AAPS PharmSciTech, Vol. 10, No. 3, September 2009 (© 2009)
DOI: 10.1208/s12249-009-9283-5

Research Article

The Enhancing Effect of Ion-pairing on the Skin Permeation of Glipizide

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Received 2 December 2008; accepted 2 July 2009; published online 28 July 2009

Abstract. The purpose of the present study was to investigate the permeation of glipizide (GP) and observe the effect of an interaction with amines as counter ions, including diethylamine, triethylamine, ethanolamine, diethanolamine, triethanolamine, *N*-(2-hydroxylethyl) piperidine. Permeation experiments were performed *in vitro*, using rat abdominal skin as a barrier. The lipophilic donor system consisting of isopropyl myristate (IPM) and ethanol (EtOH; EI system, 8:2) produced a marked enhancement of GP *flux* through rat skin. All the amines investigated in this study had performed an enhancing effect on GP *flux*, and triethylamine had the most potent enhancing effect on GP in the vehicle IPM:EtOH=8:2(*w*/*w*). In the presence of counter ions, the solubility of GP in the donor solution (IPM:EtOH=8:2) was increased and the log $K_{o/w}$ of GP was decreased, which may due to higher solubility of the GP in the IPM:EtOH=8:2(*w*/*w*). ^{13}C NMR spectroscopy was used to identify the ion-pairing formation between GP and the respective counter ion. It was surprising that all the four enhancers examined, such as isopropyl myristate, propylene glycol, *N*-methyl-2-pyrrolidone, azone, and oleic acid, had no enhancing effect on the percutaneous permeation of GP. This study showed that the formation of ion-pairs between GP and counter ions is a useful method to promote the skin permeation of GP.

KEY WORDS: amine; counter ion; glipizide; ion-pair; percutaneous absorption.

INTRODUCTION

Glipizide (GP) is a second-generation oral hypoglycemic agent that belongs to the sulfonylurea class of compounds (1,2). It is one of the most commonly prescribed drugs for the treatment of patients with type II (non-insulin-dependent) diabetes mellitus (3). GP takes effect by increasing the release of endogenous insulin as well as its peripheral effectiveness (4,5). As a weak acid (pK_a=5.9), GP is better absorbed in acidic medium, however, at very low pH values, the solubility of GP is minimal (6) and this limited aqueous solubility causes large variations in bioavailability and, in the presence of renal or hepatic insufficiency, alcohol or other drugs may incur severe and prolonged hypoglycemia (7). In addition, the absorption of GP is delayed when it is taken with a meal (8). Moreover, oral therapy may cause gastric disturbances like nausea, vomiting, heartburn, and increased appetite (4). Therefore, it is quite necessary to develop a new administration route for GP.

Transdermal drug delivery system (TDDS), one of the most significant drug delivery breakthroughs in the new millennium, is a promising alternative for oral therapy of GP to overcome the problems associated with oral administration of the drug. Nevertheless, the outermost layer of skin (the stratum corneum, SC) forms a strong barrier to most exogenous substances including drugs. A number of potential methods to enhance skin permeation of GP have been proposed, such as penetration enhancers (9), iontophoresis (10,11), and clathrate (12). Servile membrane-moderated transdermal systems of GP were also studied (13).

One method to increase the ability of penetrating biologic membrane is to form ion-pairs with counter ions. Irwin *et al.* were among the first researchers to confirm this theory (14), and many others have applied this technique to transdermal delivery since then (15–23). For example, Fang *et al.* found that the skin permeation of mefenamic acid was increased by adding diethylamine, triethylamine, ethanolamine, diethanolamine, triethanolamine, and propanolamine (24). Mohammad *et al.* studied the solubility of salicylate in isopropyl myristate (IPM) and found out that it was increased by adding either alkylamines or benzylamine as counter ions (25).

The objective of this study was to prove the significant enhancement impact of the counter ions on the permeation of GP. Six different amines, including ethanolamine, diethanolamine, triethanolamine, triethylamine, diethylamine, and *N*-(2-hydroxylethyl) piperidine were employed as counter ions. ^{13}C NMR spectroscopy was used to identify the ion-pairing formations between GP and the corresponding counter ions. The different ratios between IPM and EtOH of donor solution on the percutaneous absorption of GP were also

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ABBREVIATIONS: DA, diethylamine; DEA, diethanolamine; EA, ethanolamine; EtOH, ethanol; ER, enhancement ratios; GP, glipizide; HEPP, *N*-(2-hydroxylethyl) piperidine; IPM, isopropyl myristate; ME, menthol; NMP, *N*-methyl-2-pyrrolidone; OA, oleic acid; *P*, permeability coefficient; Q_{12}, cumulative amount of drug permeating through the skin; SC, stratum corneum; TA, triethylamine; TEA, triethanolamine.

examined. The application of penetration enhancers is a long-standing and widely used approach to increase transdermal and topical drug delivery (26). Then, we investigated the effect of chemical enhancers, such as isopropyl myristate, *N*-methyl-2-pyrrolidone, azone, and oleic acid on the *in vitro* percutaneous absorption of GP. Besides, the solubility of six ion-pairs was measured and the partition characteristics of ion-pairs in an *n*-octanol aqueous system were also examined.

