Transdermal Penetration of Vasoconstrictors—Present Understanding and Assessment of the Human Epidermal Flux and Retention of Free Bases and Ion-Pairs

Sheree E. Cross,[1] Melanie J. Thompson,[1] and Michael S. Roberts[1,2]

Received July 9, 2002; accepted October 8, 2002

Purpose. As reductions in dermal clearance increase the residence time of solutes in the skin and underlying tissues we compared the topical penetration of potentially useful vasoconstrictors (VCs) through human epidermis as both free bases and ion-pairs with salicylic acid (SA).

Methods. We determined the *in vitro* epidermal flux of ephedrine, naphazoline, oxymetazoline, phenylephrine, and xylometazoline applied as saturated solutions in propylene glycol:water (1:1) and of ephedrine, naphazoline and tetrahydrozoline as 10% solutions of 1:1 molar ratio ion-pairs with SA in liquid paraffin.

Results. As free bases, ephedrine had the highest maximal flux, Jmax = 77.4 ± 11.7 μg/cm^2/h, being 4-fold higher than tetrahydrozoline and xylometazoline, 6-fold higher than phenylephrine, 10-fold higher than naphazoline and 100-fold higher than oxymetazoline. Stepwise regression of solute physicochemical properties identified melting point as the most significant predictor of flux. As ion-pairs with SA, ephedrine and naphazoline had similar fluxes (11.5 ± 2.3 and 12.0 ± 1.6 μg/cm^2/h respectively), whereas tetrahydrozoline was approximately 3-fold slower. Corresponding fluxes of SA from the ion-pairs were 18.6 ± 0.6, 7.8 ± 0.8 and 1.1 ± 0.1 respectively. Transdermal transport of VC's is discussed.

Conclusions. Epidermal retention of VCs and SA did not correspond to their molar ratio on application and confirmed that following partitioning into the stratum corneum, ion-pairs separate and further penetration is governed by individual solute characteristics.

KEY WORDS: vasoconstrictor; transdermal; percutaneous absorption; epidermal retention.

INTRODUCTION

The extent of penetration of topically applied drugs into underlying tissues is principally determined by (i) the ability of the drug to partition into or dissolve in intercellular lipids, (ii) diffuse across the structural barrier of the epidermis, and (iii) be cleared by a combination of the underlying dermal blood supply into the systemic circulation and transport into lower tissues (1). In principle, the maximum flux is generally related to the product of the solubility of the solute in the vehicle and its diffusivity in stratum corneum lipids. This solubility can ultimately be related to the solute's melting point and octanol-water partition coefficient (1) whereas the diffusivity in the stratum corneum is related to both the solute's hydrogen bonding properties and molecular size (2). Small, polar drugs are cleared by the local blood supply. The topical route of administration is useful for potent drugs most effectively administered at a constant rate as exemplified by glyceryl trinitrate, nicotine, and scopolamine transdermal patches. In contrast, percutaneous penetration of drugs to dermis and muscle is optimized when clearance by the local blood supply is limited (1). Indeed, Singh and Roberts (3) have shown that higher concentrations are found in tissues below the application site when there is minimal blood flow to the tissues underlying the application site.

The coadministration of vasoactive agents to the dermis has been demonstrated to alter the absorption and distribution profiles of locally applied solutes from the epidermis, subcutaneous tissue, and dermis (3–6). The adrenergic VCs epinephrine and phenylephrine have traditionally been included in intradermal injections to decrease the systemic clearance of local anaesthetics from subcutaneous sites by decreasing dermal blood flow (3,7). Riviere *et al.* (5) showed that coadministration of norepinephrine significantly increased the tissue accumulation of lidocaine in a perfused porcine skin flap preparation following topical iontophoresis of both solutes. The similar, but less potent, adrenoceptor agonist phenylephrine has also been shown to increase local tissue concentrations of salicylic acid and lidocaine applied topically to the dermis of anesthetized rats (3).

