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Note

Effect of temperature on imipramine hydrochloride permeation: role of lipid bilayer arrangement and chemical composition of rat skin

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Abstract

The aim of this investigation was to study the effect of temperature on the permeation of imipramine hydrochloride (IMH) across rat skin from two different vehicles. Differential scanning calorimetry (DSC) was used to characterize the phase transitions of rat epidermis and extracted rat SC lipids, and the transition temperatures were correlated with the permeability of IMH at different temperatures. Permeability of IMH from ethanol and propylene glycol (PG) was determined at five different temperatures and observed that a significant increase in IMH permeability occurred 45 °C from both the vehicles. Further, high energies of activation for rat skin permeation suggested that IMH diffuses across intercellular lipid matrix and therefore any change in the packing of SC lipids will have an effect on IMH permeation. Three endotherms T_1, T_2 and T_3 of rat epidermis were observed in DSC thermograms at 44, 53 and 64 °C and were assigned as transitions corresponding to orthorhombic to hexagonal, hexagonal to more disordered phase and melting of lipids with high cholesterol content, respectively. The high permeability values of IMH above 45 °C were therefore reasoned to be because of orthorhombic to hexagonal phase transition in rat skin from close to that temperature.

Keywords: Stratum corneum; Temperature; Imipramine hydrochloride; Permeability; Activation energy

Stratum corneum (SC), the upper most layer of the skin, functions as protective barrier against water loss (hence, maintains body temperature) and invasion of microbes. This very protective function of the SC also makes it the main hurdle from view of drug delivery through the skin. SC is made up of lipids and proteins with its architecture being comparable to a 'brick and mortar' wall. The lipid bilayers exist as mortar in between the corneocyte pockets (bricks). These lipid bilayers of the SC impose maximum resistance to the permeation of drugs. Drugs can permeate across the skin through appendages (via hair follicles, acrine and sebaceous glands), transcellularly (via corneocytes)

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through lipid bylayer. Phase behavior studies of SC have shown that arrangement and state of lipid bilayer is changing with temperature (White et al., 1988; Krill et al., 1992; Ongpipattanakul et al., 1994). Lipid bilayer of SC can exist in crystalline gel, liquid–crystalline state or mesomorphic form depending upon the temperature.

In literature, effect of polymorphic phase behavior of human cadaver skin and pig skin, and its effect on permeability of membrane was documented very well. However, inspite of the fact that rat skin was used as model membrane in most of the research papers related to transdermal drug delivery, very few reports discuss the polymorphic phase behavior of rat skin and its implication on permeability. Since the polymorphism of the lipids of SC is critically dependent on the chemical composition, it is likely that composition changes of SC lipids affect the phase behavior and consequently the barrier property. Therefore, in this investigation rat skin was used as model membrane and effect of polymorphic phase behavior of rat SC lipid bilayer on the permeability of an amphiphilic drug e.g. IMH was studied.

^{3}H-Imipramine hydrochloride (^{3}H-IMH) and unlabelled IMH were obtained from Du Pont, Willington, DE (USA) and Sigma Chemicals (USA), respectively. All other chemicals were purchased either from Sigma Chemical Co. or E. Merck (Germany), and were used as received. Dorsal skin of Sprague–Dawely rats was used in all experiments and protocol had the approval of Institutional Animal Ethics Committee (IAEC).

Permeability studies were conducted at five temperatures, 30, 35, 40, 45 and 50 °C, as per procedure described elsewhere (Panchagnula, 1996). All temperatures shown here are displayed value on instrument rather than actual temperature of skin. It was observed that there is a 5 °C difference between instrument and skin temperature (32 °C of skin temperature is achieved when instrument is set at 37 °C). Diffusion assembly comprised of unjacketed Franz diffusion cells and dry block heater–stirrer (Permegear, USA) was used in all studies. Epidermis sheet was prepared by a procedure as described by Scott et al. (1986) and stored in desiccator over phosphorous pentoxide for 24 h before thermal analysis. Heating rate and temperature range of 1.0 °C/min and 0–80 °C were selected for differential scanning calorimetry (DSC) analysis (Metler-Toledo, USA).