MATERIALS AND METHODS

Materials

Glipizide (GP) was purchased from Shandong Boshan Pharmaceuticals Co., Ltd. (Shandong, China). Ethyl *p*-hydroxybenzoate was purchased from Tianjin Yuanhang Chemicals Co., Ltd. Diethylamine (DA), triethylamine(TA), ethanolamine (EA), diethanolamine (DEA), and triethanolamine (TEA; Fig. 1) were all supplied by Tianjin Bodi Chemicals Co., Ltd. (Tianjin, China). *N*-(2-hydroxylethyl) piperidine (HEPP) was obtained from Alfa Aesar (Johnson Matthey Company, U.S.A.). Methanol of HPLC grade and oleic acid (OA) were purchased from Shandong Yuwang Chemicals Co., Ltd. (Shandong, China). Ethanol (EtOH), isopropyl myristate (IPM), *N*-methyl-2-pyrrolidone (NMP), azone, and menthol (ME) were obtained from Tianjin Baishi Chemicals Co., Ltd. (Tianjin, China), Bodi Drug Manufacturing Co. Ltd. (Tianjin, China), China National Medicines Co., Ltd. (Shanghai, China), Beijing Chemical Industry (Beijing, China), Tianmen Kejie Pharmaceuticals Co., Ltd. (Hubei, China), and Chengdu Kelong Chemical Industry (Sichuan, China), respectively.

All other chemicals and solvents were of analytical grade.

Preparation of skin samples

Male Wistar rats weighing 180–220 g (6–8 weeks old) used in all experiments were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang China). The experiments were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and also in accordance with the guidelines for animal use published by the Life Science Research Center of Shenyang Pharmaceutical University. All efforts were made to minimize animal suffering and to limit the number of animals used. The rats were anesthetized with urethane (20% *w/v*, i.p.) and the abdomen was carefully shaved with animal hair clippers (model 900, TGC, Japan). Full thickness skin (i.e., epidermis with SC and dermis) was harvested immediately after killing the animals. The integrity of the skin was carefully confirmed by microscopic observation, and any skin that was not completely uniform in appearance was rejected. After removing the fat and sub-dermal tissue, the skin was kept frozen at −4°C until use (within 1 week). Before starting the experiments, the skin was allowed to reach room temperature for at least 10 h.

^{13}C NMR Spectroscopy

^{13}C NMR spectra were recorded at 75 MHz using a Bruker Avance 300 spectrometer (Karlsruhe, Germany). Samples were dissolved in dimethyl sulfoxide and chemical shifts for carbon resonance reported in parts per million relative to TMS.

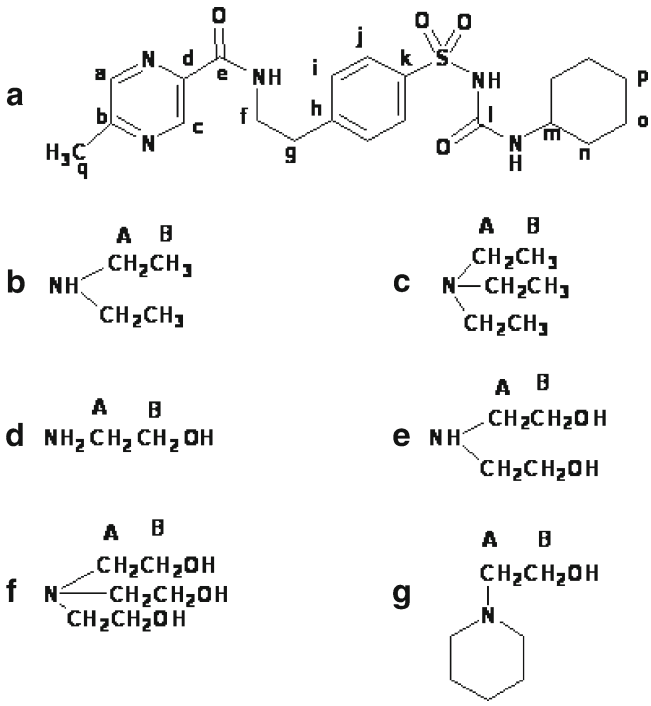

Fig. 1. Chemical structures of GP and amines. **a** Glipizide, **b** DA, **c** TEA, **d** EA, **e** DEA, **f** TEA, **g** HEPP

Estimation of the Drug

GP was estimated by HPLC. For making standard graph, working standards were prepared in methanol (0.1–30 μg·ml^{-1}) and injected into the column (20 μl). The column was eluted with the mobile phase consisting of redistilled water and methanol (65:35, adjusted to pH 3.5 by orthophosphoric acid) and the detection wavelength was 275 nm and the flow rate was set to 1 ml·min^{-1}. Consequently, the retention time recorded of GP was 7.19 min, and the retention time recorded of the internal standard ethyl *p*-hydroxybenzoate was 5.32 min. The plots of peak area *versus* respective concentration of GP were found to be linear with the correlation coefficient (*r*) of 0.9999.

Determination of Drug Solubility

The saturation solubility was determined in the individual donor vehicles, by adding excess drug to the fixed volumes of vehicle, vortexing for 2 min followed by sonication for 10 min to dissolve the drug and then equilibrating at 32±0.5°C for more than 48 h. Finally, the solutions were centrifuged at 16,000 rpm for 10 min and the suspension was passed through a membrane filter (0.45 μm). The concentration of GP in the filtrate was determined by HPLC using a calibration curve after appropriate dilution with methanol if necessary. The experiments were performed in triplicate.

Determination of *n*-Octanol/Water Apparent Partition Coefficients

The partition coefficients of GP-amines were determined in *n-octanol/water*. Initially, *n*-octanol and water were shaken

together, and then allowed to stand for 48 h to ensure mutual saturation. A precise amount of GP and an equimolar amount of each amine were dissolved in water, and then an equal volume of *n*-octanol was added. After that, the system was shaken in a water bath at 32°C for 48 h. The residual concentration in *n*-octanol was the C_w measured by HPLC. Hence, the amount partitioned into the aqueous phase was $C–C_w$. The partition coefficient ($K_{o/w}$) can be obtained using the following equation:

$$K_{o/w} = C_w/(C - C_w)$$

Preparation of Donor Solutions

GP alone or GP with additives were added to the vehicle IPM:EtOH , then vortexed for 2 min followed by sonication for 10 min to dissolve the drug, and an excess amount of solute was present throughout the experiments.