It is apparent that there are many potential therapeutic advantages to be gained from simultaneous VC therapy to increase local tissue accumulation through decreased systemic clearance of solutes in the topical treatment of local conditions such as muscle pain and inflammation. The first requisite of a dermatological formulation possessing local VC activity is that the vasoconstricting agent itself is capable of passing through the epidermis in sufficient quantities such as to elicit the desired pharmacological response on dermal blood vessels. In fact, a number of adrenoceptor agonists are already used clinically for their topical VC activity as decongestants following application to the nasal mucosa. However, the nasal mucosa has a far higher permeability to solutes than the normal epidermis as well as many other mucosal surfaces (8). Therefore, this study was designed to compare the flux of a group of potentially useful adrenergic receptor agonists through the less permeable and clinically most relevant human abdominal epidermal membranes. We were particularly interested in examining whether VC structure (Table I)—human epidermal flux relationships could be defined for this series of compounds.

Increasing the lipophilicity of solutes can increase their ability to penetrate biologic membranes. One method of achieving this for ionizable solutes is by the formation of ion-pairs with opposite-charged molecules. Irwin *et al.* (9) were among the first to confirm this theory of the gastrointestinal tract, and many researchers have since applied the technique to transdermal delivery (10–13). The VCs used in this study are ionizable species (bases), therefore we determined whether ion-pair formation with a clinically relevant anti-inflammatory agent, salicylic acid, would effectively increase the epidermal penetration of the two compounds together, or whether dissociation of the ion-pair occurred immediately following partitioning from the vehicle.

[1] Therapeutics Research Unit, Department of Medicine, University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia.

[2] To whom correspondence should be addressed. (e-mail: mroberts@medicine.pa.uq.edu.au)

0724-8741/03/0200-0270/0

Table I. Physicochemical Properties of the VCs Studied

VC	MW	Log P[a]	Mp (°C)
Ephedrine	165	1.05	79
Naphazoline	210	3.53	120
Oxymetazoline	260	4.17	179
Phenylephrine	167	–0.03	171
Tetrahydrozoline	200	3.13	113
Xylometazoline	244	4.91	132

[a] From SciFinder Scholar.

MATERIALS AND METHODS

(-)Ephedrine, naphazoline hydrochloride, oxymetazoline hydrochloride, tetrahydrozoline hydrochloride, and xylometazoline hydrochloride were purchased from Sigma Chemical Co, St. Louis, L-Phenylephrine was purchased from ICN Biochemicals, Cleveland, Ohio. Where necessary free bases were formed from the hydrochloride salts of VCs by dissolving a small amount in water in a separating funnel then adding chloroform and ammonia solutions and allowing the free base formed to partition into the chloroform layer. The chloroform layer was then run off and the ammonia layer re-extracted twice with fresh chloroform. Chloroform layers were pooled and a molecular sieve added to dry the chloroform extract, it was then decanted off the sieve into a rotary evaporator and the chloroform removed to leave the free base. Saturated solutions of each of the VCs were prepared in a 1:1 propylene glycol:water vehicle that had been mixing with excess of the solute for at least 48 hours. For the ion-pairs study, 1:1 molar ratios of the VCs ephedrine, naphazoline, tetrahydrozoline, and the amine triethylamine paired with SA base were added to a liquid paraffin vehicle to give final concentrations of 10% (w/v).

HPLC Analysis

The HPLC detection system used for analysis consisted of a model LC10AD pump, an SPD-6A variable wavelength UV detector, and SIL-9A autoinjector (Schimadzu Corp., Kyoto, Japan). Data were collected and processed using Delta version 5.0 chromatographic software. The column used was an Alltima C_{18} 150 × 4.6 mm i.d. (Alltech Assoc., Eagle Farm, Queensland), the flow rate used was 1 ml/min, injection volume 20 μl and UV detection effected at a wavelength of 215 nm. The mobile phases consisted of mixtures of acetonitrile (EM Science, New Jersey) and 0.05 M potassium dihydrogen phosphate (Sigma Chemical Co, St. Louis, US) with the addition of 0.05% heptane sulphonic acid (Sigma Chemical Co, St. Louis, US) as the ion-pair, with linearity of response confirmed for concentrations between 1–200 ug/ml. The HPLC method used for analysis of SA was published previously (14).

