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The effect of glycyrrhizin on the release rate and skin penetration of diclofenac sodium from topical formulations

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Abstract

The influence of glycyrrhizin extracted from *Glycyrrhiza glabra* var. glandulifera (licorice roots) on the percutaneous absorption of diclofenac sodium from sodium carboxymethylcellulose (NaCMC) gels or oil-in-water (o/w) emulsion was investigated. Skin permeation experiments were carried out using excised abdominal rat skin. The results showed that the efficiency of glycyrrhizin as an enhancer agent is greater in gel formulations than it is in the emulsions. The enhancer with the concentration of 0.1% w/w in gel increased diclofenac sodium flux value to tenfold compared with the control gel.

Keywords: Skin penetration; Enhancer; Glycyrrhizin; Release rate; Diclofenac sodium

1. Introduction

Diclofenac sodium is a non-steroid-type anti-inflammatory agent and is widely used clinically because of its strong analgesic, antipyretic and anti-inflammatory effects. It is known that this drug inhibits biosynthesis of the prostaglandin in vivo and in vitro and the drug is considered to have only a slightly adverse effect on the stomach and intestines [1]. It is extensively metabolized in the liver and because of its short biological half-life, the drug has to be given frequently. Therefore, developing a therapeutic system to provide a transdermal delivery is beneficial.

Transdermal delivery of drugs promises many advantages over oral or intravenous administration, but drug delivery via the skin is not a simple task. The principal barrier to topical drug delivery is the stratum corneum, the outer most layer of the skin comprising keratin-rich cells embedded in multiple lipid bilayers. Many strategies have been suggested in order to overcome the low permeability of drugs through the skin. A popular approach is the use of penetration enhancers (or accelerants) which reduce reversibly the permeability of the stratum corneum [2]. These agents partition into, and interact with, the stratum corneum constituents to induce a temporary, reversible increase in skin permeability.

As diclofenac sodium is not easily absorbed on transdermal application [3]. many compounds, such as isopropyl myristate [4], nicotinic acid esters [5], hydrogenated soya phospholipid [3], ethanol [6,7], *n*-octanol and decanol [8,9], nonionic surfactants [10] and terpens [11] have been reported to enhance the permeability of diclofenac sodium.

The objective of the present paper was to examine the influence of a natural penetration enhancer (glycyrrhizin) with different concentrations on the in vitro permeation of the drug through abdominal rat skin. NaCMC gels and emulsions (o/w) containing diclofenac sodium and the enhancer were formulated. The release characteristics of the formulations were then evaluated in vitro using dialysis tube.

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2. Materials and methods

Diclofenac sodium was provided by Industrial Sobhan Pharmaceutical (Rasht, IRAN). Lanette O (viscosity-increasing agent), Eutanol G (oily vehicle), arlacel 65 (nonionic surfactant), Tween 80 (nonionic surfactant), NaCMC (gelling agent), propylene glycol were provided from Merck (Darmstdt, Germany). The root of *Glycyrrhizin glabra* variety of glandulifera (licorice root) was collected from botanic garden of the School of Pharmacy, Tabriz Medical Sciences University.

2.1. Extraction procedure

The unpeeled and air-dried roots of licorice (500 g) were coarsely grounded and extracted with boiling water (3 l $\times$ 4). The combined solutions were filtered and then were boiled for 10 min and set aside in room temperature for 12 h. The solution filtered again to eliminate albuminoid substances. After this time the solution was acidified gradually with sulfuric acid (20% v/v) step-by-step until no precipitation was occurred. The yellow precipitation ($\sim$25 g) were separated by centrifuge and re-dissolved in little amount of ammonium hydroxide then its volume was brought to 250 ml with distilled water. Precipitation procedure with sulfuric acid repeated. The precipitated material was rinsed several times with water in order to neutralize the acid sulfuric acid. The pasty, acid insoluble precipitate was dried at 40 °C and then ground as fine powder. The yield of glycyrrhizin was 4% w/w [12]. According the previous work, the purity of the resultant glycyrrhizin was 97% [13,14].

2.2. Preparation of diclofenac sodium gels

The composition of the diclofenac sodium gel formulations used in this study is shown in Table 1. Gels were prepared by dispersing 3% w/w NaCMC in water for a period of 2 h. Diclofenac sodium was dissolved in propylene glycol and the solution was added gently to NaCMC dispersion under continuos stirring (200 rpm). The same method was employed for the preparation of gels containing the enhancer. In this case the enhancer was dissolved in the mixture of water–propylene glycol.

