Bull. Pharm. Sci., Assiut University, Vol. 30, Part 2, 2007, pp. 159-167.

LIPOSOMAL GEL OF DICLOFENAC SODIUM AS TOPICAL DELIVERY SYSTEM

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من المعروف ان عقارالدكلوفيناك صوديوم يستعمل علي نطاق واسع كمضاد للالتهاب وأن تعاطيه عن طريق الفم يؤدي الي بعض الاثار الجانبية ولهذا تتناول هذه الدراسة استخدامه موضعيا لتلافي بعض هذه الاثار الجانبية. في هذه الدراسة تم تحضير ليبوزومات ديكلوفيناك الصوديوم وتحميلها علي هلاميات البلورونك ف ١٢٧ وتمت دراسة تأثير كولات الصوديوم علي نفاذية العقار خلال جلد الفئران وكذلك دراسة النشاط المضاد للالتهاب لهذه الهلاميات. كما تم تحضير الليبوزومات بالطريقة التقليدية واستعمال الموجات فوق الصوتية لضبط حجم الليبوزومات وتم قياس حجم الليبوزومات وكمية الدواء المتحوصل داخلها. وجد أن حجم الليبوروماتٍ يتراوح ما بين ١٠٠-٧٠٠ نانوميتر وكمية العقار المتحوصل ما بين ٦٠ ٨٠%. وبدراسة نفاذية العقار من الليبوزومات والهلاميات خلال جلد الفئران في محلول الفوسفات ذو الوسط الهيدروجيني المنتظم (٧,٤) كان أعلي نفاذية للعقار في وجود كولات الصوديوم سواء لليبوزومات او الهلاميات لما لها من تأثير ونشاط سطحي. كما تناولت الدراسة النشاط المضاد للالتهاب للعقار وذلك باستعمال الهلام المحضر موضعيا للفئران. ووجد انه F6 و F8 اعطت حماية كبيرة ضد الالتهاب.

This study the preparation of diclofenac sodium in lecithin vesicles and loading in pluronic F-127 gel, the effect of sodium cholate on the diffusion of the drug through rat skin and the anti-inflammatory activity of the liposomal gel formulations. Lecithin vesicles were prepared in the presence or absence of sodium cholate by the dry film method and sonication. The size of liposomal vesicles ranged from 100-700 nm and the encapsulation efficiency of the diclofenac sodium was between 60-80%. The lecithin vesicles were loaded in pluronic F-127 gel. The highest cumulative of drug diffusion through rat skin was 19.31± 1.50 ($\mu g/cm^2$) for lecithin vesicles in the presence of sodium cholate (F4). Also the highest cumulative of drug diffusion through rat skin was 12.20± 0.50 ($\mu g/cm^2$) for liposomal gel (F8). The anti-inflammatory activity of the liposomal gel formulations was studied

Received in 5/9/2007, Received in revised form in 20/11/2007 & Accepted in 24/11/2007

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on carrageenan induced paw edema in rats. The results show that, F8 and F6 show superior anti-inflammatory activity in comparison with the other gel formulations. From the results, lecithin vesicles and liposomal gel of diclofenac sodium appear to be advantagesous for topical delivery of the drug.

INTRODUCTION

Diclofenac sodium (DFS) is a non-steroidal anti-rheumatic agent which has a potent anti-inflammatory action but has undesired side-effects on the stomach[1]. It is extensively metabolized in the liver and because of its short biological half-life, the drug to be given frequently. As the result, developing a therapeutic system to provide transdermal delivery would be beneficial. Diclofenac sodium (DFS) is not easily absorbed on transdermal application[2]. Although a major portion of commercial diclofenac is available in the form of oral medications, the drug causes serious adverse effects on the gastrointestinal tract. Gastrointestinal bleeding and ulceration are quite common due to oral therapy of diclofenac[3]. Clinical evidence suggests that topically applied non-steroidal anti-inflammatory drugs (NSAIDs) are safer and at least as efficacious as oral NSAIDs in the treatment of rheumatic diseases[4]. Gastrointestinal adverse drug reactions after topical NSAIDs use are rare when compared to the 15% incidence of serious GI events associated with oral NSAIDs[4].

