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Il Farmaco 58 (2003) 605–611
IL FARMACO
www.elsevier.com/locate/farmac

In vitro release studies of chlorpheniramine maleate from gels prepared by different cellulose derivatives

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Received 28 February 2003; accepted 12 April 2003

Abstract

The objective of this study was to evaluate the in vitro and ex vivo percutaneous absorption of chlorpheniramine maleate (CPM) from different hydrogel formulations. Various concentrations of polymers, including hydroxypropylmethylcellulose (HPMC), sodium carboxymethylcellulose (NaCMC) and methyl cellulose (MC) were used in the hydrogel formulations. All experiments were conducted using cellulose dialysis membrane. The passive permeation of CPM was affected by the polymer concentrations. The effect of each polymer on the release rate of CPM was found to be statistically different ($P < 0.05$). The formulation which exhibited maximum drug release through cellulose membrane was then used with other membranes namely polyurethane membrane, rat skin and human skin. The release rate of CPM from different membranes was found to be statistically different ($P < 0.05$). Within the different diffusional barriers rat skin was found to be best alternative to human skin. It seems suitable the use of cellulose derivatives for topical application of CPM to obtain high therapeutic concentration at the application site. The synthetic membranes can be used to assess product performance in quality assurance but give little indication of its performance ex vivo.

Keywords: Chlorpheniramine maleate; Cellulose derivatives; Percutaneous absorption; Gel; Human skin

1. Introduction

The delivery of drugs into and through the skin is recognized as an effective means of therapy for local dermatologic and systemic disease. In recent years, transdermal delivery of drugs for systemic and local effect has gained considerable attention, because they eliminate first-pass effect, provide sustained plasma levels and improve patient compliance [1].

The antihistaminic CPM is a typically cationic amphiphilic drug characterized by the hydrophobic ring structure of the molecule and the hydrophilic side chain with a charged cationic amino group. CPM is used for topical ointments, especially for skin disorders such as sunburn, urticaria, angioedema, pruritus and insect bites [2,3]. It has the known side effects of all the antihistamines when it is given orally. The most common ones are sedation varying from slight drowsiness to deep sleep, dizziness, muscular weakness and gastrointestinal disturbances. Due to the first past effect only 25–45% of the orally administered dose reaches the blood circulation. In order to bypass these disadvantages the gel formulations have been proposed as topical application. Because gel base formulations make the drug molecules more easily remove from the system than cream and ointment ones [4,5]. Over the last decade hydrogels formed from natural, semisynthetic or synthetic polymers have been confirmed as vehicles for different types of pharmaceutical applications. They have good viscosity, satisfactory bioadhesion, and are without irritating or sensitizing actions [6]. High molecular grades of several commercial polymers derived from cellulose can be used in the formation of viscid, jelly-like aqueous solutions. These include methyl cellulose, sodium carboxymethylcellulose and hydroxypropylmethylcellulose. These are water soluble derivatives of cellulose and have been used as ointment bases so called 'hydrogel bases'. Generally, hydrogel bases can be easily washed out and well adhered to mucous mem-

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0014-827X/03/$ - see front matter
doi:10.1016/S0014-827X(03)00080-6

brane or skin, wet with secreting fluid and thus these are applied to injured skin and also to eyes [7,8].

Percutaneos penetration, that is, the passage through the skin, involves the dissolution of a drug in a vehicle, diffusion of the solubilized drug from the vehicle to the surface of the skin, and the penetration of the drug through the layers of the skin, mainly the stratum corneum. This penetration may be improved by selecting the appropriate vehicle [9].

Researches up to day, have been mostly focused on preparation of the same polymers with different concentrations and their in vitro release of the active substance from the formulations [10,11]. However studies investigating the active substance release from the formulations prepared using the same polymer different viscosity derivatives are rare in the literature. Therefore, in the present study we used different viscosity derivatives of the same polymers.