2.3. Preparation of diclofenac sodium emulsions

The o/w emulsions were prepared at a phase volume ratio of unity, and the compositions of these emulsions are tabulated in Table 1. Lanette O, Eutanol G and Arlacel were placed in a beaker and heated up to 70 °C while stirring. The aqueous phase containing water, diclofenac sodium, Tween 80 and glycyrrhizin was heated to $\sim$60 °C. The oily phase was slowly added to the aqueous phase to form the emulsion under continuos stirring (200 rpm). The final emulsion was passed through a homogenizer. Each emulsion was visibly stable for the duration of the experiment. The pH of the formulations were adjusted about 7 using phosphate buffer and the concentration of the glycyrrhizin was 0.1 or 0.5% w/w in the emulsion.

2.4. Release studies from dialysis tube

Distilled water (500 ml) with pH 7.4 (adjusted with phosphate buffer) was used as the dissolution media. The rate of stirring was 50 rpm. Gel (3 g) or emulsion was used in the dialysis bag which was placed in dissolution media and maintained at 37 °C for a period of 5 h. At appropriate intervals (1, 2, 3, 4 and 5 h), 5 ml of each sample was taken and filtered. The dissolution media was then replaced by 5 ml of fresh dissolution media to maintain a constant volume. The samples were assayed at 275.6 nm by UV–Vis spectrophotometer (Shimadzu 60A). The mean of three determinations was used to calculate the drug release from each of the formulation.

Table 1
Composition (%w/w) of diclofenac sodium topical formulations

Constituents	Formulation code					
	F1	F2	F3	F4	F5	F6
Diclofenac sodium	1	1	1	1	1	1
NaCMC	3	3	3			
Propylene glycol	40	40	40			
Glycyrrhizin		0.1	0.5		0.1	0.5
Lanette O				13	13	13
Eutanol G				13.5	13.5	13.5
Arlacel 63				2	2	2
Tween 80				1	1	1
Water	66	65.9	65.5	69.5	69.4	69

Formulations F1–F3 and F4–F6 are gel and o/w emulsions, respectively.

2.5. Skin membrane preparation

The abdominal hair of wistar male rats, weighing 160±25 g, was shaved using electric and hand razors. After anesthetizing the animal with ether, the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. To remove extraneous debris and leachable enzymes, the dermal side of the skin was in contact with a saline solution for 1 h before starting the diffusion experiment.

2.6. Permeation studies

A system employing three improved Franz diffusion cells with a diffusional area of 5.3 cm^2 was used for permeation studies. The excised rat skin was set in place with the stratum corneum facing the donor compartment and the dermis facing the receptor.

The gel (0.75 g) or emulsion was placed on the skin surface in the donor compartment that was sealed from the atmosphere with plastic film (Parafilm). The receptor compartment of the cell was filled with 25 ml of phosphate buffer (pH 7.4). During the experiments, the solution in receptor side was maintained at 37±0.5 °C and stirred at 800 rpm with teflon-coated magnetic stirring bars. After application of the test formulation on the donor side, 0.5 ml aliquots were collected from the receptor side at designated time intervals (1, 2, 3, 5, 8, 12, 16, 24 and 28 h), and 0.5 ml of the phosphate buffer was added into the receptor side immediately after each sample collection. To determine the effect of the enhancer on the skin permeability as solution, the removed rat skin membranes were immersed in solution containing 0.1% w/w glycyrrhizin for 24 h, rinsed and mounted in the 'in vitro' diffusion cells. Then the topical formulation without the enhancer was used as described above (F7 and F8 in Table 2).

2.7. Analytical method

Diclofenac sodium in samples was determined using HPLC apparatus (Ceceil 1100, UK) equipped with a variable-wavelength UV detector. The column was Spherisorb C18 (150 × 4 mm, 5 μm, Hichrom, UK). Elution was carried out at room temperature with a mobile phase consisting of acetonitrile and water (50:50, v/v) adjusted to pH 2.2 with phosphoric acid (about 2.5 ml); the flow rate was 2 ml/min. Detection was performed at 276 nm and the injection volume and retention time for diclofenac solution were 20 μl and 2.4 min, respectively [11].