Liposomes are vesicles in which an aqueous core is entirely enclosed by surrounding lipid bilayers. They have been shown to be interesting as drug-delivery systems for topical delivery[5]. Due to their similarity to biological membranes - since the lipid bilayer contains natural phosphorlipid and cholesterol - liposome theoretically do not have any risk of antigenicity[6].

The purpose of the present study is to evaluate the lecithin vesicles and its liposomal gel in pluronic F-127 to deliver diclofenac sodium *in-vitro* and show their anti-inflammatory activity.

EXPERIMENTAL

Materials

Egg phosphatidylcholine (EPC) was a gift from Lipoid GmbH (Ludwigshafen, Germany). Sodium cholate, diclofenac sodium, Triton X-100 were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Pluronic F-127 was supplied from BASF (BASF, Ludwigshafen, Germany). All other chemicals used were of analytical grade.

Apparatus

A rotary evaporator (Rotavapor R E 111, Buchi, Goepingen Swistzerland). A sonicator bath (Transsonic 460/H, Elma, Germany). Photon Correlation spectroscopy

(PCS) Malvern zeta master (Malvern GmbH, Herrenberg, Germany). UV-spectrophotometer (Schimadzu, Japan). Electronic balance (Percisa 205A, Zurich, Sweitzerland). Magnetic stirrer with hotplate (Gallenkamp, England). pH-meter (Jenway, Ltd. , UK.).

Methods

Preparation of EPC vesicles of diclofenac sodium

Four lecithin vesicle formulations of diclofenac with compositions shown in Table 1 were prepared. Egg phosphatidylcholine alone or with sodium cholate was dissolved in a 1:1 mixture of methanol and chloroform, organic solvents were evaporated using a rotary evaporator The dried film was hydrated with the diclofenac solution and agitated for 30 min. F3 and F4 were sonicated for 5 min in a bath sonicator.

Liposomal size determination

The liposomal size was analyzed by Photon Correlation spectroscopy (PCS) using a Malvern zeta master. The measurements were made from a 90°C angle at room temperature and the average of three measurements was calculated.

Encapsulation efficiency determination

The liposome-encapsulated diclofenac was separated from unentrapped drug by dialysis method[7]. Liposomes were lysed with Triton X-100 in a concentration 0f 0.5% (w/v). Diclofenac was spectrophotometrically assayed at 250 nm[8]. The percent of encapsulation efficiency (EE %) was then calculated according to the following equation:

EE% =
{drug content / total drug added} X 100

Each result is the mean of three separate experiments.

Gel preparation and liposome loading

Pluronic F-127 gel (25% w/v) was prepared by the cold method described by Yong *et al.*[9]. The weighed amount of the polymer was slowly added to water with gentle mixing. The mixture was left in refrigerator (4°C) overnight to complete dissolution of the polymer. After the formation of clear viscous solution the liposome suspension was added and mixed very gently with glass rod. The mixture was left at room temperature until a clear gel is formed. Table 2 shows the code of each formula.

Table 1: Composition of the EPC vesicles of diclofenac.

Ingredient	Formula			
	F1	F2	F3	F4
Diclofenac sodium	100 mg	100 mg	100 mg	100 mg
Egg phosphatidylcholine	440 mg	440 mg	440 mg	440 mg
Sodium cholate	--	150 mg	---	150 mg
Distilled water	10 ml	10 ml	10 ml	10 ml

Table 2: Description of gel formulations.

