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Effect of ion-pairing and enhancers on scutellarin skin permeability

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Abstract

The aim of this work was to investigate the effect of enhancers and organic amines on the in-vitro percutaneous absorption of the major pharmacologically active compound, scutellarin, obtained from breviscapine extract. The donor vehicle consisted of isopropyl myristate–ethanol in a ratio of 4:1. Percutaneous absorption across full thickness rat skin was investigated in-vitro using 2-chamber diffusion cells, with reverse-phase HPLC for quantification of the permeating scutellarin. Organic amines increased scutellarin permeation by ion-pair formation. We also found that the cumulative amount of scutellarin over a period of 12 h of scutellarin was inversely related to the molecular weight of organic amines ($r = 0.9134$), as well as the logarithm of scutellarin permeability coefficient inversely related to the partition coefficient of organic amines ($r = 0.8929$). All the permeation enhancers tested increased the cumulative amount of scutellarin over a period of 12 h, and the order of this increase was n-methyl-2-pyrrolidone, oleic acid, menthol or Azone. Drug solubility in donor phase was markedly increased by Azone and n-methyl-2-pyrrolidone, and reduced by menthol and oleic acid. The combined effects of ethanolamine plus Azone, ethanolamine plus menthol, and Azone plus menthol were also investigated. Azone plus menthol had a synergistic effect on the cumulative amount of scutellarin over a period of 12 h.

Introduction

Scutellarin (Figure 1), 4′,5,6-trihydroxy flavone-7-*O*-β-D-glucuronide, is the major active constituent of breviscapine, which is derived from the traditional herb *Erigeron breviscapus* (Vant.) Hand-Mazz. It is used in the treatment of cerebrovascular diseases such as hemiplegia, coronary heart disease and hypertension. Pharmacological studies have shown that breviscapine can significantly reduce blood viscosity, improve blood flow, reduce vascular resistance and inhibit platelet aggregation and thrombosis formation. In addition, it has recently been reported that scutellarin has a neuroprotective effect on rat neuronal damage (Hu et al 2005), an anti-HIV-1 effect (Zhang et al 2005) and antioxidant effects (Liu et al 2002).

It has been reported that the absolute bioavailability of oral scutellarin in dogs and mice is 0.25~0.75% and 5.0%, respectively (Ge et al 2003; Liu et al 2003). The relative bioavailability of oral scutellarin in rats is also very low ($10.67 \pm 4.78\%$) (Huang et al 2005). The main reason for the low oral bioavailability of scutellarin is its degradation in the gastrointestinal tract, rather than hepatic first-pass elimination (Hao et al 2005a). The rapid elimination of scutellarin in dogs, rabbits and rats has also been reported by some authors (Jiang et al 2003; Li et al 2003; Hao et al 2005b).

Some studies have been conducted to overcome this unfavourable pharmacokinetic profile by synthesizing ester prodrugs (Cao et al 2006) and scutellarin–PEG conjugates (Zhou et al 2006), and preparing freeze-dried scutellarin–cyclodextrin complexes (Cao et al 2005). It has also been reported that breviscapine liposomes, administered intravenously to beagle dogs or rabbits, produce much higher concentrations in plasma and exhibit sustained release (thereby improving the pharmacokinetic properties of scutellarin) (Lo et al 2006; Lv et al 2006).

In addition to these approaches, percutaneous absorption may be another way of improving the bioavailability of scutellarin, since transdermal formulations can transport compounds across the skin and into the systemic circulation, thereby avoiding poor

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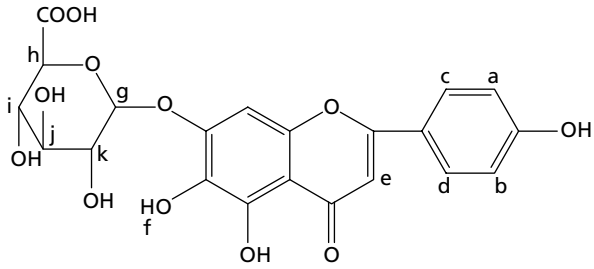

Figure 1 Chemical structure of scutellarin.

absorption from the gut and hepatic first-pass metabolism (Willams 2003). Scutellarin is a highly hydrophilic drug with a log *P* value of −2.56 (Cao et al 2006). Nowadays it is believed that polar molecules penetrate via the shunt routes of the skin.