2.8. Data treatment

According to Fick's second law of diffusion, the total amount of drug (Q_t) appearing in the receptor solution in time t is expressed as:

$$Q_t = AKLC_0[(Dt/L^2) - (1/6) - (2/\pi^2)\sum(-1)^n/n^2)\exp(D^n 2\pi^2 t/L^2)] \quad (1)$$

where A is the effective diffusion area, C_0 represents the drug concentration which remains constant in the vehicle, D is the diffusion coefficient, L denotes the thickness of the membrane and K is the partition coefficient of the drug between membrane and vehicle. At steady-state, Eq. (1) is expressed as follows:

$$Q_t/A = KLC_0[(Dt/L^2) - (1/6)] \quad (2)$$

The flux, J, was determined from the slope of the steady-state portion of the amount of the drug permeated divided by A versus time. The lag time values were determined from the x-intercept of the slope at steady-state.

From Eq. (2) the flux is expressed as:

$$J = C_0KD/L = C_0K_p \quad (3)$$

where K_p is the permeability coefficient.

Table 2
Diclofenac sodium skin permeation parameters for various topical formulations

Formulation code	Steady-state flux (μg cm^{-2} h^{-1})	K_p ($\times 10^4$) (cm h^{-1})	Lag time (h)	ER
F1	0.266±0.08	0.354±0.10	0.19	1.00
F2	2.834±0.15	3.788±0.18	2.46	10.70
F3	0.456±0.07	0.608±0.13	2.72	1.72
F4	0.647±0.15	0.862±0.19	1.41	1.00
F5	0.605±0.10	0.806±0.20	3.70	0.94
F6	0.697±0.12	0.929±0.21	2.88	1.08
F7 [a]	2.050±0.25	2.733±0.30	2.23	7.72
F8 [b]	0.774±0.19	1.032±0.25	2.91	1.20

[a] The composition is the same as F1, the rat skin is rinsed with the solution containing 0.1% w/w glycyrrhizin for 24 h.
[b] The composition is the same as F4, the rat skin is rinsed with the solution containing 0.1% w/w glycyrrhizin for 24 h.

The enhancer ratios (ER) were calculated from following equation [15].

$$ER = (K_p \text{ with enhancer}/K_p \text{ without enhancer}) \quad (4)$$

The values reported are mean ratios from a minimum of three replicates.

3. Results and discussion

Naturally occurring terpenes which are non-toxic and non-irritant towards the skin are great interest as skin penetration enhancers. Glycyrrhizin is one such chemical which may act as a penetration enhancer. Fig. 1 shows the effect of various concentrations of the enhancer on the release rate of diclofenac sodium from gels. The presence of 0.1% w/w glycyrrhizin within gels (F2) significantly increased ($p < 0.05$) the release rate of the drug in comparison with formulation containing no enhancer (F1). The Figure also shows that enhancement of the concentration of the enhancer from 0.1 to 0.5% w/w results in a release rate of diclofenac sodium which is similar to the release rate of the drug from F1.

The effect of the enhancer on the release rate of diclofenac sodium from the emulsions is shown in Fig. 2. It can be seen that the presence of the enhancer with a concentration of 0.1% w/w had no significant effect on the release of the drug ($p > 0.05$), whereas an increase in the concentration of the enhancer from 0.1 to 0.5% w/w results in a considerable reduction ($p < 0.05$) in the release rate of the drug from the emulsion.

The permeation profiles of diclofenac sodium through rat skin from NaCMC gels (formulations F1–F3) and emulsions (formulations F4–F6) containing different concentrations of glycyrrhizin as penetration enhancer are given in Figs. 3 and 4, respectively. The flux, J, permeability coefficient, K_p, lag time and ER for each of the different concentrations of the enhancer according to Eqs. (2)–(4) are listed in Table 2. Diclofenac sodium mean flux values at steady-state from control gel (F1) and control emulsion (F4) were 0.266 and 0.647 μg cm^{-2} h^{-1}, respectively. The results show that glycyrrhizin highly increases diclofenac sodium flux in gel