Gel Code	Description
F5	F1 loaded in pluronic F-127 gel
F6	F2 loaded in pluronic F-127 gel
F7	F3 loaded in pluronic F-127 gel
F8	F4 loaded in pluronic F-127 gel

***In-vitro* skin permeation studies**

Skin permeation studies with DFS-containing liposomal formulations (liposome suspension and liposomes containing Pluronic F-127 gel) were carried out using abdominal skin of female mice. To obtain skin, animals were sacrificed. Hair on the dorsal side of the animal was removed with the help of a 0.1 mm animal hair clipper, in the direction of tail to head. Dermis part of the skin was wiped three times with a wet cotton swab soaked in isopropanol to remove any adhering fat material. Then, 6 hrs. treatment of the dermal side with phosphate buffer pH 7.4, to equilibrate the membrane, was done before starting the diffusion experiment. Skin was stretched over the end of an open-ended glass tube. The tube was immersed in a 400 ml beaker containing 100 ml of phosphate buffer pH 7.4 and kept in vertical position so that the membrane was just below the surface of the buffer solution. The surface area available for diffusion was 2.51 cm^2. The tube (donor) and beaker (receptor) were maintained at 37°C in thermostatically controlled shake water bath. The donor compartment was filled with diclofenac formulations (1.5 ml in case of formulations F1-F4 and diclofenac equivalent quantities - in gm- in case of formulations F5-F8). At time interval (up to 12 hrs) samples of 2.0 ml were withdrawn from the receptor and analyzed spectrophotometrically at 250 nm. The experiment was repeated three times and the average of reading was calculated.

Flux calculation

Amount of diclofenac sodium released in successive time intervals was obtained from the cumulative amount released at different time interval and the flux was calculated per unit area and time. Cumulative amount of diclofenac sodium released at the end of 12 hr and the maximum flux values for different formulations were compared using student t-test at 0.05 level of significance. Lag times were obtained by extrapolating the cumulative amount of diclofenac sodium released versus time curves to the time axis.

Anti-inflammatory activity of diclofenac liposomal gel

Acute inflammatory activity model, carrageenan induced rat paw edema method was applied in this study[10&11].

The rats weighing about 200 gm were divided into 5 groups each group has 4 rats. The animals of group 1 received placebo gel and group 2, 3, 4, 5 received formula F5, F6, F7, F8 respectively. Inflammation was produced in the rats using 0.1 ml of 1.0% w/v carrageenen solution in saline. This was injected subcutaneously into left hind paw. To evaluate the topical anti-inflammatory activity of the gel formulations, paw edema was examined. Thirty minutes later, 200 mg of each gel was applied topically on the edematous paw. The increase in the paw thickness was measured before carrageenen injection (time 0) and 2, 3, 4, 5 and 6 hrs after carrageenen adminstration using a dial micrometer. The percentage swelling of the paw and the percent inhibition of edema were calculated. The data were reported as mean ± SD (n=4).

Statistical analysis for the obtained results

Statistical analysis for the obtained results was carried out by the student t-test at 0.05 level of significance.

RESULTS AND DISCUSSION

Characterization of liposomes

Four different liposome formulations were prepared by dry film method in the presence or absence of sodium cholate and sonication for F2 and F4. The main characteristics of liposomes prepared are presented in Table 3. The size of prepared liposome is ranged from 500 to 700 nm for MLVs with low degree of homogeneity (0.25 polydispersity) but the size of the sonicated liposome is from 100-180 nm with high homogeneity (0.1 polydispersity).

The encapsulation efficiency of the drug in the prepared liposome, either MLVs or SUVs is from 63-80%.

Skin permeation studies

In this study, the *in-vitro* release of diclofenac sodium through rat skin using lecithin vesicles of the drug was evaluated and compared with that from the loaded liposomal gel formulations. Based on the cumulative amount of the drug released at 12 hrs, the formulations evaluated in this study showed a decreasing order for their ability to deliver the diclofenac sodium across rat skin as follows F4 > F2 > F3 > F1 for liposomal formulations and F8 > F6 > F7 > F5 for liposomal gel (Table 4). Figures 1-2 show the mean cumulative amount of diclofenac sodium diffused ($\mu g/cm^2$) through rat skin for lecithin vesicles and liposomal gel formulations.