Epidermal Penetration Studies

The penetration of VCs from saturated solutions of the base form of each in a propylene glycol:water (1:1) vehicle was determined using segments of a heat-separated human female abdominal epidermal membrane (single donor, age 33) mounted in Franz-type horizontal glass diffusion cells (surface area approx. 1.3 cm^2). Receptor chambers contained 20% ethanol: 80% water, approx. 3.5 ml (volume determined for each cell to accurately calculate penetrating concentrations) which was degassed and continuously stirred at 32°C. Membranes were equilibrated in diffusion cells with receptor fluid overnight and 500 μl of donor vehicle was added at t = 0 with the receptor chamber sampled by complete emptying and refilling with fresh receptor medium at approx. 4, (ephedrine only) 8, 24, 32, 48, and 55 h. The steady state flux, Jss (μg/cm^2/h), was estimated as the slope of the linear regression between cumulative amount of VC or SA penetrating into the receptor compartment and time.

At the end of the penetration study the amount of VC or SA remaining in the epidermis was also quantified. Membranes were removed, blotted dry, swabbed with methanol solution, air dried on the stratum corneum side, and stripped once with adhesive tape (which was discarded) and weighed. Samples were then minced with scissors in 400 μl of receptor medium, sonicated on ice for 30 s, centrifuged for 15 min at 13,000 g and the supernatant removed for analysis. All VC and SA concentrations in receptor fluid and epidermal samples were determined in duplicate by HPLC.

Data Analysis

Stepwise multiple regression was used to examine solute physicochemical properties: molecular volume, melting point, lipophilicity (defined as octanol-water partition coefficient), solubility in vehicle, solubility parameter as well as skin retention as possible determinants of maximum flux of the free bases from the PG:water vehicle.

RESULTS

The maximum flux of each VC through the isolated human epidermis is shown in Table II. It can be seen that there was over a 100-fold difference observed in this parameter, with ephedrine having the fastest rate of penetration, followed by tetrahydrozoline, xylometazoline, phenylephrine, naphazoline, and oxymetazoline. The variability in the skin retention of the VCs, however, was much lower than that of the maximum fluxes, with only a 2-fold difference exhibited between the highest (ephedrine) and lowest (xylometazoline) levels retained (Table II).

Stepwise linear regression identified melting point (°C) as the only significant ($p < 0.05$ (Pearson correlation one-tailed)) predictor of VC maximum flux (Jmax) from the series of physicochemical properties tested (Fig. 1): Log J max = 3.008 – 0.0147mp $r^2 = 0.643$.

The epidermal penetration and retention characteristics

Table II. Epidermal Transport of Various Adrenergic VCs following Topical Application to Human Epidermal Membranes *in Vitro*

VC	Epidermal maximum flux (μg/cm^2/h)	Skin retention (μg/mg)
Ephedrine	77.4 ± 11.69	60.29 ± 4.66
Naphazoline	8.08 ± 1.75	55.68 ± 7.27
Oxymetazoline	0.68 ± 0.23	37.25 ± 8.34
Phenylephrine	12.88 ± 1.39	58.54 ± 6.87
Tetrahydrozoline	22.62 ± 5.47	48.57 ± 3.56
Xylometazoline	19.85 ± 3.96	33.77 ± 1.76

Note: Data represents mean ± SD, n = 6.