Permeation Experiments

Skin permeation experiments were performed according to the method of Fang *et al.* (22). A diffusion cell consisting of two half-cells with a water jacket connected to a water bath at 32°C was used. Each half-cell had a volume of 2.5 ml and an effective area of 0.95 cm^2. The dermis side of the skin was in contact with the receiver compartment and the SC was in contact with the donor compartment. The donor compartment was filled with the drug suspension and the receiver compartment with pH 7.4 PBS. During all the experiments, the excess drug was maintained in the donor compartment. Both donor and receiver compartments were stirred with a star-head bar driven by a constant-speed synchronous motor at 600 rpm. At predetermined time intervals, a 2.0-ml sample of receptor solution was taken from each receiver compartment for analysis and replaced with the same volume of fresh solution to maintain sink condition. The drug concentration was determined by reversed-phase HPLC using a calibration curve.

Data Analysis

The cumulative amount of each drug permeating through the skin was plotted as a function of time. The skin flux was determined from Fick's law of diffusion:

$$Js = dQr/Adt \quad (1)$$

Where *J*s is the steady-state skin flux in micromole per square centimeter per hour; *dQ*r is the change in quantity of the drug passing through the skin into the receptor compartment in micromole; *A* is the active diffusion area in square centimeter and *dt* is the change in time. The flux was calculated from the slope of the linear portion of the profiles. The lag time was determined by extrapolating the linear portion of the curve to the abscissa (27).

The permeability coefficient (*P*) was calculated as

$$P = Js/Cs \quad (2)$$

*C*s is the saturated solubility of drug in the donor solution (28).

To evaluate the promoting activity of each amine, the enhancement ratios (ER) were calculated as skin parameters (*P* and Q_{12}) for the amine-containing group divided by the same parameter for the Control (no amine present). Controls were assigned a value of 1.00. All parameters were reported as the mean±SE.

RESULTS AND DISCUSSION

The Effect of EtOH and IPM at the Different Ratios on the Permeation of GP

The effect of IPM and EtOH on the permeation of GP was examined at the ratios of 10:0, 9:1, 8:2, 7:3, and 5:5. The solubility and permeation parameters (*flux*, *lag time*, *P*, and Q_{12}) are shown in Table I, and the permeation profiles obtained are presented in Fig. 2. When comparing the cumulative permeating amounts of GP using different ratios of EtOH and IPM at 12 h, a wide fluctuation was observed. As can be seen from Table I, when the vehicle was IPM, the Q_{12} and flux were the lowest one of those studied; When IPM:EtOH=8:2 (EtOH concentration was 20%), the Q_{12} and flux values were much higher than that of the others. These results suggested that the concentration of EtOH was important for GP transdermal delivery through rat skin.

Hori *et al.* indicated that the combination of vehicles from two different groups was better than one vehicle (29). Since EtOH and IPM belong to two different groups, the combination of these two solvents could exhibit a synergistic effect on penetration enhancement (30–33).

It also showed that the enhancement of the skin permeability of a drug by EtOH at a low concentration was due to the action on skin lipids (34,35) and solvent drag effect (36–38). The former could be characterized as a rather "static effect" mainly governed by the integral amount of EtOH acting on the skin lipids. This effect mainly modified the barrier function of the stratum corneum (SC). On the other hand, the latter was characterized as a "dynamic" or "kinetic effect" exerted by the flow of EtOH through the skin. The reason why the Q_{12} and *flux* values of GP decreased with the

Table I. Permeation Parameters of GP Through Rat Abdominal Skin Using an Identical Receiver phase (PBS) and Donor Phases Consisting of IPM:EtOH (9:1), IPM:EtOH (8:2), IPM:EtOH (7:3; *w*/*w*; *n*=3)

Donor solution	Solubility (mg/100 ml)	Q_{12} (μg/cm^2)	Flux (μg/cm^2/h)	Lag time (h)	$P\times10^3$ (cm/h)
IPM	0.12	18.18	1.46±0.04	0.26	1.83±0.12
IPM:EtOH=9:1	7.67	23.01	2.14±0.11	0.70	1.20±0.01
IPM:EtOH=8:2	18.28	225.14	18.26±0.77	3.69	1.16±0.02
IPM:EtOH=7:3	24.26	129.03	11.41±0.96	0	1.12±0.05
IPM:EtOH=5:5	45.63	81.85	8.16±0.38	1.95	0.86±0.32

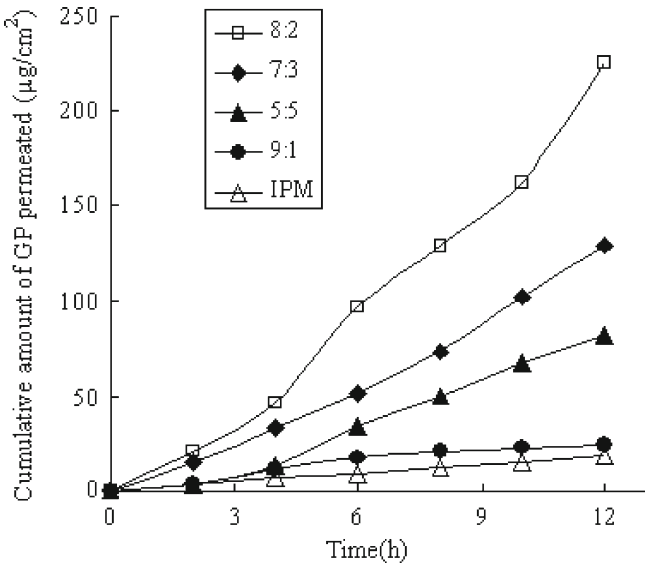

Fig. 2. Effect of IPM and EtOH with different ratios on the permeation of GP through rat abdominal skin