It is important to note the effect of the addition of sodium cholate to lecithin on the permeation of diclofenac sodium through rat skin either for vesicles or liposomal gel formulations. Addition of sodium cholate to lecithin resulted in enhancing the amount of diclofenac sodium diffused, (for liposomal suspension F2 > F1, and for liposomal gel F6 > F5).

Table 3: Liposome size and encapsulation efficiency.

Preparation	Size, mean ± SD (nm)	Polydispersity	Encapsulation efficiency %
F1	600 ±100	0.25	65 ± 5
F2	550 ± 50	0.25	60 ± 3
F3	150 ± 30	0.10	75 ± 4
F4	120 ± 20	0.10	70 ± 5

Table 4: Lag time, cumulative amount of diclofenac sodium and flux values for different diclofenac topical preparations.

Formula	Lag time Mean ± SD (h)	Cumulative amount of drug diffused mean± SD (µg/cm^2)	Flux of drug Mean± SD (µg//h.cm^2)
F1	1.811 ± 0.100	8.20 ± 0.25	1.10 ± 0.20
F2	1.675 ± 0.150	17.50 ± 0.50	2.20 ± 0.15
F3	1.755 ± 0.110	10.10 ± 1.10	1.26 ± 0.25
F4	1.360 ± 0.120	19.31 ± 1.50	2.41 ± 0.32
F5	1.955 ± 0.150	7.15 ± 0.30	0.89 ± 0.11
F6	1.740 ± 0.110	9.52 ± 0.25	1.19 ± 0.20
F7	1.856 ± 0.120	8.51 ± 0.31	1.05 ± 0.12
F8	1.675 ± 0.100	12.20 ± 0.50	1.52 ± 0.25

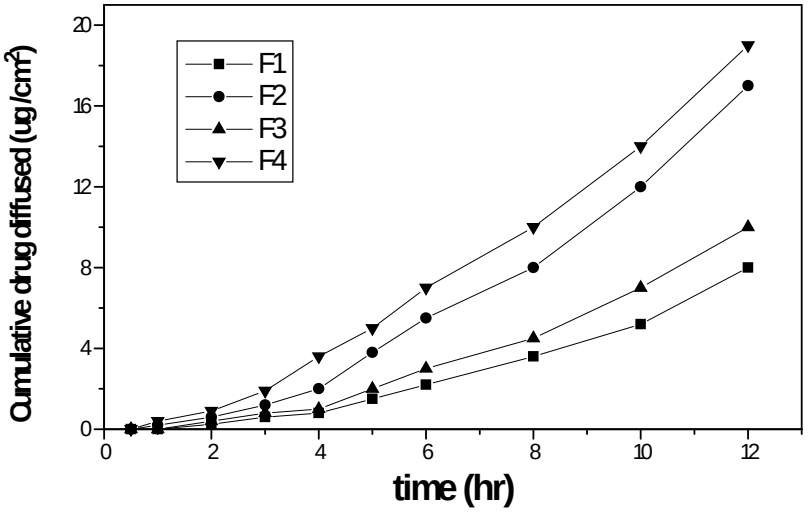

Fig. 1: Time course of the diffused amount of diclofenac sodium from different lecithin vesicles.