Two methods are usually used to enhance drug permeation—one involves changes in the physicochemical properties of the drug by prodrug or ion-pair formation, while another involves altering the properties of the stratum corneum by disrupting its intercellular lipid structure or modifying lipid packing or presence as pools in the ordered stratum corneum. The latter approach mainly involves chemical enhancers. In this study, five different amines (ethanolamine (EA), diethanolamine (DEA), triethanolamine (TEA), triethylamine (TETA) and diethylamine (DETA)) were employed with the aim of forming ion-pairs with the acidic model drug, scutellarin (pK_a 3.29) (Zhong et al 2005). To investigate the effects of chemical enhancers on scutellarin penetration through full thickness rat skin, n-methyl-2-pyrrolidone (NMP), oleic acid (OA), menthol (MT) and Azone (AZ) were used. The combined effect of EA with AZ and MT, and the combined effect of AZ and MT were also investigated. Scutellarin was purified before starting the studies since it is difficult to rationalize its transdermal delivery from complex mixtures, such as plant extracts, as the behaviour of each constituent can potentially influence that of the others.

Materials and Methods

Materials

Scutellarin was purified in our laboratory from breviscapine extract, which was purchased from the Dali Wanrong Pharmaceutical Factory. AZ, MT, OA and NMP were obtained from Hubei Ketian Pharmaceutic Co. Ltd, Anhui Taidao Bohe Pharmaceutic Co. Ltd, the Fifth Shenyang Reagent Factory and Tianjin Jizhun Chemical Reagent Co. Ltd, respectively. EA, DEA, TEA, DETA, TETA, PEG 400, acetoacetate, acetonitrile (HPLC grade) and glacial acetic acid were all supplied by Tianjin Bodi Chemical Reagent Co. Ltd. Potassium dihydrogen phosphate (KH_2PO_4), isopropyl myristate (IPM), methanol (HPLC grade) and absolute ethanol were purchased from Guangdong Shantou Longxi Chemical Plant, Shenyang Guoyao Group Chemical Reagent Co. Ltd, Shandong Yuwang Chemical Industry Co. Ltd and Tianjin Bashi Chemical Industry Co. Ltd, respectively. All reagents were either of analytical grade or chromatographic grade.

Double-distilled water was purified using a SZ-96 water-purified system (Yarong Biochemical Instrument Factory, Shanghai, China).

All solutions were prepared using double-distilled water.

Purification of scutellarin

The purification was carried out by column chromatography. Regardless of the permanent absorption of the drug in the static phase, silica gel with particle diameter of 60–120 μm was used and elution was carried out using an acetoacetate–methanol–1% glacial acetic acid solution. In this study, glacial acetic acid was used to reduce drug absorption by silica gel and prevent tailing. The purity of scutellarin was increased from 70% to 95% (determined by reverse-phase HPLC) by that procedure.

Solubility studies

The solubility of scutellarin in the vehicle, with or without incorporating various adjuvants, was determined. The vehicle consisted of 20% ethanol and 80% IPM solution by weight. Excess scutellarin was added to the vehicle, with and without incorporating an equimolar amount of organic amines. For OA, AZ, MT and NMP, the amount added was 5% of the weight of vehicle. The suspensions were agitated in a water bath at 32°C for 24 h. The amount of scutellarin in the vehicles was determined by HPLC after centrifugation and appropriate dilution with methanol. All determinations were performed in triplicate.

Skin preparation

Abdominal skin of male Wistar rats, 180–220 g, obtained from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China), was used for the permeation studies. The experiments were performed in accordance with the guidelines for animal use in the Life Science Research Center of Shenyang Pharmaceutical University.

The rats were anaesthetized with 20% (w/w) ethylurethane, the abdominal hair was removed using electric clippers and the skin then carefully shaved using a shaver. Then the rats were sacrificed. Full thickness skin samples were cut, removed and washed with water. Fat and connective tissues were carefully excised with surgical scissors. The thickness of the skin was measured by a micrometer and was in the range 0.3 ± 0.1 mm. The rat skin was used immediately.