Rat epidermal sheets were extracted sequentially in the following solvents: (1) hexane–chloroform–acetone (8:90:2); (2) chloroform–acetone–methanol (76:8:16); (3) hexane–chloroform–acetone–methanol (6:80:10:4); (4) chloroform–acetone–methanol (76:4:20); (5) hexane–diethyl ether–ethyl acetate (78:18:4), to extract the lipids of different polarities. These solvent systems have been used as ceramide development system by Ponec et al. (2000). Vacuum dried lipids were used for DSC study and were heated at 5 °C/min from 0 to 200 °C (Metler-Toledo, USA).

The lipid bilayer of SC is heterogeneous in nature (Klausner et al., 1980; Bouwstra et al., 2000) and is assumed that three different phases of varied size exist in lipid bilayers, which are bound together with short-range co-operativity. These phases in SC are classified as: (a) highly ordered or crystalline phase comprised of straight, saturated fatty acyl chains packed tightly together (Bouwstra et al., 2000); (b) gel phase (β) made up of a 'condensed complex' of cholesterol and ceramides (Radhakrishnan and McConnell, 2000); (c) fluid region-comprised of unsaturated fatty acids with *cis* double bonds in their acyl chain (Bouwstra et al., 2000). Further, molecular dynamics simulations indicated that cholesterol is absent and saturated fatty acyl chain lipids (*trans*-conformation) exist in orthorhombic phase (Holtje et al., 2001).

DSC of dried epidermis of rat skin shows three endotherms T_1, T_2 and T_3 consistently at 44, 53 and 64 °C, respectively as shown in Fig. 1, which is in agreement with the previous findings (Barry et al., 1998). On the basis of recent findings of Velkova and Lafleur (2002), polymorphism of rat skin lipids could be explained as follows: T_1 belongs to transition from the crystalline orthorhombic phase to hexagonal phase; T_2 is transition of hexagonally arranged low cholesterol content lipid domain to more disordered phase (not lamellar); and T_3 belongs to lipid domain with higher cholesterol content. Below phase transition temperature (T_1), which depends on chemical composition of membrane, lipids are arranged in