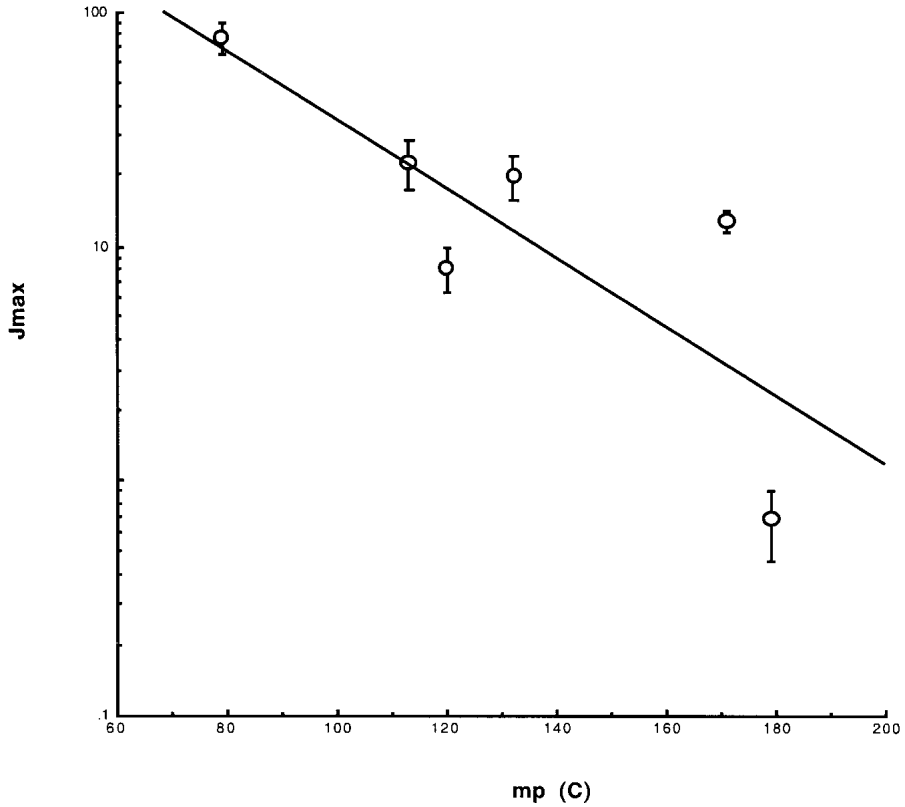

Fig. 1. Relationship between Log maximum flux (Log Jmax) and VC melting point. Data represents mean ± SE, n = 6.

of the combination of VCs with SA as ion-pairs applied in liquid paraffin are shown in Table III. It can be clearly seen that following absorption into the membrane the ion-pairs do not appear to penetrate together, with unequal molar ratios of VC and SA present in either or both the receptor phase and the epidermal membrane. Figure 2 gives a schematic representation of the distribution of the VC and SA components of the ion-pairs at the end of the study period.

DISCUSSION

Much research effort has been focused on increasing the penetration of topically applied drugs through the epidermis to attain higher local tissue concentrations. The inclusion of a range of chemicals capable of enhancing absorption rates by altering the physiology of the stratum corneum has been popular (15). The process of inducing vasoconstriction of the local dermal vasculature and resultant decrease in systemic clearance of drug from an applied site appears to be a more physiologic approach to achieving the desired effect. The constriction of dermal blood vessels by topically applied agents has been used pharmacologically as a treatment of nasal congestion for some time. It has been shown that topical VCs can be absorbed through the nasal mucosa and reduce local blood flow by over 50%, as demonstrated by Bende (16).

The adrenergic VC xylometazoline has been shown to decrease the systemic absorption of nicotine administered from a nasal spray through its ability to reduce dermal blood flow and subsequent clearance by the blood from the application site (17). Using the isolated perfused porcine skin flap model with iontophoresis to enhance transdermal absorption,

Table III. Epidermal Flux and Tissue Retention of VCs and SA following Application of Ion-Pairs

	Epidermal flux ($\mu g/cm^2/hr$)		Skin retention $\mu g/mg$	
VC	VC	SA	VC	SA
Ephedrine	11.5 ± 2.3	18.6 ± 0.6	10.0 ± 0.4	4.2 ± 0.7
Naphazoline	12.0 ± 1.6	7.8 ± 0.8	20.7 ± 6.0	3.5 ± 1.1
Tetrahydrozoline	2.9 ± 0.5	1.1 ± 0.1	3.7 ± 0.6	2.8 ± 1.1

Note: Mean ± SE. n = 6.