increasing EtOH concentration (from 20% to 50%) can be explained as follows: the relative higher concentration of EtOH removed the water from the SC, thus it reduced the level of hydration. It is well known that the percutaneous absorption of most substances is increased by increasing the water content of the stratum corneum (SC) and therefore dehydration should reduce the permeation of such substances. Blank *et al.* estimated that the diffusion of water within the SC was halved as the relative humidity dropped from 93% to 46% (39). A similar result has been obtained by Megrab *et al.* (40). Therefore, the ratio of IPM:EtOH=8:2 was chosen to perform subsequent experiments.

The Effect of Counter-ions on the Permeation of GP from the Vehicle IPM:EtOH=8:2

The effects of counter ions on the skin permeation of GP were examined using a suspension of the mixture of equimolar amounts of GP and each amine (GP-amine), and GP sodium salt (a suspension of the mixture of equimolar amounts of GP and NaOH) as control. The solubility and permeation parameters (*flux*, *lag time*, *P,* and ER) are shown in Table II and the permeation profiles are shown in Fig. 3. As shown in Table II, 2.4-fold to 14.2-fold increases in *flux* of GP-amines compared to the control was observed. TA had the greatest enhancing effect on the permeation of GP, followed by EA, DA, DEA, HEPP, and TEA in a descending order. One possible explanation is that ion-pair is formed in the non-aqueous mixture. The descending order observed above can be explained from the perspective of structure and physicochemical properties of the different amines.

First, some researchers have found that the alkylamine counter ions affected salicylate penetration through human epidermis in the following order: tertiary>secondary>primary (17). Fung *et al.* compared tertiary amines with their corresponding secondary amines to obtain a group contribution values for the *N*-substituted methyl moiety (41). Our studies complied well with this research and the *flux* of GP-TA was higher than that of GP-DA.

Second, EA, DEA, and TEA contain different numbers of hydroxyl groups in this study. The *flux* of GP decreased with the increase of the number of –OH in amines. It has been reported that H-bonding potentials within the permeants caused a decrease in diffusion coefficient (D), suggesting that the permeants are bound by these forces to the immobilized polar regions of the SC lipid leading to a negative effect on D (42). It is well known that the intercellular space contains a complex array of lipids which are mainly composed of long-chain fatty acids, ceramides, and cholesterol in roughly equimolar proportions (43). The most obvious interaction seems to be H-bonding between the permeants and the –COOH of the fatty acids, the –OH, or amide groups of the ceramides. The flux of GP decreased as the number of hydroxyl groups increased, which indicated that the hydroxyl group had a negative effect on the permeation of GP, and the H-bonding between the hydroxyl groups of amines and the immobilized polar regions of the SC lipid played an important role in GP diffusion through skin (44,45).

Third, as we can see from Table II, GP-EA had a relative higher *flux* than GP-HEPP. This may result from the steric hindrance between GP and HEPP. As shown in Fig. 1, both EA and HEPP have one hydroxyl group and HEPP also has a ring. The similar results had been reported by Fini *et al.* and James *et al.* (46,47).

The increase in the solubility of GP in the presence of the amines, compared with GP sodium salt may be due to

Table II. Permeation Parameters of GP in the Presence of Various Counter- ion Through Rat Skin from IPM:EtOH (8:2, *w*/*w*), and Physicochemical Properties of Various Counter ions

Permeants	Solubility (mg/100 ml)	log$K_{O/W}$	Q_{12} (μg/cm^2)	Flux (μg/cm^2/h)	Lagtime (h)	ER	$P\times10^3$ (cm/h)	pK_a	Molecular weight
GP-Na salt	21.33	0.06	234.19	19.85±0.23	0.58	–	1.86±0.34	–	–
GP-EA	344.39	−0.22	2791.34	282.75±9.86	0	14.2	1.14±0.05	9.5	61.08
GP-DEA	168.53	−1.12	1351.76	121.87±20.35	33.70	6.1	1.11±0.03	8.96	105.14
GP-TEA	43.36	−0.36	524.51	47.53±7.11	10.73	2.4	0.50±0.31	7.76	149.19
GP-DA	156.05	−0.90	1589.40	156.05±39.09	1.43	7.9	1.09±0.03	11.1	73.14
GP-TA	68.93	−0.48	4592.32	470.75±43.01	1.50	3.6	1.10±0.02	10.8	101.19
GP-HEPP	74.35	−0.56	912.86	81.33±11.12	0.73	4.1	1.17±0.06	9.66	129.20

Data are given as average±SE (n=4/n=3)
ER is the enhancement ratio calculated as follows: ER = Flux (with enhancer)/Flux (without enhancer)
Data of pK_a were obtained from SRC PhysProp Database