Permeation experiments

Before use, each skin specimen was visually inspected for integrity. The equipment used for the in-vitro studies was a 2-chamber horizontal diffusion assembly (Fang et al 2002). The volume of the cell was 2.5 mL. The skin samples were placed between two diffusion cells with the desired section centred on the cell to give an effective area for diffusion of 0.9 cm^2. The receiver solution consisted of 40% PEG 400 and 60% phosphate buffer solution (pH 7.4) by weight. The donor

cells, which the stratum corneum was arranged to face, were filled with IPM–Enthol solution in a ratio of 4:1 (w/w). The arbitrary dose of the drug was 10 mg per donor cell. The amount of the drug exceeded its solubility in the donor vehicle. The stoichiometric ratio of drug to various amines used as potential absorption promoters was 1:1, while chemical enhancers, such as AZ, MT, OA and NMP, were added at a concentration of 5% by weight. The donor and receiver solutions were then stirred at 600 rev min^{-1} with a magnetic bar driven by a synchronous motor and maintained at 32°C by a water jacket surrounding the cell. Then, 2-mL volumes of the receptor solution were withdrawn at regular intervals over a period of 12 h. The volume withdrawn was always replaced with an equal volume of fresh receptor solution and, consequently, sink conditions were maintained. Throughout the experiments, the cells were sealed with stoppers to prevent the solution from evaporating. The sampling port was also covered with aluminium foil for the same purpose. All the experiments were performed at least in quadruplicate.

High-performance liquid chromatography

Drug concentrations were determined by reversed-phase HPLC. The HPLC system consisted of an L-7110 pump (Hitachi High-Technologies Corporation, Tokyo, Japan), an L-7420 variable-wavelength ultraviolet absorbance detector (Hitachi High-Technologies Corporation, Tokyo, Japan) and a CTO-10A column oven (Dalian, China). A computer employing T-2000L (Tianmei techcom, China) software controlled the Hitachi HPLC system. Separation of compounds was carried out on a Diamonsil C18 reversed-phase column (200 × 4.6 mm i.d., 5 μm; Dikma Technologies, Beijing China), which was protected by a guard column (10 × 4.6 mm) packed with the same material. Elution was performed at 40°C and the UV detector was set at 334 nm. The mobile phase used consisted of methanol–acetonitrile–phosphate buffer (20 mmol mL^{-1}, pH 2.5) in a ratio of 30:15:55. The HPLC flow rate was 1 mL min^{-1} and the injection volume was 20 μL. Under these conditions the retention time of scutellarin was 7.2 min.

NMR spectroscopy

^{1}H NMR spectra were recorded at 300 MHz using a Bruker Avance 300 spectrometer (Karlsruhe, Germany). Samples were dissolved in dimethyl sulfoxide (DMSO) and chemical shifts for hydrogen resonance reported in ppm relative to TMS.

Data analysis

The cumulative amount of scutellarin permeated through the skin was plotted as a function of time. The slope of the linear portion of the plot was calculated as the steady-state flux (J) (μg cm^{-2}h) and the χ-intercept of the linear portion of the plot was the lag-time (T_{lag}). The steady-state permeability coefficient (K_p) of the drug was calculated using the following equation:

$$K_p = J/C \quad (1)$$

where J is the flux and C is the concentration of scutellarin in the vehicle. The penetration-enhancing effect of the adjuvant was calculated in terms of the enhancement ratio (ER), using the following equation:

$$ER = Q_{12,\text{ with enhances}}/Q_{12,\text{ control}} \quad (2)$$

Statistical analysis was performed using Kruskal–Wallis one-way analysis of variance followed by Dunn's post-hoc test. $P \leq 0.05$ was considered significant.

Results and Discussion

NMR spectroscopy

The goal of the ^{1}H NMR measurement was to obtain evidence for the presence of ion-pair formation between scutellarin and respective organic amines from the chemical shift changes to protons near the carbonyl group. For the ion-pair formation potential of AZ and scutellarin according to the literature (Hadgraft et al 1985), the interaction between scutellarin and AZ was also measured.