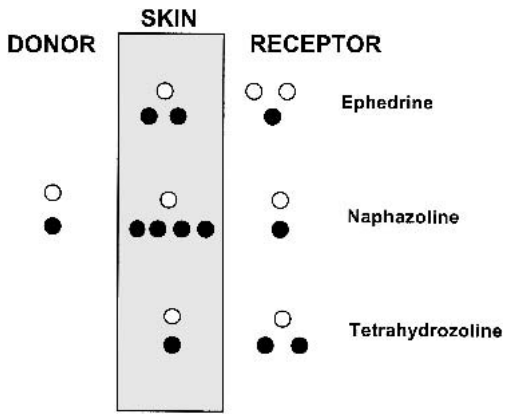

Fig. 2. Schematic representation of the approximate relative distribution of each part of the VC (closed symbols) and SA (open symbols) ion-pair applied to human epidermal membranes.

Riviere *et al.* (5) showed that norepinephrine increased the depth of penetration of lidocaine whereas its clearance by the local blood vessels into outflowing perfusate was significantly decreased. In anaesthetized rats, Singh and Roberts (3) showed that phenylephrine increased the depth of penetration of lidocaine and salicylic acid into underlying muscle when applied directly onto the exposed dermis. Unfortunately, these studies examining abdominal skin have either used iontophoresis to overcome the permeability barrier of the stratum corneum or removed the epidermis and studied only kinetics following direct dermal application.

The present compared the passive transepidermal flux of a range of VCs through human abdominal membranes with the aim of identifying which solutes have the highest potential for use on human peripheral skin sites. Our results show that ephedrine clearly has the highest permeation rate through human epidermis at 77.4 ± 11.7 $\mu g/cm^2/h$ being at least 4- to 100-fold faster than the other agents studied. (-)Ephedrine has also been one of the most potent VCs on cutaneous vasculature (18), suggesting that it is the most suitable candidate for future topical formulation studies.

The dominance of the VC melting point as a determinant of Jmax is consistent with earlier studies. Kaplun–Frischoff (19) and Stott (20) considered the effect of permeant melting point depression on transdermal penetration in terms of the concept that the lower the melting point of a substance the greater its solubility in a given solvent, including skin lipids. Consistent with this hypothesis, Larsen *et al.* (21) observed a linear correlation between log molar solubility of compounds in oil solutions and the melting point of the solutes. In their examination of the flux of 35 substituted pyridine derivatives through polydimethylsiloxane membranes, Hu and Metheson (22) observed that compounds with a higher melting point had a lower steady-state flux. However, for the compounds used, a higher melting point was also correlated with both higher polarity, and therefore less favorable partitioning, and higher molecular volume. Therefore, it should be expected to correlate with steady-state flux.

Other studies with larger numbers of solutes confirm that solute size and lipophilicity may also be determinants of maximum flux. Kai *et al.* (23) observed linear relationships between Jmax and both melting point and lipophilicity for the penetration of beta-blockers through rat abdominal skin and hamster cheek pouch. In a study examining the transfer rate of 13 drugs from transdermal patches into rat skin, the transferred percentage of drug was lower the higher the melting point, lipophilic index and molecular weight. In addition, the difference between transferred drug percentages to stripped and intact skin, which could be regarded as the regulatory contribution of the stratum corneum, tended to be larger, the lower the drugs melting point and lipophilic index (24). Ghosh and Reddy (25) observed a relationship between the reciprocal of melting point and the steady-state flux of five antihypertensive drugs and concluded that drug derivatives of low melting point and good aqueous solubility could be favorable candidates for topical delivery.

