A. Al-Suwayeh

يستخدم دواء الإزراديبين، أحد صادات قناة الكالسيوم، في علاج الذبحة الصدرية وضغط الدم المفرط. ولقد تم اقتراح توصيل هذا الدواء عبر الأدمة للتغلب على إتاحته الحيوية الضعيفة. في هذه الدراسة، جرى تقييم تأثير السواغات المختلفة معملياً على انتشار الإزراديبين عبر جلد الأرنب، وهي تحديداً البروبيلين جليكول، والبولي إيثيلين جليكول 400 والإيثانول. ولقد تبين أن أكبر كمية من الدواء (الكمية التراكمية على مدار 24 ساعة) اخترقت الجلد كانت في وجود الإيثانول. بينما كانت الكمية التي عبرت الجلد باستخدام البروبيلين جليكول 1.7 ضعف الكمية العابرة باستخدام البولي إيثيلين جليكول 400 مما يدل على أن البروبيلين جليكون قد يكون سواغاً فعالاً في توصيل الإزراديبين عبر الأدمة. وتمت أيضاً دراسة تأثير تراكيز الإزراديبين 10 و 20 و 40 مغ/مل في وجود البروبيلين جليكول كسواغ، فأظهرت النتائج علاقة خطية بين التركيز والكمية التراكمية المنتشرة مع تركيز 40 مغ/مل الذي أعطى أكبر كمية من الدواء تخللت الغشاء.

Isradipine is a calcium channel blocker approved for the treatment of angina pectoris and hypertension. Transdermal delivery is proposed for this drug to overcome its poor oral bioavailability. In this study, the effect of different vehicles namely: propylene glycol, polyethylene glycol 400 and ethanol was evaluated for in vitro transdermal diffusion of isradipine through rabbit skin. The highest amount of isradipine (cumulative amount over a 24 hour period) penetrated the skin was achieved in presence of ethanol. While the amount of the drug transported across the excised skin using propylene glycol was about 1.7 fold that observed using polyethylene glycol 400 indicating that propylene glycol could be a good and effective vehicle for transdermal delivery of isradipine. The effect of isradipine concentration was also studied at drug concentration of 10.0, 20.0 and 40.0 mg/ml in presence of propylene glycol as a vehicle. Results showed that there is a linear relationship between isradipine concentration and cumulative amount diffused with 40 mg/ml giving the highest amount of drug permeated through the membrane.

Key Words: Isradipine, transdermal delivery, rabbit skin, vehicle, propylene glycol, polyethylene glycol 400, ethanol.

Introduction

Isradipine (ISRA) is a potent dihydropyridine calcium antagonist with higher affinity for calcium channels in arterial smooth muscles than those in the myocardium. It is used in the treatment of ischemic heart disease, systemic hypertension (1) and chronic angina (2). It undergoes extensive first pass metabolism resulting in bioavailbilty of about 16 to 18 % (3). Therefore, transdermal delivery of ISRA is proposed as an alternative route of drug administration to overcome its poor oral bioavailability. In spite of the barrier function of the skin, transdermal delivery of drugs remains an important alternative route of drug administration. It maintains therapeutic blood levels for longer period of time and minimizes first-pass metabolism. Several factors are known to influence the rate and extent of absorption through the skin. These are: mode of application, condition and site of the skin, type of vehicle used and drug concentration and its physicochemical properties. By keeping other factors constant, the effect of vehicle and drug concentration on the percutaneous absorption of drugs can be investigated. Vehicles play an important role in transdermal delivery of drugs. Mollgaard and Hoelgaard (4) studied the effect of propylene glycol and polyethylene glycol 400 on transdermal delivery of metronidazole. Sathyan *et al*.

Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.
E-mail ssuwayeh@ksu.edu.sa

(5), Cross *et al*. (6) and Gabiga *et al*. (7) studied the effect of propylene glycol and ethanol on the permeation of tarcine, hydrocortisone and isosorbide dinitrate, respectively. Ethanol is a solvent known to modify the barrier properties of the skin (8,9). It was used in different transdermal delivery systems for oestradiol (10), nitroglycerin (11) and fentanyl (12).