Comparison of the proton NMR spectra of scutellarin with a mixture of scutellarin and organic amine in DMSO showed some significant shift differences (Table 1). The proton chemical shift attribution was performed based on the literature (Cui et al 2005). The largest changes observed were in the protons (h) on the carbons attached to carboxyl group, then in glycon terminal group protons (g) and phenolic hydroxyl group protons (f). The protons on the carbon adjacent to carboxyl group are recognized by their upfield position in the mixture as compared with the scutellarin, which is indicative of ion-pair formation (Sarveiya et al 2004, 2005). In the case of the mixture of scutellarin and AZ, there was no significant chemical shift differences compared with scutellarin alone, which suggests no ion-pair formation in that mixture.

Effect of various organic amines on scutellarin penetration across rat skin

A variety of organic amides (EA, DEA, TEA, TETA and DETA) were employed in this investigation in an attempt to increase scutellarin permeability. The Log P, pK_a and molecular weight (MW) of these amines are listed in Table 2. The permeation parameters of scutellarin through rat skin from various vehicles are shown in Table 3.

All the organic amines employed in this study, except TEA, significantly increased the cumulative amount of scutellarin penetrating through rat skin over 12 h (Q_{12}) compared with control. The rank order of the relative enhancement was as follows: EA > DETA > TETA > DEA > TEA. The Q_{12} of scutellarin plus organic amines was 1.66- to 7.11-fold higher than that of the control (Table 3), depending on the adjuvant used. No significant difference in Q_{12} was observed between DEA and DETA ($P > 0.05$), which may be attributed to the very close MW. The order of the K_p of scutellarin in the presence of organic amines was DEA > EA > TEA > DETA > TETA, which was different from that of the flux. In general, an alkylol amine was a more

Table 1 ^{1}H NMR chemical shifts of scutellarin and its ion-pairs for proton on carbon

	Scutellarin	EA	DEA	TEA	DETA	TETA	AZ
a	7.95	7.90	7.91	7.94	7.89	7.92	7.95
b	7.92	7.87	7.88	7.91	7.86	7.89	7.92
c	6.99	6.96	6.96	6.98	6.96	6.98	6.99
d	6.95	6.92	6.93	6.95	6.91	6.94	6.96
e	6.93	6.89	6.90	6.92	6.89	6.91	6.93
f	5.21	4.95(–0.26)	4.97(–0.24)	5.10(–0.11)	4.97(–0.24)	5.01(–0.20)	5.21(0)
g	5.28	4.98(–0.30)	4.99(–0.29)	5.16(–0.12)	4.99(–0.29)	5.03(–0.25)	5.29(+0.01)
h	4.03	3.59(–0.44)	3.63(–0.40)	3.80(–0.23)	3.60(–0.43)	3.68(–0.35)	4.04(+0.01)
i	overlap	overlap	overlap	overlap	overlap	overlap	overlap
j	overlap	overlap	overlap	overlap	overlap	overlap	overlap
k	overlap	overlap	overlap	overlap	overlap	overlap	overlap

Table 2 The Log P, pK_a and MW of various amines

Amine	Log P	pK_a	MW
DETA	0.58	11.10	73.14
TETA	1.45	10.80	101.19
EA	–1.31	9.50	61.08
DEA	–1.43	8.96	105.14
TEA	–1.00	7.76	149.19

efficient permeability enhancer than an alkyl amine under our experimental conditions.

Organic amines have been employed in some studies to alter the physicochemical properties of acidic drugs and promote drug permeation. The drug permeation enhancing effect of organic amines is usually attributed to the alteration in the physicochemical properties of a drug produced by the formation of an ion-pair. An ion-pair behaves as a single unit during the process of penetration. Therefore, drug permeation behaviour should be correlated with the physicochemical properties of the organic amine. Consistent with the results of the study on mefenamic acid (Fang et al 2003), addition of an organic amine resulted in a reduction in drug solubility in the IPM–ethanol system, which could result from the hydrophilic properties of the organic amine, implying a reduction in the polarity of the ion-pair compared with the drug. The in-vitro permeability of scutellarin across full thickness rat skin was enhanced. This suggests an enhancing effect of all the organic amines independent of their ability to increase the drug saturation concentration, except in the case of TETA. Also, the log K_p value of scutellarin increased monotonically with the reduction in the log P value of the organic amine ($r = 0.8929$). In addition, the Q_{12} of scutellarin was inversely dependent on the MW of the organic amine ($r = 0.9134$).