The result of the ion-pairing of VCs with SA in liquid paraffin, chosen as a non-polar solvent and therefore in which ion-pairing should be maximal, for topical application shows that the ion-pairs appear to separate on entry into the epidermal membrane. The difference in the molar ratios of VC to SA detected in the receptor fluid and remaining in the membrane at the end of the study confirms that the solutes are distributing according to their individual characteristics. The concept of ion pairing was applied to transdermal delivery in accordance with the pH-partition hypothesis, which suggested that only the unionized forms of drugs are able to pass through lipoidal membranes (26). Absorption studies with SAs, lignocaine and carboxamine supported this hypothesis, however, evidence provided by later work has supported the existence of parallel penetration pathways and shunt routes within the stratum corneum for ionized species (27). In this study, the evidence suggests that the VC and SA ion-pairs formed in the liquid paraffin vehicle rapidly separate in the environment of the epidermis and diffuse and distribute separately. Unfortunately, it was not determined whether the ratios of the retained amounts of VC and SA were the same for both the lipoidal stratum corneum and more polar viable epidermis as the tissue was measured as a single sample. Further studies to examine this possible difference in penetration ratios are required to shed light on where the separation occurs. The data does confirm, however, that the concept of application of VC bases together with a drug of clinical interest, such as an anti-inflammatory non-steroidal agent or a local anesthetic could be an effective topical combination therapy without the need for ion-pairing or chemical bonding of the two drugs to increase penetration and pharmacological efficacy.

CONCLUSIONS

In conclusion, the combination of, high permeability shown in this study and known relative potency, therefore warrant the further investigation of ephedrine as an adjuvant to topical creams and products designed for the treatment local conditions, such as muscle pain and inflammation, where an ability to deliver high concentrations of drug to underlying tissues and not the systemic circulation is desired.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Dr W. John Pugh in the preparation of free bases of the VCs. This research was supported by the National Health and Medical Research Council of Australia.

REFERENCES

1. M. S. Roberts, S. E. Cross, and M. A. Pellett. *Skin Transport in Dermatological and Transdermal Formulations* ed KA Walters Marcel Dekker, New York (2002) pp 89–195.
2. W. J. Pugh, M. S. Roberts, and J. Hadgraft. Epidermal permeability-penetrant structure relationships: 3. The effect of hydrogen bonding interactions and molecular size on diffusion across the stratum corneum. *Int. J. Pharm.* **138**:149–167 (1996).
3. P. Singh and M. S. Roberts. Effects of vasoconstriction on dermal pharmacokinetics and local tissue distribution of compounds. *J. Pharm. Sci.* **83**:783–791 (1994).
4. C. S. Canepa, S. H. Miller, D. C. Buck, R. J. Demuth, and M. Miller. Effects of phenylephrine on tissue gas tension, bleeding, infection and lidocaine absorption. *Plast. Reconstr. Surg.* **81**:554–560 (1988).
5. J. E. Riviere, B. Sage, and P. L. Williams. The effects of vasoactive drugs on transdermal lidocaine iontophoresis. *J. Pharm. Sci.* **80**:615–620 (1991).
6. J. E. Riviere, N. A. Monterio-Riviere, and A. O. Inman. Determination of lidocaine concentrations in skin after transdermal iontophoresis: Effects of vasoactive drugs. *Pharm. Res.* **9**:211–214 (1992).
7. T. R. P. Ford, M. A. Seare, and F. McDonald. Action of adrena-