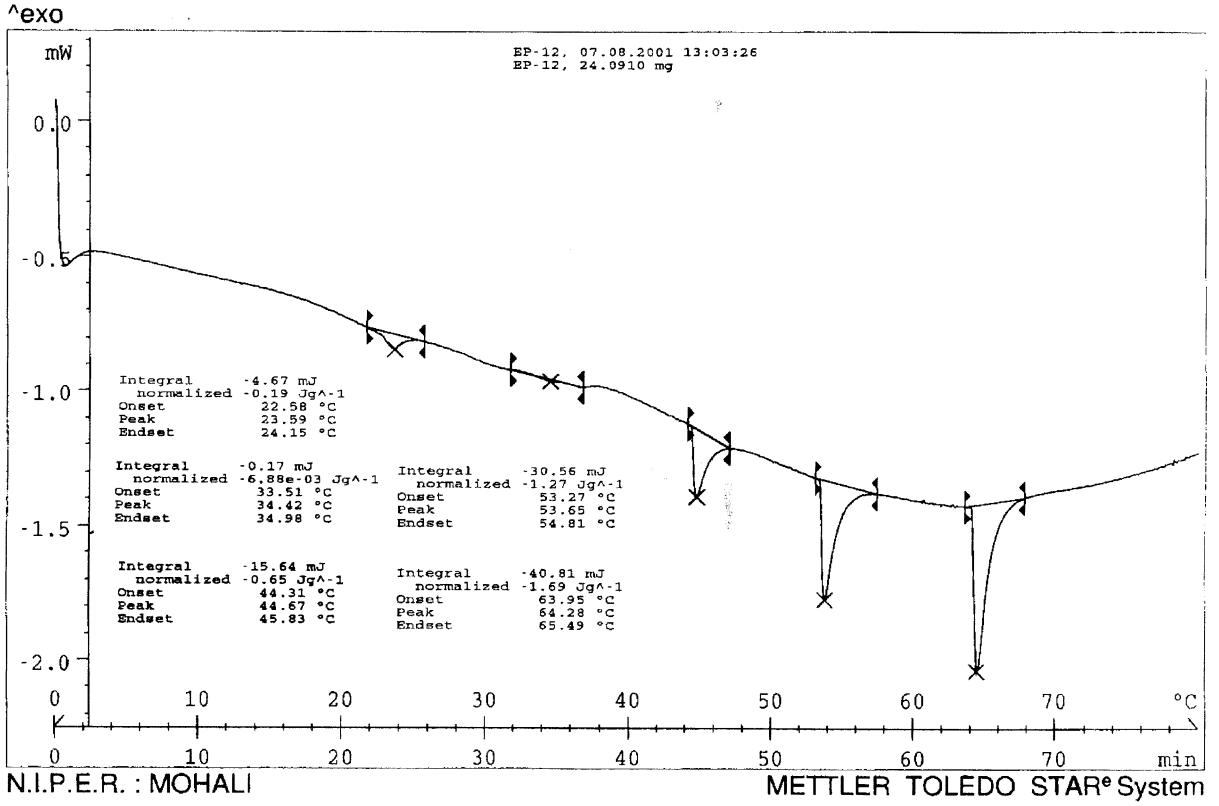

Fig. 1. DSC endotherm of desiccated epidermis of rat skin.

orthorhombic phase has been established by wide range of biophysical methods like X-ray (White et al., 1988), FT-IR (Cameron et al., 1980, 1981) and ESR (Ogiso et al., 1996b). It was observed that the enhancement ratio (ER) of IMH with ethanol and propylene glycol was approximately 16 at 50 °C (Table 1). Below 45 °C, the ER of IMH in both solvents was 1–2. This significant increase in flux can be attributed to phase transformation of lipid bilayer from orthorhombic phase to hexagonal arrangement, termed as solid–solid transition, which results in increase in free volume in SC due to loose packing of lipids as reported by Krill et al. (1992).

It has been reported earlier using molecular dynamics simulations that after annealing process, fatty acyl chains of orthorhombic phase exhibit a tilt in the bilayer plane which apparently seems to exhibit hexagonal gel phase (Holtje et al., 2001). In this phase, conformations of fatty acyl chains remain in *trans* conformation which suggest that fatty acyl chains are tightly packed and only polar head groups are disoriented. This implies that in the absence of significant conformational disorderness in fatty acyl chains of lipid bilayer, permeability through bilayer can be enhanced through the loosened head groups. The same phenomenon of phase transition of SC lipids has been explained in terms of decreased microviscosity and density of lipid fatty acyl chains of SC bilayer by Krill et al. (1992). Both these phenomenon can be attributed to the enhanced permeation of IMH. Further, the increment in flux of IMH as a result of temperature induced solid–solid transition in lipid bilayer is in agreement with the results reported by Ogiso et al. (1996b). Similarity in the curves observed in permeability vs temperature plot of IMH (Fig. 2a) in all our studies to that of Ogiso et al. (1996b) with lipophilic drugs (Fig. 2b) suggests that IMH preferably follow intercellular

Table 1
Effect of temperature on permeation parameters of IMH [mean (SD), $n = 4$]

	PG					EtOH				
	30 °C	35 °C	40 °C	45 °C	50 °C	30 °C	35 °C	40 °C	45 °C	50 °C
Lag time (h)	24.74 (2.51)	24.97 (13.22)	24.48 (13.82)	40.16 (11.47)	27.35 (9.20)	16.94 (9.80)	13.52 (2.93)	15.83 (6.36)	6.41 (1.01)	1.66 (0.89)
Flux (μg/cm^2/h)	16.33 (2.51)	12.00 (5.16)	24.33 (6.65)	27.00 (17.06)	249.75 (40.35)	64.66 (30.03)	51.00 (10.64)	109.01 (16.43)	502.5[a]	884.50 (116.58)
k_p (cm/h, $\times 10^4$)	2.18 (0.33)	1.60 (0.68)	3.24 (0.88)	3.60 (2.2)	33.30 (5.5)	7.30 (3.4)	6.80 (1.4)	14.50 (2.1)	67.00[a]	117.93 (15.5)
ER	–	0.73	1.48	1.65	15.29	–	0.78	1.68	7.17	13.67
E_a			24.82					21.24		

E_a, activation energy of transport (kcal/mol); k_p, permeability coefficient.
[a] $n = 2$.