In this study, in order to evaluate the feasibility of percutaneous of CPM, we evaluated the in vitro and ex vivo permeation studies through synthetic and natural diffusion barriers. Furthermore, we assessed to which extent the vehicle composition can affect the drug release from gel formulations and we also wanted to choose the best alternative for human skin.

2. Materials and methods

2.1. Materials

The following materials were used as received: CPM, carboxymethylcellulose sodium (high viscosity, medium viscosity and low viscosity), hydroxypropylmethylcellulose (50, 100 and 4000 cps), methyl cellulose (25 and 4000 cps) (Sigma Chemical Company, USA), cellulose membrane (Travenol Lab. Inc., USA), polyurethane membrane (Omiderm, Omicron Scientific Ltd. Israel).

2.2. Preparation of CPM gels

All the cellulose derivatives were used at the percentages of 2–6–10. Hydrophilic cellulose derivative gel base was taken in a 100-ml beaker and wetted by water for 24 h [12]. CPM and thimerosal were dissolved in some water and this solution was added little by little to the wetted gel base and mixed (stirrer, Heidolph SO 111, Germany) well. Prepared formulations are given in Table 1.

2.3. Stability studies

Hydrogels were stored in glass containers, well stoppered, for 6 months at room temperature (23 ± 1 °C) and in the dark. They were checked after preparation and throughout a 6-month period with a

Table 1
Composition of the formulations (w/w%)

Code of Form.	HPMC 50 cPs	HPMC 100 cPs	HPMC 4000 cPs	CMCNa L	CMCNa M	CMCNa H	MC 25 cPs	MC 4000 cPs	TMS	CPM	Distilled water q.s.
F1	10								0.01	1	100
F2	6								0.01	1	100
F3		6							0.01	1	100
F4			2						0.01	1	100
F5				10					0.01	1	100
F6					6				0.01	1	100
F7						2			0.01	1	100
F8							6		0.01	1	100
F9								2	0.01	1	100

HPMC, hydroxypropylmethylcellulose; CMCNa, carboxymethylcellulose sodium salt, MC, methylcellulose; TMS, Thimerosal; L, low viscosity; M, medium viscosity; H, high viscosity; q.s., quantum sufficit.

bimonthly frequency. Physical evaluation of the samples' stability was carried out by visual inspection and rheological tests. Chemical stability was evaluated by pH measurements and spectrophotometric analysis of the drug content.

2.3.1. Viscosity measurements

A viscosimeter (Brookfield digital viscosimeter DV II RVTDV-II USA) was used to measure the viscosities (in cps) of the gels. The spindle (numbers of 2, 3, 7, TF 96) was rotated at 10 rpm. Samples of the gels were to settle over 30 min at the assay temperature (25 ± 1 °C) before the measurements were taken.

2.3.2. pH measurements

The pH was measured in each gel, using a pH meter (Philips PW 9422, UK), which was calibrated before each use with buffered solutions at pH 4, 7 and 10.

2.3.3. Drug content studies

Drug content of the gels was determined by dissolving an accurately weighed quantity of gel (about 100 mg) in about 50 ml of pH 6.0 phosphate buffer saline. These solutions were quantitatively transferred to volumetric flasks and appropriate dilutions were made with the same buffer solution. The resulting solutions were then filtered 0.45 μm membrane filters [13] before subjecting the solution to spectrophotometric analysis for chlorpheniramine maleate at 261 nm.