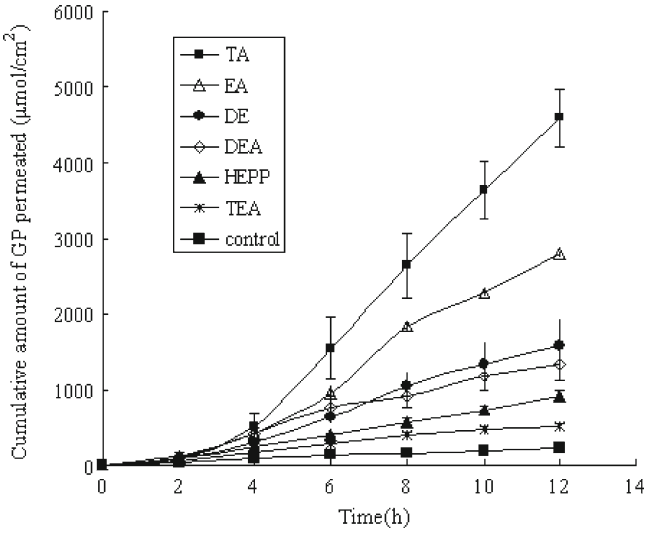

Fig. 3. Effect of different counter ions on the permeation of GP in IPM:EtOH=8:2 through rat abdominal skin

higher solubility of the GP ionized form in the non-aqueous mixture. The decrease in the *n*-octanol–water partition coefficient ($\log K_{o/w}$) of GP in the presence of the amines, compared with GP sodium salt, was also a result of the higher water solubility of the GP ionized form. However, the *flux* of GP-amines was in the following order: GP-EA>GP-DEA>GP-DA>GP-HEPP>GP-TA>GP-TEA. Minghetti *et al*. have reported that the high solubility does not indicate a high permeation, and it is often too simple a parameter for comparing difference among diclofenac salts, unless the activity coefficient of the permeant is taken into account (48).

The physicochemical properties of the different amines were summarized in Table II. It can be seen that the molecular weight and pK_a of the amines can significantly affect their permeation-enhancing performance.

The Q_{12} of GP increased with the decreasing organic amine molecular volume, expressed as molecular weight (for many compounds, the molecular weight is often a reasonable approximation of the molecular volume, except for TA). As the molecular weight of the counter ions decreased, the Q_{12} of GP was increased to produce an inverse relationship between the Q_{12} and the molecular weight (Fig. 4). The importance of the molecular size of the amines for the

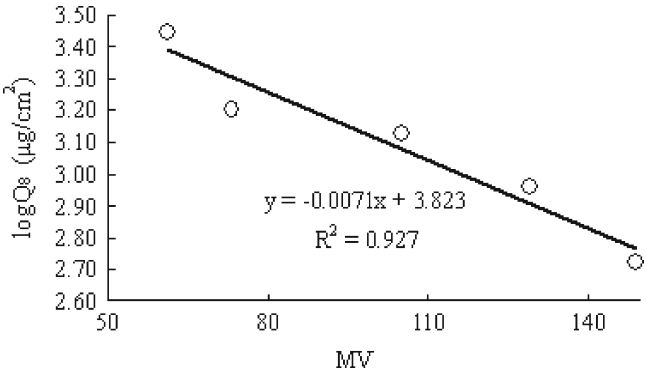

Fig. 4. The relationship between the LogQ_{12} and the molecular weight (MV) of the amines

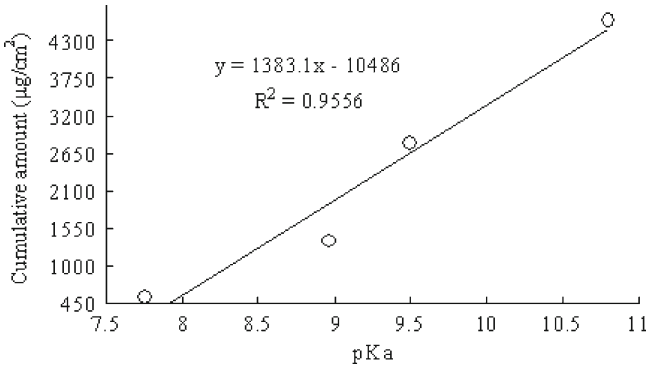

Fig. 5. The relationship between the cumulative amount (Q_{12}) and the pK_a of the amines

penetration of drugs through rat skin has been highlighted (18,19,49).

The Q_{12} of GP was found to increase as a function of the pK_a value of the amines (Fig. 5), with the exception of DA and HEPP. A high pK_a generally results in a high Q_{12}. The present results indicated that the enhancement of counter ions is related to their alkalinity.

^{13}C NMR Spectroscopy

Solvents with high dielectric constants encourage the complete dissociation of the electrolytes whereas in solvents of low dielectric constant (ϵ), considerable ion-pairing occurs (50). A common method to calculate the ϵ of mixed solvents is the weighted average of the mixture components by assuming a simple additive function of the concentration of

Table III. ^{13}C NMR Chemical Shifts in the Part of Amines of Amines, Amine Hydrochloride, and GP-amine

	^{13}C chemical shift	
Amine	A	B
EA	43.3	63.8
DEA	51.1	60.9
TEA	56.9	59.3
DA	44.1	15.5
TA	46.7	12.0
HEPP	58.3	61.2
Amine hydrochloride		
EA	42.4(−0.9)	58.7(−5.1)
DEA	49.8(−1.3)	57.4(−3.5)
TEA	55.9(−1.0)	56.2(−3.1)
DA	43.2(−0.9)	11.8(−3.7)
TA	47.6(0.9)	9.5(−2.5)
HEPP	57.3(−1.0)	59.3(−1.9)
GP-amine		
EA	42.1(−1.2)	59.0(−4.8)
DEA	49.7(−1.4)	57.5(−3.4)
TEA	56.9	58.7(−0.5)
DA	42.5(−1.6)	11.1(−4.4)
TA	45.8(−0.9)	10.3(−1.7)
HEPP	57.4(−0.9)	60.1(−1.0)

Data of amines and amine hydrochloride were obtained from Sadtler Spectral Handbooks

the solvents (51–53). The dielectric constants of IPM and EtOH are 3.31 and 24.13, respectively (17). The ϵ of the vehicle consisting of IPM:EtOH=8:2(*w*/*w*) employed in this study was 9.47 using the calculation method mentioned above. Bjerrum's equation (54), which describes a critical separation distance for the formation of ion-pairs, pointed out the importance of the ϵ: a solvent with a high ϵ such as water (ϵ=78.5) is unfavorable for ion-pair formation, while the interaction becomes increasingly important in solvents with ϵ<40 (55). Therefore, the vehicle of IPM:EtOH=8:2(*w*/*w*) is quite suitable to evaluate the effect of ion-pairing in the percutaneous absorption of ionic drugs.