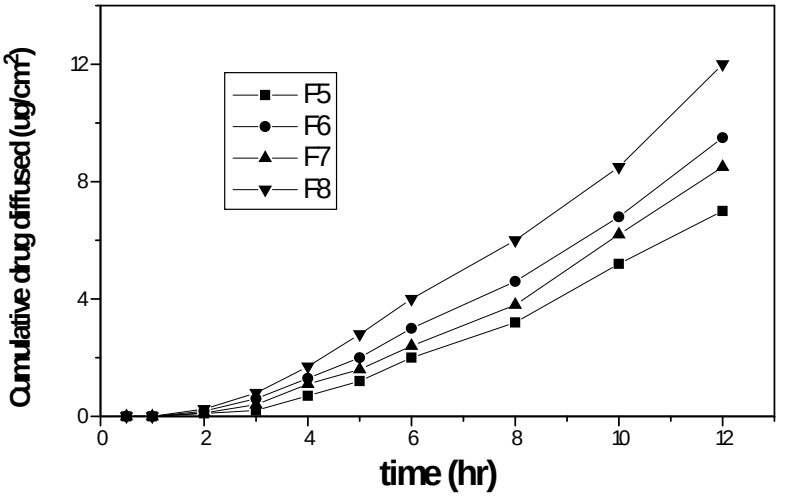

Fig. 2: Time course of the diffused amount of diclofenac sodium from different liposomal gel formulations.

Also addition of sodium cholate reduces the lag time either for liposomal vesicles or liposomal gel formulations (Table 3). The sonicated liposomal vesicles resulted in improving the diffusion of diclofenac sodium through rat skin either in the presence or absence of sodium cholate.

The effect of sodium cholate on the enhancing of diclofenac sodium permeation through rat skin is in agreement with EL Maghraby *et al.*[12] who reported that, cholate containing vehicles increased the transepidermal flux of oestradiol and reduced the time of maximum flux marginally compared with the saturated aqueous control. Also Cevc *et al.*[13] reported that, the presence of sodium cholate imparts flexibility to the bi-layer lipid membrane of lecithin vesicles, thus enabling them to pass through pores many folds smaller than the vesicle size spontaneously under the influence of trans-epidermal water activity gradient.

Anti-inflammatory activity studies using paw edema

Topical anti-inflammatory activity of semisolid preparations has been reported when applied 1 and 2 hours before carrageenan treatment as mentioned by Hiramatsu *et al.*[14]. Clinically, it seems more reasonable to apply the anti-inflammatory topical preparations after the inflammation stimulus. Table 5 illustrate the anti-inflammatory activity of liposomal gel formulations on the hind paw of the rats. It is shown that, all the liposomal gel formulations have significant effect ($p < 0.05$) as anti-inflammatory vehicle and this effect was in the following order F8 > F6 >

Table 5: Anti-inflammatory activity of diclofenac sodium using different liposomal gel formulations.

Rat group no	Formulation	% Swelling of induced edema after				
		2 h	3 h	4 h	5 h	6 h
1	Control	100±0.5⋆	99.0±0.5	98.0±1.0	99.0±0.6	100±0.5
2	F5	70.0±0.5 (30.3)	68.1±0.6 (31.2)	60.2±0.5 (38.6)	50.1±0.4 (49.3)	40.5±0.3 (59.5)
3	F6	55.1±0.4 (45.9)	50.5±2.0 (48.9)	45.2±1.5 (54.2)	40.3±1.2 (58.9)	30.5±1.1 (69.5)
4	F7	70.2±0.8 (29.8)	65.3±0.5 (34.1)	60.2±0.7 (38.5)	50.1±0.5 (49.4)	40.4±1.5 (59.6)
5	F8	50.2±1.5 (49.8)	40.2±2.0 (59.4)	35.5±1.2 (64.5)	30.3±0.9 (69.5)	20.5±2.5 (79.5)

⋆ Mean ± SD

- The value between parentheses indicates the % inhibition of edema.

F7 > F5. This order is similar to that appear in the permeation of drug through rat skin. This means, that there is a correlation between the amount of drug diffused from the liposomal gel formulations and the anti-inflammatory activity.

Conclusions

This study demonstrated that, the presence of sodium cholate either in lecithin vesicles or liposomal gel shows enhancement of diclofenac sodium diffusion through skin rat and superior effect on anti-inflammatory activity of drug.

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