2.4. In vitro release studies

CPM release rates from different gels were measured through cellulose asetate dialysis membrane (Travenol Lab. Inc., USA) employing three improved Franz diffusion cells (Ildam, Turkey) with a diffusional area of 1.86 cm^2 and a receiver compartment volume of 14 ml. The receptor compartment contained pH 6.0 phosphate buffer saline, to allow the establishment of the 'sink condition' and to sustain permeant solubilization, was stirred and thermostated at 37 ± 0.5 °C (constant temperature thermostatic water bath and circulators, GCA Precision Scientific, USA) during the experiments. 1 g of each gel formulations was placed on the diffusion barrier in the donor compartment and the latter was covered with parafilm. At appropriate time intervals samples (300 μl) of receiving solution were withdrawn and replaced with fresh solution. This dilution of the receiver content was taken into account when evaluating the penetration data. The samples were analyzed spectrophotometrically (UV spectrophotometer, Schimadzu 2100 S, Japan) at a wavelength of 261 nm and the concentration of chlorpheniramine maleate in each sample was determined from a previously calculated, standart curve. The total amount of CPM penetrating through the unit membrane surface and diffusing into the receptor medium was calculated and plotted as a function of time. The linear portion of the plot was estimated as steady-state flux (J_{ss}). The permeability coefficient (K_p) was calculated as $K_p = J_{ss}/c_v$, where c_v is the total donor concentration of the gel [14]. Statistical comparisons were made using one way ANOVA and Kruskal Wallis varyans analysis. The level of significance was taken as $P < 0.05$. Each data point represents the average of three determinations. In vitro release studies have been observed for a 4-h period.

2.5. Different diffusion barriers and ex vivo permeation studies

The sample with optimum drug release through the cellulose membrane was further studied with different diffusion barriers. For this aim we preferred the polyurethane membrane because its usage of synthetic skin for burns. As a natural membrane rat skin was chosen since it appears to be as good as pig skin in modeling absorption through human tissue [15]. All animal procedures were carried out under approved institutional protocols. Rat skin and human skin were obtained as follows.

Male Sprague–Dowley rats weighing 180–200 g, were anesthetized with intraperitoneal injection of ketamine HCl and their hairs were removed with an electric clipper. For regenerating of the damaged skin we have waited 1 week. At the end of this period the rats again anesthetized with the same method and their skin was carefully excised. The adhering fat and debris were carefully removed from the skin samples.

Samples of adult human skin were obtained from breast reduction operations. Subcutaneous fat was carefully trimmed and the skin was immersed in distilled water at 60 °C for 2 min after which stratum corneum and epidermis were removed from the dermis by blunt dissection [15].

The skin samples were then soaked in normal saline solution and waited in the deep freeze until used in the diffusion studies.

The skin samples were mounted on the receptor compartment with the stratum corneum side facing upward into the donor compartment and the dermal side facing downward into the receptor compartment. In vitro release studies with the excised rat and human skin lasted for 24 h.

3. Results and discussion

3.1. Stability studies

Cellulose derivatives gels presented good stability. Therefore no macroscopical physical changes were observed during storage. Viscosity, pH and drug content

values of the formulations were carried out 6 months showed no significant difference (data not shown).

3.2. In vitro release studies

Because the absorption process of a drug is conditioned by the nature of the vehicle and other components of the formulation by effecting the release rate or by influencing the partitioning behaviour of the drug between vehicle and the skin. CPM diffusion through the different gels was examined using cellulose dialysis membrane.

The active substance released from the formulations decreases as the polymer concentration increases when a semi-solid formulation in a unique type polymer with different concentrations is used. It is possible that at the higher polymer concentrations the active substance is trapped in smaller polymers and it is structured by its close proximity to that polymer molecules. This increases the diffusional resistance by more than expected. Also, the density of chain structure which has been observed in gels' microstructure increases at the higher polymer concentration and this limits the active substance's movement area [10,11,16,17]. In our study we have found the same results as shown in Table 2 for the formulations F1 and F2. The steady-state flux and permeability coefficient values were also found lower for the formulation in which polymer concentration was kept high (Table 2). In addition, generally as polymer concentration increases, viscosity increases as well. The ability of a hydrogel system to serve as a reservoir for drug delivery is influenced by the macro and micro rheological properties of the matrix. Viscosity is the most widely utilized reference for the characterization of polymer structure, although it is not sufficiently comprehensive for the full determination of hydrogel strength [18]. Viscosity is negatively related to release of active substance from formulation and its penetration through the diffusion barriers. This decrease in the release could be attributed to increased microviscosity of the gel by increasing polymer concentration. Thus, both high concentration of polymer and high viscosity complete each other in decreasing the release of active substance release from the formulation [19,20].