The carbamido group of GP is in the chemical equilibrium of keto–enol tautomerism $-NH-C=O \leftrightarrow N=C-OH$. The model of Huyskens and Zeegers–Huyskens predicts that a difference of 3.6–6 orders of magnitude between the acid dissociation constants of the base and the acid leads to an almost complete shift of the proton-transfer equilibrium of the $O-H\cdots N \leftrightarrow O^{-}\cdots H-N^{+}$ system (56).

The difference of pK_a between amines (Table II) and GP (pK_a=5.9) were almost in the range of 3.6–6, except for TEA. This indicted that it is possible to form ion-pairs between amines and GP.

The aim of ^{13}C NMR spectroscopy was to obtain the evidence of ion-pair formation between GP and each amine from the chemical shift changes. The ^{13}C NMR of GP and each GP-amine spectroscopy are shown in Figs. 6 and 7. Comparison of the carbon NMR spectra of amines, amine hydrochlorides, and GP-amines showed a significant change in carbon chemical shift in amines. As shown in Table III, with respect to the corresponding amines, the carbon NMR spectra in the amine and GP-amines showed a significant upfield shift: carbon "a" (0.9 to 1.6 ppm shift) and carbon "b"(1.0 to 4.8 ppm), except for TEA. But smaller chemical shift differences were observed in the amine of GP-amines compared with the amine hydrochloride. These values indicated the degree of charge neutralization in the mixture of GP and organic-amines was similar to amine hydrochloride.

Comparison of the carbon NMR spectra of GP and GP-amines showed a significant change in carbon shift differences in GP. As shown in Table IV, a 1.10 ppm to 6.16 ppm downfield shift was observed on the carbon "k". This signal

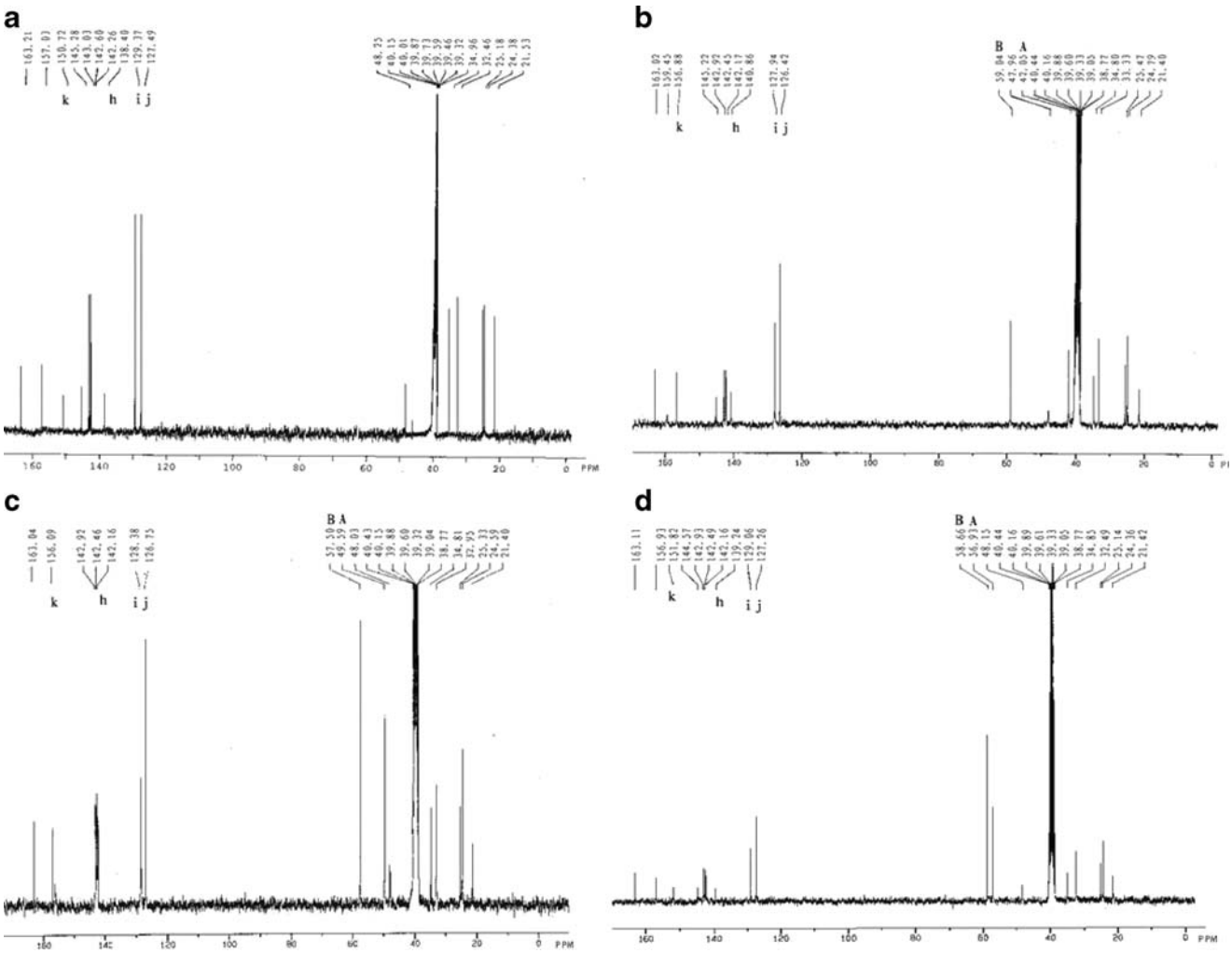

Fig. 6. The NMR spectra of GP and amines. **a** GP, **b** GP-EA, **c** GP-DEA, **d** GP-TEA