In the present study, in order to develop a transdermal delivery system for ISRA, different vehicles including propylene glycol (PG), polyethylene glycol 400 (PEG 400) and ethanol were evaluated for in vitro transdermal diffusion of ISRA through excised rabbit skin using improved Franz-diffusion cells. The skin was used without hydration as hydration would disturb the lipophylicity of the skin (13). In addition, PG was used as a vehicle to study effect of ISRA concentration prepared at 10.0, 20.0 and 40.0 mg/ml on its percutaneous absorption. We believe this work provides valuable data that could be utilized for development of transdermal formulation for a high potential drug that is commercially not available as a transdermal formulation. In addition, calculation of the diffusion parameters for isradipine adds to the value of this work since these parameters are being reported here for the first time ever.

Materials and Methods

Materials

Isradipine was gratefully gifted by Novartis Pharma (Basle, Switzerland). Propylene glycol and polyethylene glycol 400 (Winlab LTD., Maidenhead, Berkshire, UK), ethanol (Riedel–Dehaen AG, Seelze, Hanover, Germany), acetonitrile and glacial acetic acid (BDH Chemicals, LTD., Poole, UK) were used as supplied.

Methods

Analytical method

The concentration of ISRA was analyzed by a sensitive and validated HPLC method developed in our laboratory (14). The liquid chromatography system consisted of a constant flow pump, LC-10AD (Schimadzu), SPD-10AV UV visible variable wavelength detector set at 325 nm, computing integrator C-R4A chromatopac (Schimadzu Corporation, Koyoto Japan), Rheodyne Injector (Rheodyne Model 70 injector, Rheodyne Inc., Catati, CA, USA) and stainless steel column Kromasil C8 5µ 150 x 4.6 mm (Restek Corporation, 110 Benner Circle, Bellefonte, PA, USA). The mobile phase consisted of 50% water, 50% acetonitrile adjusted to pH 4.00 by glacial acetic acid and pumped isocratically at a flow rate of 1.8 ml /minute. The method was reproducible and precise for a range of concentration between 5-200 ng/ml.

Permeation procedure

Dorsal full–thickness skin of male rabbit (white New Zealand, n=4-5, weighing 3-4 kg) was used as a permeation membrane. The skin was carefully removed from animals and the hair was clipped using Diato electric machine without damaging the skin. The fat was removed with the aid of a scissor and the skin was stored at –20ºC before starting the permeation studies. The permeation experiments were performed at 37.5º C using Franz diffusion cells (Crown Glass Company, Somerville, NJ, USA). A full thickness dorsal skin was mounted in the diffusion cell having across sectional area of 3.14 cm^2. The skin was tightly secured between the donor and receptor compartments. The upper surface of the membrane was exposed to solution of drug formulation. The receptor compartment was filled with 14.5 or 12 ml of isotonic phosphate buffer pH 7.4. The donor compartment (sample volume of 0.5 ml) was sealed with parafilm to prevent evaporation of the test formulation. The Franz diffusion cells were placed into especially designed Franz diffusion cell drive console which was connected to thermostatic circulating water bath (Haak company, Frankfurt, F.R.G.) through stainless steel pipes allowing circulation of water through the water jacket surrounding each cell. A volume of 1.0 ml was withdrawn from the receptor compartment from each cell after 3, 6, 9, 12 and 24 hours after application of the test formulation. The withdrawn sample was immediately replaced by freshly prepared buffer solution and the samples were analyzed for ISRA by HPLC.

Preparation of test solutions:

For effect of vehicle studies, ISRA (10 mg/ml) was prepared in PEG 400, PG and ethanol after which 0.5 ml of the prepared solution was applied to the donor compartment of the diffusion cell. Different concentrations of isradipine (10 mg/ml, 20mg/ml and 40 mg/ml) were prepared in PG and 0.5 ml was applied to the donor compartment for effect of drug concentration studies.