It is generally accepted that skin penetration is better for a drug with balanced hydrophilicity–lipophilicity. The drugs that permeate most easily are small molecules of moderate lipophilicity. A parabolic dependence has been found between skin permeation and the octanol–water partition coefficient (P) with an optimum value of log $P \approx 2$ (Hinz et al 1991; Hadgraft 1999). Ion-pair formation is often used to facilitate drug permeation. Therefore, in the case of a hydrophilic drug, such as 5-aminolevulinic acid (log P –0.82) (Auner et al 2003) and methotrexate (log P_{app} –2.15) (Trotta et al 1996), a lipophilic counter-ion should be used to achieve this goal, and a hydrophilic counter-ion should be used for lipophilic drugs, such as benzydamine (log P 2.08 (pH 7.6)) (Sarveiya et al 2005) and mefenamic acid (log P 3.31) (Fang et al 2003). However, in our study, a hydrophilic counter-ion

Table 3 The permeation parameters of scutellarin with different enhancers

Enhancer	S (μg mL^{-1})	Q_{12} (μg cm^{-2})	J (μg cm^{-2} h)	T_{lag} (h)	K_p (cm h^{-1})	ER
Control	16.50 ± 0.08	69.20 ± 7.82	12.70 ± 0.99	6.64 ± 0.20	0.77 ± 0.06	1.00
EA	3.14 ± 0.04	492.0 ± 40.3	55.10 ± 5.65	1.86 ± 0.85	17.50 ± 1.80	7.11
DEA	1.23 ± 0.03	255.0 ± 21.9	36.90 ± 4.80	5.10 ± 0.07	30.00 ± 3.56	3.68
TEA	1.23 ± 0.05	115.00 ± 20.56	17.23 ± 3.07	5.06 ± 0.62	14.00 ± 2.50	1.66
DETA	11.60 ± 0.07	295.0 ± 30.8	29.80 ± 3.19	1.78 ± 0.31	2.56 ± 0.27	4.76
TETA	18.50 ± 0.06	265.0 ± 18.4	22.80 ± 0.46	2.73 ± 0.46	1.23 ± 0.26	3.83
AZ	233.00 ± 4.43	390.0 ± 46.9	42.40 ± 4.61	1.72 ± 0.29	0.18 ± 0.02	5.64
OA	11.20 ± 0.05	180 ± 13	17.10 ± 1.17	1.12 ± 0.50	1.53 ± 0.11	2.60
MT	7.46 ± 0.03	227.0 ± 30.1	27.80 ± 2.04	3.51 ± 0.67	3.73 ± 0.27	3.28
NMP	494.00 ± 2.56	95.5 ± 13.1	14.90 ± 2.74	5.43 ± 0.70	0.03 ± 0.01	1.38
EA + AZ	5.19 ± 0.05	208.0 ± 18.2	24.90 ± 2.03	3.31 ± 0.17	4.81 ± 0.39	3.01
EA + MT	1.82 ± 0.02	295.0 ± 30.9	29.80 ± 3.19	1.38 ± 0.32	16.30 ± 1.75	4.26
AZ + MT	194.00 ± 4.26	739.0 ± 79.2	81.20 ± 7.65	2.86 ± 0.26	0.42 ± 0.04	10.7

Values represent the mean ± s.e.m., n = 4.

had a greater effect on skin permeability compared with a lipophilic one. This suggests that scutellarin might have a different interaction site in the stratum corneum compared with the drugs referred to above.

The actual mechanism governing the permeation of scutellarin is unclear. One possibility is that scutellarin penetrates the skin through polar or porous routes, which has been modelled or assumed to explain the permeation behaviour of some hydrophilic or ionic drugs, such as n-alkanols (Scheuplein & Blank 1971; Behl et al 1983), or the hydrophilic ingredient of an extract of guarana (Sznitowska et al 1995; Heard et al 2006).