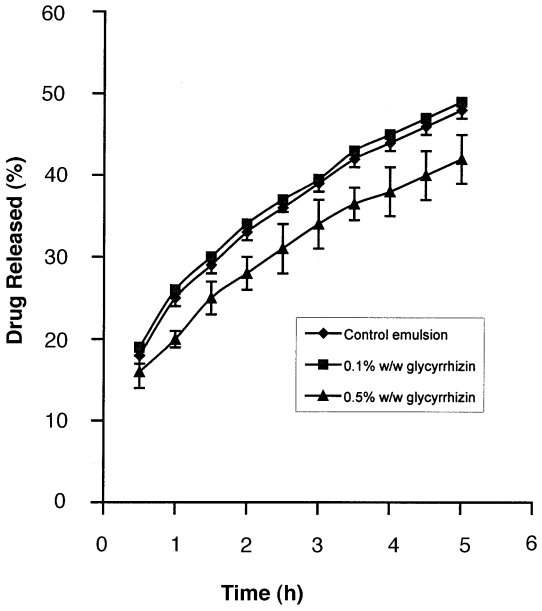

Fig. 2. The effect of glycyrrhizin on the release rate of sodium diclofenac from emulsion formulations.

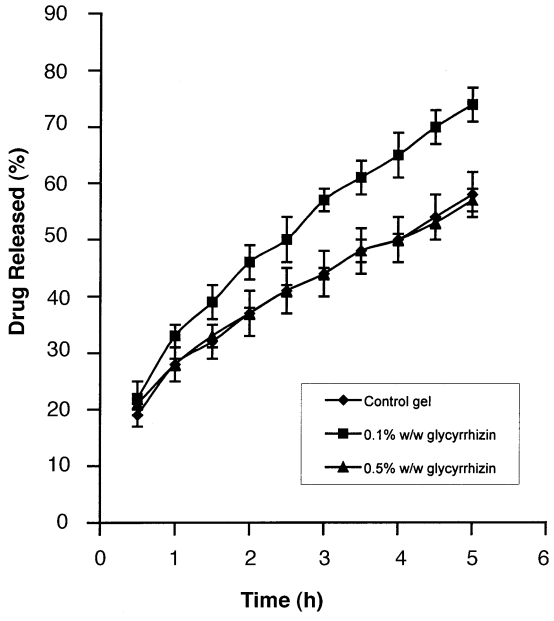

Fig. 1. The effect of glycyrrhizin on the release rate of sodium diclofenac from gel formulations.

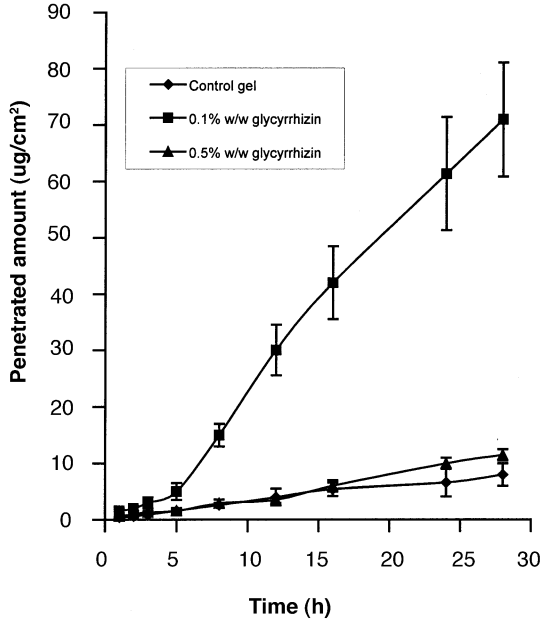

Fig. 3. Permeation profiles of sodium diclofenac from gel formulations in presence of glycyrrhizin through rat skin.

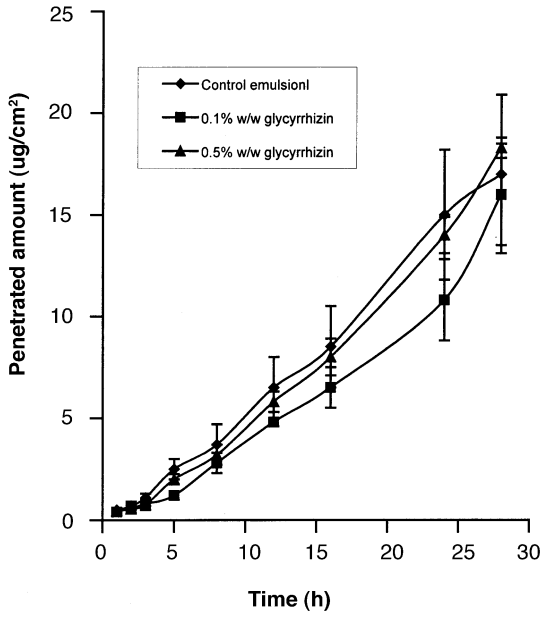

Fig. 4. Permeation profiles of sodium diclofenac from emulsion formulations in presence of glycyrrhizin through rat skin.