Calculation of the cumulative amount diffused

The cumulative amount penetrated through rabbit skin was calculated as follows:

Receptor compartment volume = VR

Sample volume withdrawn = 1 ml

Sample #1(3 hrs), #2 (6 hrs), #3 (9 hrs), #4 (12 hrs), #5 (24 hrs)

Concentration C1 (3 hrs), C2 (6 hrs), C3 (9 hrs), C4 (12 hrs), C5 (24 hrs)

Cumulative amount at sample #1 (3 hrs) = VR X C1

Cumulative amount at sample #2 (6 hrs) = VR X C2 + 1ml X (C1)

Cumulative amount at sample #3 (9 hrs) = VR X C3 + 1ml X (C1 + C2)

Cumulative amount at sample #4 (12 hrs) = VR X C4 + 1ml X (C1 + C2 + C3)

Cumulative amount at sample #5 (24 hrs) = VR X C5 + 1ml X (C1 + C2 + C3 + C4)

Calculation of the diffusion parameters of isradipine

The diffusion parameters of ISRA were determined in presence of PG at a drug concentration of 40 mg/ml (i.e. 20 mg dose) using the lag time method originally described by Aguiar and Weiner (15). The Diffusion coefficient was calculated according to the following formula: $D = h^2/ 6.LT$

where D is the diffusion coefficient, h is the skin thickness measured as 0.088 cm, LT is the lag time which is calculated as the intercept of the linear portion of the graph at y = 0

The permeability coefficient (P) was calculated by dividing steady state flux by the drug concentration in the donor compartment (Cd). The steady state flux was calculated by dividing slope of the linear portion of the graph by the cross sectional area of the skin (3.14 cm^2) according to the equation: P = slope/ A.Cd

The skin/ vehicle partition coefficient (K) was calculated as follows:

K = P.h / D

Statistical analysis

The data were reported as mean ± SEM (n=4-5) and statistical analysis of the cumulative amount of ISRA diffused over a 24 hour period was carried out using one way ANOVA followed by Duncan's Multiple Comparison Test at a level of significance of $p \leq 0.05$.

Results and Discussion

Effect of vehicle:

The cumulative amount of ISRA penetrated from the different vehicles over 24 hours was found to be 333.2 ± 21.18, 570.2 ± 22.56, and 13768 ± 1906 ng (Mean ± SEM) for PEG 400, PG and ethanol, respectively as illustrated in Figure 1. It can be clearly seen from the data presented in Figure 1, that the amount of ISRA permeated across the membrane in presence of ethanol was significantly greater than PG and PEG 400. This can be explained by the fact that ethanol can serve as a penetration enhancer leading to extensive diffusion of drug molecules through the skin. Several studies have reported the effect of ethanol as a vehicle and a penetration enhancer. Obata *et al*. (16) observed that ethanol at high concentrations increases the permeation of drugs through lipid pathways by attacking the dense barrier structure of the skin. Optimum enhancement of salicylate ion permeation through human skin has been observed using ethanol (17). Similar results were observed for oestradiol by Megrab *et al*. (18), which suggested that ethanol influences the partition coefficient in the stratum cornium and enhances permeation of drugs in vitro through human cadaver skin. This enhancement was attributed to the ability of ethanol to alter the diffusional resistance of the stratum cornium and hence increasing the solubility of the drug in the stratum cornium. In presence of PG, the cumulative amount of ISRA diffused over 24 hours was higher than that produced by PEG 400 approximately 1.7 fold (Figure 1). This finding is in good agreement with other reports that have described PG as an effective vehicle for transdermal delivery of different drugs: oestradiol and metronidazole (4), minoxidil (19), corticsteroids (20), tarcine (5, 21) and piroxicam (22). The ability of PG to enhance transdermal delivery of drugs could be explained by the ability of PG to solvate the keratin structure of the cells and as a result, PG could be involved in the intracellular diffusion enhancement (23) and by disordering the lamellar lipid structure (24). It can also be seen from Figure 1 that the amount of the drug diffused through the rabbit skin from PEG 400 was about 58% of the amount obtained by using PG. This result can be attributed to the lower penetration ability of PEG 400 (4). In addition PEG 400 is less effective in maintaining hydration of the skin surface as dehydrated skin is more resistant to penetration (25).