Effect of chemical enhancers on scutellarin penetration through rat skin

As suggested in the former section, the polar route should be mainly responsible for scutellarin permeation. This polar pathway is located intercellularly and consists of aqueous regions surrounded by polar lipids (Sznitowska et al 1998). A discontinuity in the polar pathway is also indicated by the long lag-time of the drug permeating through that pathway. Accordingly, we can assume that a chemical enhancer that can disrupt the packing order of lipids surrounding the aqueous regions of the stratum corneum may promote the continuity of the polar pathway, consequently facilitating drug penetration across rat skin. AZ, MT and OA have all been reported to disrupt the packing order of lipids (Goodman & Barry 1985; Ongipattanakul et al 1991; Williams & Barry 1992; Diez-Sales et al 1996; Fujii et al 2003; Obata et al 2006).

Not only does the route of skin penetration of the drug depend on its physicochemical properties, but also enhancers will occupy the preferential site according to their physicochemical properties, and alter the barrier function. Therefore, the enhancing effect of various potential enhancers should be specific on drug penetration.

The percutaneous permeation parameters of the tested formulations are presented in Table 3. Some of these agents significantly increased the scutellarin Q_{12} ($P<0.05$), such as AZ, MT and OA with an ER (Q_{12}) of 5.64, 3.28 and 2.60, respectively. In the case of NMP, although the drug solubility in the donor phase was increased from 16.45 μg mL^{-1} to 494.22 μg mL^{-1}, no significant effect ($P>0.05$) was observed on Q_{12}.

AZ and NMP, with the highest drug solubility in the donor phase, produced poor skin permeability for scutellarin (Table 3). The K_p of scutellarin from saturated solutions containing AZ and NMP was, respectively, 0.18 cm h^{-1} and 0.03 cm h^{-1}, much lower than that of control (0.77 cm h^{-1}). In the case of OA and MT, an increase in the permeability coefficient was observed, and the highest permeability coefficient of scutellarin was obtained in the presence of MT (K_p of 3.73 cm h^{-1}). Generally, a high drug solubility resulted in a low K_p.

The influence of enhancer on drug solubility was complicated. The solubility in a solvent depends on the interactive effect of the solvent and solute molecules (i.e., an intermolecular effect). As we can see from Figure 1, scutellarin is a polyhydric compound, and the hydroxyl group is located in the outer region of the molecule. Therefore, a hydrogen bond may be the main interaction between solvent molecules and drugs. The number of the active sites in the mixed solvent for binding to hydroxyl groups was the most important factor affecting drug solubility in our study. Besides the competitive action between the molecules of enhancer and drug for hydrogen-bond binding sites of solvent molecules, MT and OA preferentially formed intermolecular and intramolecular hydrogen bonds with autochthonous molecules resulting in a decrease in the number of binding sites of hydrogen bonds, reducing the drug solubility. However, there is no intermolecular or intramolecular hydrogen bond between AZ molecules or NMP molecules, because there is no H atom linked to an N atom or O atom in their chemical structure. This increases the number of active sites in the donor solution and the drug solubility. The exact reason for drug solubility changes induced after enhancer addition has been not clarified yet, so further study should be conducted.

Administration of scutellarin along with various chemical enhancers appeared to be able to shorten the lag-time of drug penetration. The lag-time of AZ, MT, OA and NMP was 1.72 h, 3.51 h, 1.12 h and 5.43 h, respectively, while that of the control was 6.64 h. According to the data listed in Table 2, the most effective lag-time reducer was OA with a lag-time of approximately one-sixth that of the control. The reduction in lag-time also indicated an increase in the diffusion coefficient caused by disruption of the lipid lamella of the stratum corneum.

The highest ER (Q_{12}) was obtained with AZ. AZ is a more efficient enhancer for hydrophilic drugs than lipophilic ones (Diez-Sales et al 1996). The action of AZ in this study may involve a combination of removal of skin lipids (Goodman & Barry 1985), increasing the fluidity of skin lipids and hydration of the stratum corneum (Sugibayashi et al 1992). The enhanced Q_{12} may be mainly attributed to the enhanced diffusion by enhancers in this study.

In the case of OA, the synergistic effect of OA and ethanol proposed by other authors, may also be present in our systems (Aboofazeli et al 2002; Femenía-Font et al 2005).