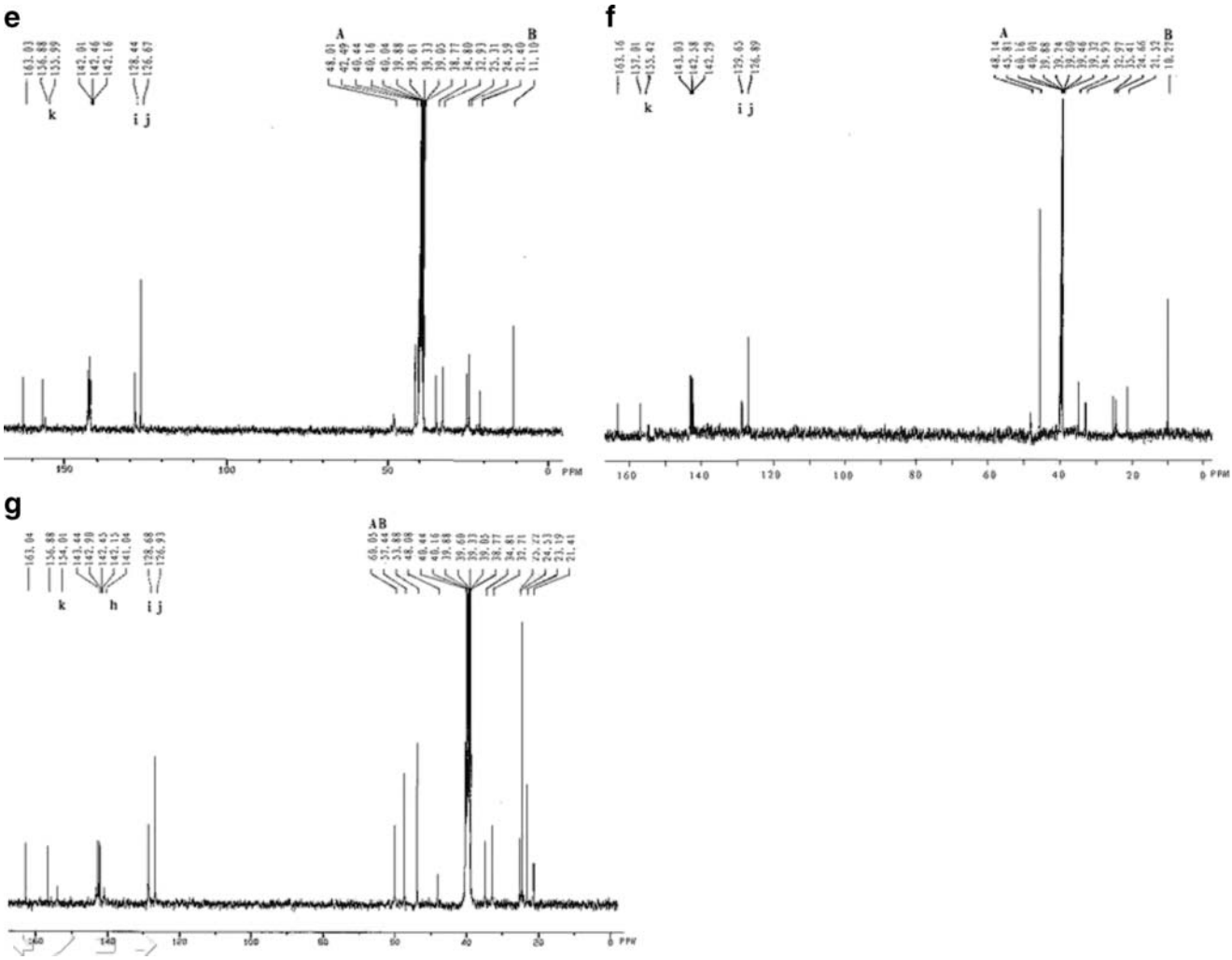

Fig. 7. The NMR spectra of GP and amines. **e** GP-DA, **f** GP-TA, **g** GP-HEPP

Table IV. ^{13}C NMR Chemical Shifts in the Part GP of GP and GP-amines

	GP	GP-EA	GP-DEA	GP-TEA	GP-DA	GP-TA	GP-HEPP
a	142.60	142.45	142.46	142.49	142.46	142.58	142.45
b	145.28	145.22	overlap	144.57	overlap	overlap	143.44
c	143.03	142.92	142.92	142.93	142.91	143.03	142.90
d	142.26	142.17	142.16	142.16	142.16	142.29	142.15
e	157.03	159.45(2.42)	156.89	156.93	156.88	157.01	156.88
f	39.93	39.88	39.88	39.89	40.04	40.01	39.88
g	34.96	34.80	34.81	34.85	34.80	34.93	34.81
h	138.40	140.86(2.46)	overlap	139.24(0.84)	overlap	overlap	141.04(2.46)
i	129.37	127.94(−0.137)	128.38(0.99)	129.06(0.31)	128.44(0.83)	128.65(0.62)	128.69(0.68)
j	127.49	126.42	126.75	127.26	126.67	126.89	126.93
k	150.72	156.88(6.16)	155.74(5.02)	151.82(1.10)	155.99(5.27)	155.42(4.70)	154.01(3.29)
l	163.21	163.02	163.04	163.11	163.03	163.16	163.04
m	48.25	47.96	48.03	48.15	48.01	48.14	48.08
n	32.46	33.33	32.95	32.50	32.93	32.97	32.71
o	25.18	25.47	25.33	25.14	25.31	25.41	25.22
p	21.53	21.40	21.40	21.42	21.40	21.52	21.41
q	24.38	24.79	24.59	24.36	24.60	24.66	24.53