Furthermore, PEG 400 was reported not to alter skin barrier properties (4, 26) and it may form complexes with drugs in the receptor compartment and therefore slows their diffusion (27).

Effect of drug concentration:

Propylene glycol was chosen as a suitable vehicle in this study for permeation of ISRA, a similar conclusion was observed for the permeation of metronidazole (28) and dihydroergotamine (29) using PG as a vehicle. It can be seen from the data in Figure 2 for the permeation of ISRA from PG using different drug concentrations that the cumulative amount of the drug penetrated the skin over 24 hours from drug concentrations of 10, 20, and 40.0 mg/ml were 570.2 ± 22.56 ng, 794.2 ± 74.56 ng and 2243.1 ± 788.03 ng (Mean ± SEM), respectively. The data show that as the concentration increased, the amount penetrated across the skin was also increased according to a linear relationship with a correlation coefficient of 0.978 and the 40 % drug concentration was significantly higher than the other two concentrations. This result suggests that the ISRA diffusion through the skin followed the passive diffusion mechanism. Wester and

gure 1. Cumulative amount (Mean ± SEM, n=5) of sradipine penetrated through excised rabbit skin form

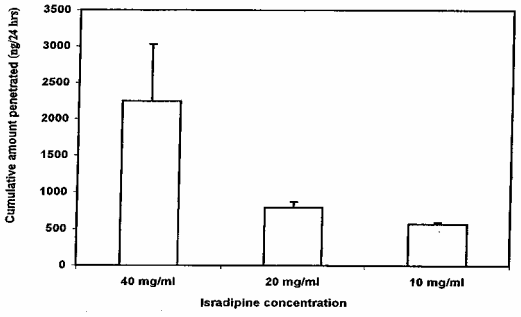

Figure 2. Cumulative amount (Mean ± SEM, n=4-5) of isradipine penetrated through excised rabbit skin from different concentrations in PG.

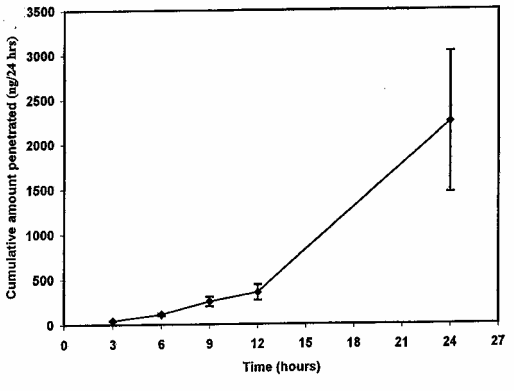

Figure 3. Cumulative amount (Mean ± SEM, n=4) of isradipine penetrated through excised rabbit skin from PG at drug concentration of 40 mg/ml.

Maibach (30) investigated the relationship between topical dose applied and percutaneous absorption for several drugs in Rhesus monkeys and in man. They concluded that as concentration of the drug increased the absolute amount of drug absorbed always increased. A similar result was reported by Scheuplein and Ross (31) for minoxidil.

Calculation of the diffusion parameters of isradipine

The mean cumulative amounts of isradipine penetrated through skin from propylene glycol at drug concentration of 40 mg/ml were plotted as shown in Figure 3 to determine diffusion parameters namely: diffusion coefficient (D), permeability coefficient (P) and partition coefficient (K). The calculated diffusion parameters are presented as

Table 1: Diffusion parameters of isradipine in presence of propylene glycol at drug concentration of 40 mg/ml.