formulations when used in small concentrations, while incorporating the enhancer in emulsions slightly increases the flux only in the concentration of 0.5% w/w. When the concentration of the enhancer in emulsion was 0.1% w/w the flux decreased slightly compared with control emulsion. As the concentration of the enhancer increased from 0.1 (F2) to 0.5% w/w (F3) in gel formulations, the flux of diclofenac sodium significantly decreased. The most outstanding penetration enhancer concentration was 0.1% w/w in gel formulation, providing an almost tenfold increase in diclofenac sodium permeability coefficient or ER (Table 2).

The enhancement of the skin transport occurs at low concentrations of the enhancer, but this is seen to decrease at higher concentrations, generally above the critical micelle concentration (CMC) of the enhancer. The increase in flux at low enhancer concentrations is normally attributed to the ability of glycyrrhizin molecules to penetrate the skin and increase its permeability. Reduction of rate of transport of the drug present in enhancer systems is attributed to the ability of the surfactant to form micelles and is normally observed only if interaction between micelle and the drug occurs. Solubilization of the drug species by glycyrrhizin micelles decreased the termodynamic activity of the drug and, hence, decreases the driving force of the drug absorption. Therefore, the overall effect of a surfactant on the rate of drug permeation across a membrane will be a combination of the influence of these two opposing effects. Although 1% w/w is above CMC of the glycyrrhizin in pure water (0.025% w/v), but it has been shown that presence of propylene glycol increased the CMC of the nonionic surfactants (emulsifier) up to ten times where the concentration of propylene glycol was 40% v/v [16].

As glycyrrhizin acts as a nonionic surfactant, it is able to reduce surface tension [17]. This activity is based on the peculiar structure having a triterpene moiety as a lipophilic group and glucuronic acid moiety as a hydrophilic group. Dalvi and Zatz [18] found that skin permeability was not increased by nonionic surfactants in purely aqueous media. However, Shahi and Zatz [19] did report that Tween 80 was responsible for enhancement of hydrocortisone penetration from isopropyl alcohol: water mixtures [19]. It is apparent that propylene glycol and Tween 80 interact to affect the skin barrier so as to promote the penetration of diazepam [20]. It has been shown that nonionic surfactants increased the skin penetration of chloramphenicol [21] diazepam [20], 5-fluorouracil [22] and haloperidol [23]. The authors hypothesized that the nature of the medium could influence the interaction between nonionic surfactants and the skin barrier. Further investigations employing lidocaine solutions in propylene glycol-water vehicles supported this assumption [16]. It was evident from surface tension studies that the addition of propylene glycol raised the critical micelle concentration of the nonionic surfactants by approximately a factor of 10. The increase in monomer concentration might be an explanation for observed synergistic effect of propylene glycol and Tween 80.

Table 2 shows that while the addition of the enhancer increased diclofenac sodium flux, diffusional lag times were not reduced. It is likely that increased lag times were due to gradual increases in membrane permeability produced by the distribution of the enhancer within the stratum corneum and consequently a conditioning of the membrane in early stages of the diffusion process. Similar delayed onsets of action have been reported with some other enhancers [24–26].

Table 2 also shows that when the enhancer is added to the solution and the rat skin is rinsed for 24 h in the solution containing the enhancer, the ER of diclofenac sodium from gel formulation is 7.72 which is lower than the gel formulation containing the same concentration of the enhancer (compare the data of F2 and F7 in Table 2). In the emulsion the ER slightly increased (from 0.94 to 1.2) when the skin was rinsed in the solution containing the enhancer instead of using the same concentration in the emulsion formulation (compare the data of F4 and F8 in Table 2).

In conclusion, the results showed that glycyrrhizin is able to increase the flux of diclofenac sodium up to tenfold in gel formulations. The results also show that the concentration of the enhancer in formulation and the type of formulation (gel or emulsion) have remarkable effect on the skin permeation of diclofenac sodium.

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