Table V. Permeation Parameters of GP in the Presence of 3% Each Enhancer and GP-TA Through Rat Skin from IPM:EtOH (8:2, w/w)

Enhancer	Q_{12} (μg/cm^2)	Flux (μg/cm^2/h)	Lagtime (h)	$P\times10^3$ (cm/h)
control	225.14	18.26±0.77	3.69	1.20±0.01
1%Azone	181.86	0.049±0.005	2.93	0.86±0.01
3%Azone	200.16	0.046±0.005	4.94	0.82±0.02
5%Azone	183.73	0.047±0.03	3.60	0.93±0.09
1%ME	124.75	0.11±0.008	7.95	0.61±0.04
3%ME	178.13	0.093±0.01	9.37	0.75±0.06
5%ME	191.74	0.074±0.002	8.88	0.74±0.02
10%ME	175.80	0.064±0.01	8.58	0.83±0.06
15%ME	145.17	0.076±0.004	7.44	0.89±0.01
3%NMP	167.06	0.087±0.009	7.54	0.90±0.01
3%OA	48.12	0.24±0.003	1.93	0.76±0.03

was the most obvious change in the GP carbon, followed by carbon "i"(−1.37 ppm to 0.99 ppm shift) and "h" (0.84 ppm to 2.46 ppm downfield shift, the carbon chemical shift of GP-DEA, GP-DA, GP-TA were overlapped). Shifts in the other carbons of GP could be negligible. The carbon atoms "h", "i", and "k" belong to the benzenesulfonyl group. The changes in the chemical shifts of carbon provided an evidence of ion-pair formation (40–42). The location of changes in the GP molecule revealed that the binding site was adjacent to the benzenesulfonyl group. In addition, carbamido group was connected to the benzenesulfonyl group. This observation provided the evidence of the binding site was carbamido group and the amino group of amines.

The Effects of Chemical Enhancers on the Permeation of GP

The effects of chemical enhancers on the skin permeation of GP were examined using a suspension of GP in the donor solution (IPM:EtOH=8:2) with the enhancers (NMP, OA, azone, ME) concentration of 3%. The permeation parameters (*flux*, *lag time*, *P,* and ER) are shown in Table V. It is surprised that the enhancers showed inhibiting effects on the percutaneous absorption of GP though rat skin, rather than enhancing effects. Among the four enhancers, 3% azone and 3% ME exhibited relative weak inhibition effects on the permeation of GP. Considering that the enhancing effect of enhancer may change with the concentration, 1% azone, 5% azone, and 1% ME, 5% ME, 10% ME, 15% ME were chosen to carry out the experiment. As shown in Table V, the changes in the concentration levels of both Azone and ME did not make their inhibiting effects converse. The smallest inhabitation concentrations of Azone and ME were 3% and 5%ME, respectively.

As a chemical enhancer, NMP altered the solubilizing ability of the aqueous regions of SC between the polar lipid head groups of the bilayers thereby promoting drug partition into skin (57); Azone probably exerted its penetration-enhancing effects through interactions with the lipid domains of the SC (58); OA could disrupt intercellular lipid domain of SC or coexisting as pools in the ordered SC lipid structure (59,60). Recently, some studies displayed ME formed hydrogen bonds with the ingredients of SC, breaking the network of lipid bilayers and disrupting barrier property of SC. GP might have a different interaction site in the stratum corneum compared with enhancers referred above. The actual mechanism governing the permeation of GP is not clear so far, therefore, further research is also needed.

However, it is obvious that enhancers cannot enhance the penetration of penetrate in all cases. Rather, it is likely that the formation of ion-pairs between GP and the amines is a useful method to promote the skin permeation of GP in present study comparing with the method of using enhancers.

Our previous studies have studied the GP emugel (61). GP-DA emugel 2 g (the dosage was 5 mg/g) was applied on the abdominal skin of the rat. After 12 h, the blood drug level was 445.26 μg·ml^{-1}, and the blood glucose of initial concentration is 36.7% (n=6). Kahn and Shechter have suggested that a 25% reduction in blood glucose levels is considered a significant hypoglycemic effect (62). So, this emugel would show a biological activity. The formation of ion-pairs between GP and the amines was a promising approach to enhance the skin permeation of GP.

CONCLUSION

The results of this investigation showed that the formation of ion-pairs significantly increased the flux of GP through rat skin. TEA, in particular, was the most effective amine to increase the permeation of GP in IPM:EtOH=8:2(w/w). The formation of ion-pair with each counter ion was confirmed by ^{13}C NMR spectroscopy. The four chemical enhancers all inhibited the permeation of GP though rat skin. The formation of ion-pairs between GP and the amines was a promising approach to enhance the skin permeation of GP.

ACKNOWLEDGMENT

The authors are grateful to Professor Yasunori Morimoto (Faculty of Pharmaceutical Sciences, Josai University, Japan) for his kind gift of two-chamber diffusion cells and a synchronous motor.

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