PARAMETER	MEAN	SEM
Lag time, LT (hour)	6.3	0.521
Diffusion Coefficient, D (cm^2/second)	5.82×10^{-8}	5.29×10^{-9}
Permeability Coefficient, P (cm/second)	5.51×10^{-10}	2.04×10^{-10}
Partition Coefficient, K	0.92×10^{-3}	0.39×10^{-3}

(Mean ± SEM) in Table 1. A lag time of 6.3 ± 0.521 hour was calculated for ISRA which is higher than that reported by Niazy *et al* (29) for dihydroergotamine. Consequently, lower diffusion coefficient ($5.82 \times 10^{-8} \pm 5.29 \times 10^{-9}$ cm^2/sec), lower permeability coefficient ($5.51 \times 10^{-10} \pm 2.04 \times 10^{-10}$ cm/sec) and lower partition coefficient ($0.92 \times 10^{-3} \pm 0.39 \times 10^{-3}$) are calculated for ISRA, which can be attributed to the lower lipophilicity of ISRA compared with dihydroergotamine.

Conclusions

In conclusion, our data suggest that the permeability of ISRA could be effective at applied concentration of 10.0 mg/ml in presence of PG, PEG 400 and ethanol as vehicles, with ethanol giving significantly higher amount of drug diffused through skin owing to its ability to serve as a vehicle and a penetration enhancer. In addition, the present study shows that increasing the concentration of the drug can significantly increase the cumulative amount of the drug permeated across the skin over a period of 24 hours. Knowing that the skin has limited capacity for drug transport beyond which further increase in transdermal delivery is difficult to obtain without using penetration enhancers. Therefore, additional studies are currently under investigation to study the effect of different penetration enhancers and the ones with highest potential to increase drug penetration will be utilized in an attempt to develop a transdermal formulation for isradipine.

Acknowledgments

The author would like to thank Mr. Abubakar El-Gorashy for the technical assistance. This study was supported by a grant from the Research Center, College of Pharmacy, King Saud University (# CPRC54).