lipid bilayer route. On the basis of present study findings and previous literature reports, it can be hypothesized that disorientation in polar heads of SC lipid bilayer due to phase transition from orthorhombic to hexagonal phase will result in direct access of permeant to fatty acyl chains or resistance imposed by tight packing of polar heads is minimized. Further, this is also supported by higher values of E_a (Cornwell and Barry, 1993). Since the structure of IMH does not contain any free functional group, higher value of E_a of IMH represents the energy that is solely required to induce 'free space' or disorientation in tightly packed lipid polar groups of SC bilayer. The hypothesis regarding the role of disorientation, in polar head groups of SC lipid bilayer, in enhanced permeation of lipophilic or amphiphilic drug could further be supported by similar type of study with hydrophilic polar drug. In our laboratory, permeation studies of zidovudine across the rat skin were carried out over the temperature range of 27–47 °C and similar results were observed as in case of IMH. It has been reported that in mammalian SC lipid bilayer orthorhombic perpendicular cells of ceramides (HFA, hydroxy fatty acid ceramide or NFA, non-hydroxy fatty acid ceramide) exists till 60 °C and main transition of gel to liquid crystalline phase occurs at approximately 80–85 °C. However, in rat SC lipid bilayer both transitions were found to be at lower temperatures. This may be due to the presence of small percentage of phospholipids in rat skin. In 1979, Elias et al. (1979) have found approximately 10% of total lipids as phospholipids in rat SC. Later Ogiso et al. (1996a) reported 3.37 ± 0.35 μg/cm^2 of phospholipids in Wistar rat SC. Recently, it has been observed that the presence of ceramides in phospholipid mixture can shift and cause broadening of transition temperature of phospholipid (Veiga et al., 1999). Since molecular dynamics simulation studies have shown that the orthorhombic phase is devoid of cholesterol (Holtje et al., 2001), the possibility of existence of phospholipids along with ceramides in orthorhombic lipid domain cannot be excluded. Recently, Imura et al. (2001) observed that at a 0.13 molar ratio of ceramide 3 in phospholipid ($T_m = 41$ °C) imparts thermostability to liposomes as reflected by shift in

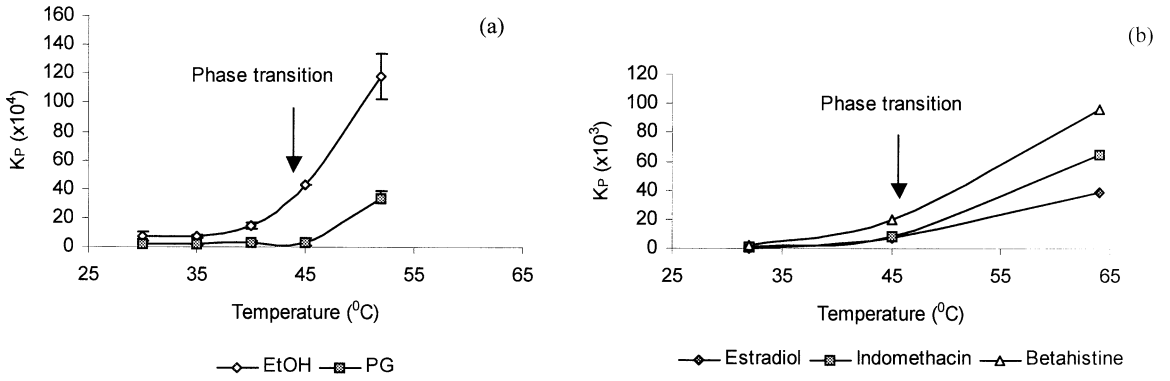

Fig. 2. (a) Permeability of IMH at different temperatures; (b) permeability of estradiol, indomethacin and betahistine (reproduced from Ogiso et al., 1996b).

transition temperature of phospholipid towards higher temperature (46 °C). This is very similar to the first transition of rat skin in DSC endotherms, which substantiate the presence of phospholipids and ceramides in orthorhombic phase. Phase behavior studies using extracted lipids have shown that the skin lipids melt between 30 and 50 °C as indicated by broad peaks in fractions 1–3 (Fig. 3).