However, there is a dilemma in choosing the proper polymer concentrations when the same type polymer with different viscosity derivatives,

1) High polymer concentration with low viscosity
2) Low polymer concentration with high viscosity

In this case, the question is which formulation would yield better release of the active substance. Also another question is whether polymer concentration or viscosity would be more effective in releasing the active substance. F2, F4 and F5, F6, F7 formulations fit the case above. Optimum results were observed in formulations of F4 and F7 with low polymer concentration in spite of their high viscosity values (Table 2, $P < 0.05$). In this case high viscosity derivative for the same polymer with different viscosities should be preferred. Because semi-solid dosage forms of low concentration of these ones can be prepared. The J_{ss} and pK_a values were found higher at the usage of low polymer concentration (Table 2, $P < 0.05$). Thus polymer concentration of a formulation is more important than viscosity value when we use the same type polymer with different viscosity derivatives.

The formulations prepared with two different viscosity derivatives of methyl cellulose exhibited the expected results that lower polymer concentration and viscosity values resulted in higher drug release, J_{ss} and pK_a values (Table 2, $P > 0.05$). This result indicates that the viscosity of hydrogels plays an important role in controlling the release of the drug if the diffusion of drug through the polymer matrix is a rate determining steps [18].

The drug release characteristic of all the formulations were found to be different when compared with each other (Fig. 1, $P < 0.05$) CMCNa formulations exhibited

Table 2
Polymer concentration, viscosity and drug release parameters (by using cellulose dialysis membrane at the end of a 4-h period) of the formulations. Each reading is an average of three determinations

Code of formulation	Polymer con. (w/w%)	Viscosity (10^3 cPs) ± SD	Spindle number	Amount of drug released (mg/cm^2)	J_{ss} ± SD (mg/cm^2/h)	pK_a ± SD (cm/h)
F1	10	9.6 ± 0.00013	7	2.2308 ± 0.1857	0.3408 ± 0.0138	0.03408 ± 0.0013
F2	6	1.28 ± 0.00821	4	2.6294 ± 0.1362	0.8560 ± 0.0164	0.08560 ± 0.0016
F3	6	5.50 ± 0.00019	6	2.4860 ± 0.0934	0.9018 ± 0.0231	0.09018 ± 0.0023
F4	2	4.84 ± 0.00012	5	2.6453 ± 0.1952	0.9594 ± 0.0174	0.09594 ± 0.0017
F5	10	15.6 ± 0.00041	7	1.6482 ± 0.1869	0.5691 ± 0.0234	0.05691 ± 0.0023
F6	6	13.8 ± 0.00011	TF 96	1.6845 ± 0.1651	0.5708 ± 0.0193	0.05708 ± 0.0019
F7	2	33.6 ± 0.00051	7	2.2630 ± 0.1457	0.8867 ± 0.0157	0.08867 ± 0.0015
F8	6	8.40 ± 0.00196	7	2.4613 ± 0.1632	0.6913 ± 0.0201	0.06913 ± 0.0020
F9	2	2.56 ± 0.00213	TB 92	2.5418 ± 0.1321	0.8966 ± 0.0189	0.08966 ± 0.0018

J_{ss}, steady-state flux; pK_a, permeability coefficient; SD, standard deviation.