Effect of combined use of enhancers on scutellarin penetration through rat skin

The enhancing effect of the combined use of EA and AZ, EA and MT, and AZ and MT on scutellarin penetration was also investigated and the permeation profiles are shown in Figure 2. EA plus AZ decreased the J value of scutellarin compared with EA ($P<0.05$) or AZ ($P>0.05$) alone, though its J value was higher than that of the control ($P>0.05$) (Figure 2A). The lag-time was significantly increased compared with that obtained with separate use ($P<0.05$) (Table 2). Both the decreased J and the increased lag-time resulted in reduced Q_{12}. This suggests that the combined use of EA and AZ did not have a synergistic effect.

The Q_{12} value of EA plus MT was between that of EA and MT alone (Figure 2B) and no significant difference in Q_{12} between EA plus MT and MT alone was observed. However, a synergistic action on the K_p of scutellarin was obtained (Table 2). Compared with EA alone, the J value of scutellarin was significantly reduced by EA plus MT ($P<0.05$). The lag-time of scutellarin of EA plus MT was shorter than that of either EA ($P>0.05$) or MT alone ($P<0.05$).

As can be seen from Figure 2C, a synergistic action on Q_{12} was obtained by the combined use of AZ and MT, as the

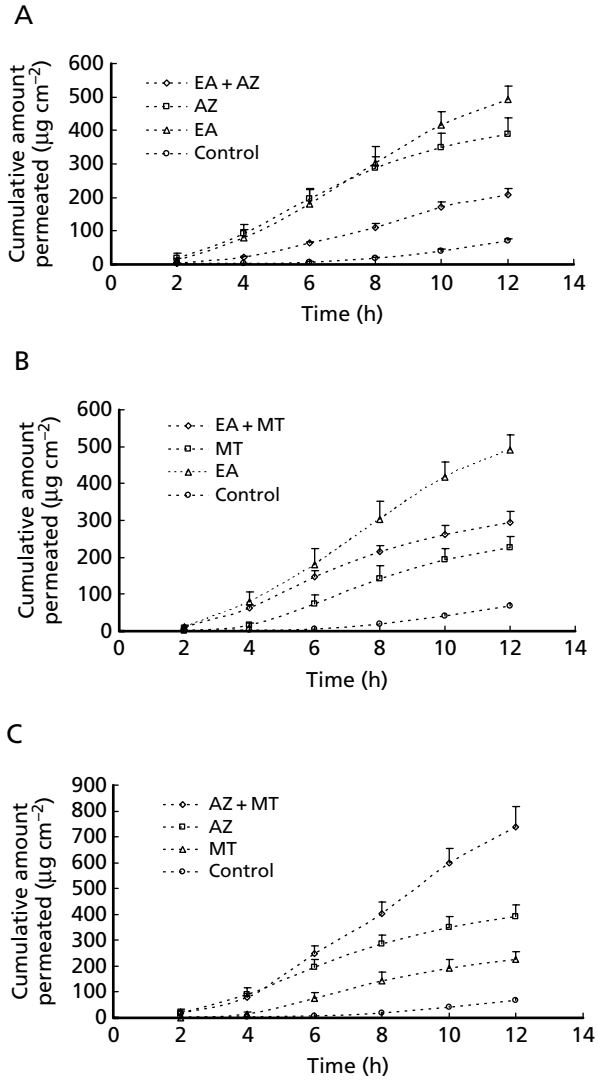

Figure 2 Effect of combination use of EA and AZ (A), EA and MT (B) and AZ and MT (C) on percutaneous absorption of scutellarin. Error bars represent the s.e.m., n = 4.

Q_{12} of enhancer combined use was greater than that of each enhancer used separately. AZ plus MT significantly increased the J of scutellarin compared with AZ or MT alone ($P < 0.05$), which benefited Q_{12} improvement. There was no significant difference in lag-time among the three groups of AZ plus MT, AZ and MT.

Conclusion

The enhancing effect of alkylolamines on scutellarin permeability was much greater than that of alkylamines. Two inversely dependent effects were observed between the log K_p value of scutellarin and the log P value of the amine, and between the ER (Q_{12}) and the MW of the amine. Azone was the most promising chemical enhancer in this study, because of its ability to promote drug permeation flux and reduce drug lag-time significantly. In addition, a synergistic action on ER (Q_{12}) was obtained with AZ plus MT.

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