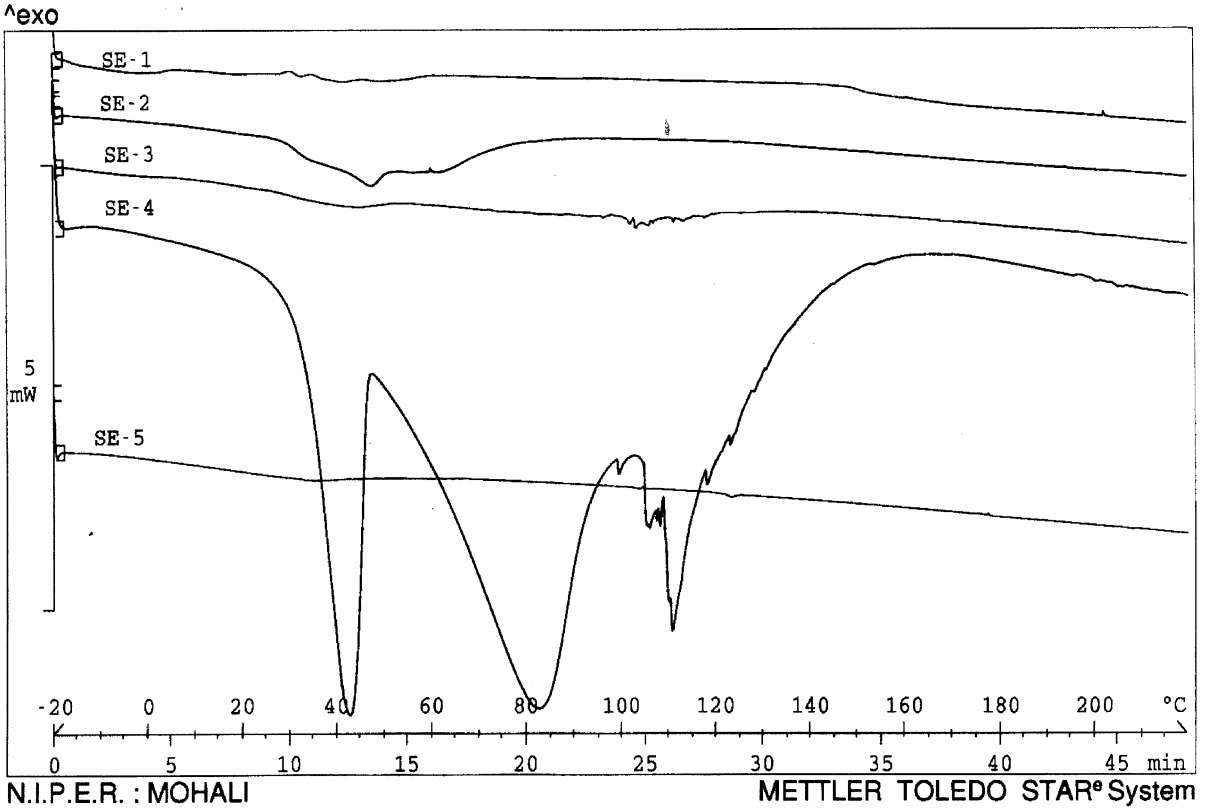

Fig. 3. DSC thermograms of lipids extracted from rat skin. Key: SE-1, lipids of fraction 1; SE-2, lipids of fraction 2; SE-3, lipids of fraction 3; SE-4, lipids of fraction 4; SE-5, lipids of fraction 5.

In fraction 4, broad intense endotherm was observed at 42 °C, which matches the first endotherm (T_1) of DSC (Fig. 1). As phospholipids melting point is known to vary from 40 to 60 °C, depending upon the fatty acyl chain, endotherm of extracted lipids at 42 °C can be attributed to phospholipids. The endotherm peak observed with extracted lipids and rat skin between 40 and 45 °C corresponds to inflection temperature in permeability (k_p) vs temperature plot. This imply that the melting of lipids (phospholipids) in orthorhombic phase facilitate the transformation of lipid acyl chain packing of skin towards hexagonal phase. Since this phase is loosely bond and less dense, drastic increase in permeability of IMH was observed.

With the DSC and permeation results of this study, and integrating the data from other studies, suggests that in rat skin one of the forms of lipid arrangement is in orthorhombic phase. Chemically, this phase may be comprised of ceramides, free fatty acids and phospholipids. Phase transition of orthorhombic to hexagonal gel phase has significant impact on permeability of drugs especially drugs which preferably follow lipophilic route as found by our results. Effect of temperature on permeation of drugs is not limited to academic interest but has potential for commercial implications as evident from the recent emergence of drug delivery technology based on control heat aided drug delivery (CHADD, http://www.zars.-com). In CHADD, skin temperature is raised by transdermal patch through oxidation reaction that results in enhanced transdermal permeation of drug. However, further studies are required to confirm the presence of phospholipids in rat skin and their contribution in phase behavior and permeability of the skin.

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