References

1. Frishman WH and Michalson MD. Use of calcium antagonists in patients with ischemic heart disease and systemic hypertension. Am J Cardiol 1997; 22: 33-38.
2. Doat M, Hoct JP, Pavin D, Righetti A. Cardiac function improvement 24 hours after isradipine SRO in patients with chronic stable angina: a double-blind randomized study. Acta Cardiol 1996; 51: 155-164.
3. Sandoz Index Pharmaceutical Product Information, International Edition. 1997: 83-92.
4. Mollgaard B and Hoelgaard A. Vehicle Effect on topical drug delivery: II. Concurrent skin transport of drugs and vehicles components. Acta Pharm Suec 1983; 20: 443-450.
5. Sathyan G, Ritschel WA, Hussain A. Transdermal delivery of tarcine: I; Identification of suitable delivery vehicle. Int J Pharm 1995; 114: 75-83.
6. Cross SE, Pugh WJ, Hadgraft J, Roberts MS. Probing the effect of vehicles on topical delivery: understanding the basic relationship between solvent and solute penetration using silicone membranes. Pharm Res 2001; 18: 999-1005.
7. Gabiga H, Cal K, Janicki S. Effect of penetration enhancers of isosorbide dinitrate penetration through rat skin from a transdermal therapeutic system. Int J Pharm 2000; 199: 1-6.
8. Ghanem AH, Mahmoud H, Higuchi WI, Rohr UD, Bordia S, Liu P, Fox JL, Good WR. The effects of ethanol on the transport of B-estradiol and other permeants in hairless mouse skin: II. A new quantitative approach. J Controlled Release 1987; 6: 75-83.
9. Berner B, Mazzenga GC, Otte JH, Steffens RJ. Ethanol: water mutually enhanced transdermal therapeutic system: II. Skin permeation of ethanol and nitroglycerin. J Pharm Sci 1989; 78: 402-407.
10. Campbell SK, US patent # 437945, 1983.
11. Gale RM and Berggren RG. US patent # 4615699, 1986.
12. Gale RM, Lee ES, Taskocich LT, Yum SI. US patent # 458858, 1986.
13. Poulsen BJ, Coquilla E, Kataz M. Effect of topical vehicle and composition on in vitro release of flucinilone acetonide and its acetate ester. J Pharm Sci 1968; 67: 928-931.
14. Al-Suwayeh SA. Quick, simple and sensitive HPLC method for determination of isradipine in plasma and its application in pharmacokinetic studies. Analytical Letters 2002; 35: 1205-1213.
15. Aguiar AJ and Weiner MA. Percutaneous absorption of chloramphenicol solutions. J Pharm Sci 1969; 58: 210-215.
16. Obata Y, Takayama K, Maitani Y, Machida Y, Nagai T. Effect of ethanol on the permeation of ionized and nonionized diclofenac. Int J Pharm 1993; 89: 191-198.
17. Kurihera-Bergstorm T, Knuston K, DeNoble J, Goates CY. Percutaneous absorption enhancement of ionic molecules by ethanol–water systems in human skin. Pharm Res 1990; 7: 726–766.
18. Megrab AN, William AC, Barry BW. Oestradiol permeation across human skin, silastic and snake skin membranes: the effect of ethanol water co-solvent systems. Int J Pharm 1995; 116: 101–112.
19. Tasi JC, Flynn GL, Weiner N, Ferry JJ. Effect of minoxidil concentration on the deposition of drug and vehicle into the skin. Int J Pharm 1993; 96: 111-117.
20. Bedas B, Schmalfub U, Neubert K. Influence of propylene glycol as cosolvent on mechanism of drug transport from hydrogels. Int J Pharm 1995; 116: 19-30.
21. Kim JH, Cho YJ, Choi HK. Effect of vehicles and pressure sensitive adhesives on the permeation of tarcine across hairless mouse skin. Int J Pharm 2002; 196: 105-113.
22. Okyama H, Ikeda Y, Imamori K, Takayama K, Nagai T. Influence of non-ionic surfactants, pH and propylene glycol on percutaneous absorption of piroxicam from cataplasm. Int J Pharm 1999; 186: 141-148.
23. Goodman M and Barry BW. Action of penetration enhancers on human stratum cornium as assessed by differential scanning colorimetry. In: Bronaugh RL and Maibach H, eds. Percutaneous absorption: mechanisms-methodology, drug delivery, Dekker, New York, 1989: 567-93.
24. Bouwstra JA, Devries MA, Gooris GS, Bras W, Brussee J, Ponec M. Thermodynamic and structural aspects of the skin barrier. J Controlled Release 1991; 15: 209-220.

25. Sarpotdar PP, Gaskill JL,Giannini RP. Effect of polyethylene glycol 400 on the penetration of drugs through human cadaver skin in vitro. J Pharm Sci 1986; 75(1): 26-28.
26. Tojo K, Chiang CC, Chien YW. Influence of donor solution upon skin permeation of drugs. J Chem Eng Japan 1986; 19: 153-155.
27. Watkinson AC, Joubin H, Green DM, Brian KR, Hadgraft J. The influence of vehicle on permeation from saturated solutions. Int J Pharm 1995; 121: 27-36.
28. Wotten P, Mollgaard B, Hadgraft J, Hoelgaard A. Vehicle effect on topical drug delivery. III. Effect of azone on cutaneous permeation of metronidazole and propylene glycol. Int J Pharm 1985; 24: 19-26.
29. Niazy EM, Molokhia A M, El-Gorashi AS. Effect of vehicle and drug concentration on transdermal delivery of dihydroergotamine using excised animal skin. Drug Dev Ind Pharm 1990; 16: 1697-1715.
30. Wester RC and Maibach HI. Relationship of topical dose and percutaneous absorption in Rhesus monkey and man. J Invest Dermatol 1976; 67: 518-520.
31. Scheuplein RJ and Ross LW. Mechanism of percutaneous absorption, V. Percutaneous absorption of solvent deposited solids. J Invest Dermatol 1974; 62: 353-360.