line on the effect of dental local anaesthetic solutions. *Endodon. Dent. Traumatol.* **91**:31–35 (1993).
8. Y. W. Chien and S.-F. Chang. Intranasal drug delivery for systemic medications. *CRC Crit. Rev. Drug Carrier Syst.* **4**:66–194 (1987).
9. G. M. Irwin, H. B. Kostenbauder, L. W. Dittert, R. Staples, A. Misher, and J. V. Swintosky. Enhancement of gastrointestinal absorption of a quaternarry ammonium compound by trichloroacetate. *J. Pharm. Sci.* **58**:313–315 (1969).
10. D. J. W. Grant and T. Higuchi. Ion pairs and solubility behaviour. In: W. H. Saunders (ed.) *Solubility Behaviour of Organic Compounds*, Vol XXI of the Techniques of Chemistry Series, Wiley, New York, 1990 pp. 399–439.
11. S. Matschiner, R. Neubert, and W. Wohlrab. The use of ion-pairing to facilitate percutaneous penetration of drugs. In: E. W. Smith, and H. I. Maibach (eds.), *Percutaneous Penetration Enhancers*. CRC Press, New York, 1995 pp. 407–417.
12. M. Kadono, K. Kubo, H. Miyazaki, N. Tojyo, S. Nakagawa, K. Miyashita, T. Imanishi, J. H. Rytting, and T. Mayumi. Enhanced in vitro percutaneous penetration of saliclyate by ion pair formation with alkylamines. *Biol. Pharm. Bull.* **21**:599–603 (1998).
13. S. A. Megwa, S. E. Cross, H. A. E. Benson, and M. S. Roberts. Ion-pair formation as a strategy to enhance topical delivery of salicylic acid. *J. Pharm. Pharmacol.* **52**:919–928 (2000).
14. S. A. Megwa, H. A. E. Benson, and M. S. Roberts. Percutaneous absorption of SAs from some commercially available topical products containing methylsalicylate or salicylic acid salts in rats. *J. Pharm. Pharmacol.* **47**:891–896 (1995).
15. K. A. Walters and J. Hadgraft. Pharmaceutical skin penetration enhancement. *Drugs in the Pharmaceutical Sciences*, Vol. 59. Marcel Dekker, New York, 1993.
16. M. Bende. The effect of topical decongestant on blood flow in normal and infected nasal mucosa. *Acta. Otolaryngol. (Stockh.)* **96**:523–527 (1983).
17. E. Lunell, L. Molander, and M. Andersson. Relative bioavailability of nicotine from a nasal spray in infectious rhinitis and after use of a topical decongestant. *Eur. J. Clin. Pharmacol.* **48**:71–75 (1995).
18. M. C. Koss. Characterization of adrenoceptor subtypes in cat cutaneous vasculature. *J. Pharmacol. Exp. Ther.* **254**:221–227 (1990).
19. Y. Kaplun-Frischoff and E. Touitou. Testosterone skin permeation enhancement by menthol through the formation of a eutectic with drug and interaction with skin lipids. *J. Pharm. Sci.* **86**:1394–1399 (1997).
20. W. Stott, A. C. Williams, and B. W. Barry. Transdermal delivery from eutectic systems: enhanced permeation of a model drug, ibuprofen. *J. Control. Release* **50**:297–308 (1997).
21. D. B. Larsen, H. Parshad, K. Fredholt, and C. Larsen. Characteristics of drug substances in oily solutions. Drug release rate, partitioning and solubility. *Int. J. Pharm.* **232**:107–117 (2002).
22. M.-W. Hu and L. E. Matheson. The development of a predictive method for the estimation of flux through polydimethylsiloxane membranes. III. Application to a series of substituted pyridines. *Pharm. Res.* **10**:732–736 (1993).
23. T. Kai, T. Isami, K. Kobota, Y. Kurosaki, T. Nakayama, and T. Kimura. Keratinised epithelial transport of beta-blocking agents. I. Relationship between physicochemical properties of drugs and the flux across rat skin and hamster skin cheek pouch. *Chem. Pharm. Bull.* **40**:2498–2504 (1992).
24. T. Izumoto, A. Aioi, S. Uneoyama, K. Kuriyama, and M. Azuma. Relationship between transference of a drug from a transdermal patch and the physicochemical properties. *Chem. Pharm. Bull.* **40**:456–468 (1992).
25. B. Ghosh and L. H. Reddy. Effect of physicochemical parameters on skin permeability of antihypertensive. *Indian J. Exp. Biol.* **39**:710–714 (2001).
26. A. Pardo, Y. Shiri, and S. Cohen. Kinetics of transdermal penetration of an organic ion pair: physostigmine salicylate. *J. Pharm. Sci.* **81**:990–995 (1992).
27. J. C. Smith and W. J. Irwin. Ionization and the effect of absorption enhancers on transport of salicylic acid through silastic rubber and human skin. *Int. J. Pharm.* **210**:69–82 (2000).