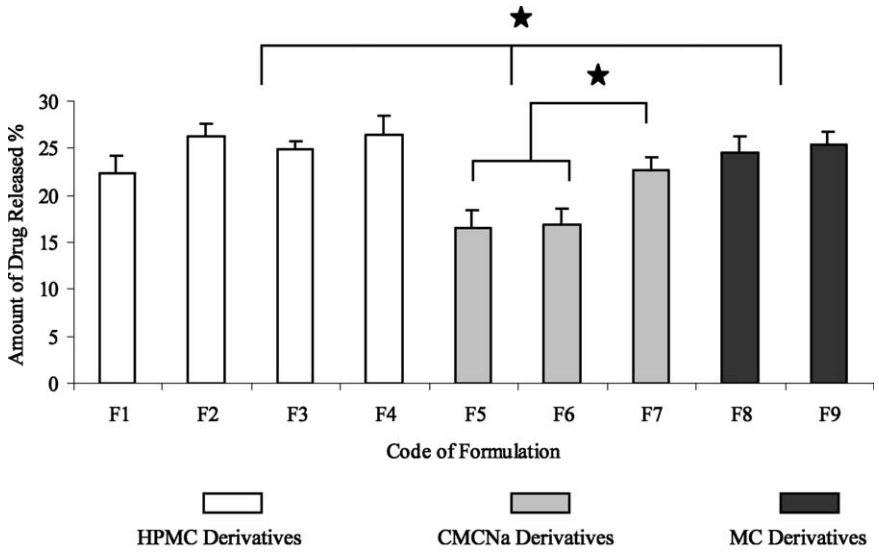

Fig. 1. Cumulative amount of drug released (%) from the tested formulations at the end of the 4-h period by using cellulose dialysis membrane (★ statistically different, $P < 0.05$)

lower active substance release ($P < 0.05$) when compared with the other cellulose derivatives. This can be explained with their higher viscosity values (Table 2) and drug polymer interactions may change the CPM partition to diffusion barrier thus changing the permeation of the drug [21].

3.3. Different diffusion barriers and ex vivo permeation studies

The F4 coded formulation which exhibited maximum drug release through the cellulose membrane was further studied for the drug release by using polyurethane membrane, rat skin and human skin. Of all the different diffusion barriers polyurethane membrane was found to be the most permeable to the active substance (Table 3, $P < 0.05$). This can be explained with its hydrophilic nature as the active substance and its thinnest structure (about 20 μm). J_{ss} and pK_a values was found as too high as compared with the other membranes (Table 3, $P < 0.05$). Thus polyurethane membrane wasn't an ideal membrane for mimicking the human skin although it can be used as synthetic human skin in burns [22].

Permeation studies with excised rat skin and human skin were performed to see if they confirmed the results in artificial membranes. The permeation of CPM from natural membranes was lower than those seen with artificial membranes (Table 3, $P < 0.05$). It is known that excised rat skin has generally been considered more permeable than human skin [15]. As expected the flux through rat skin was higher than that through human skin. The reason of this difference is that the thickness of the stratum corneum varies from species to species and the skin rodent lacks the sweat glands and abounds in hair on hair follicles which is the important pathway for many drugs penetrated through skin barrier [23]. CPM has ionic structure and with the general acceptance ionized materials penetrate the skin poorly. Therefore, there is a possibility that the ionized species could rapidly penetrate through the follicles and the aqueous layer of intercellular spaces of the stratum corneum [24–26]. On the other hand the usage of rat skin gave the closest results to the human skin when compared with the other membranes (Table 3, $P < 0.05$). From this point of view we can conclude that the usage of natural membranes such as rat skin was essential for estimating the real drug release characteristics.

Table 3
Amount of drug released by using different diffusion barries (all values are statistically different $P < 0.05$ when compared with each other). Each reading is an average of three determinations

Code of formulation	Diffusion barrier	Amount of drug released (mg/cm^2)	$J_{ss} \pm$SD (mg/cm^2/h)	p$K_a \pm$SD (cm/h)
F4	cellulose membrane	2.6453 ± 0.1952	0.9594 ± 0.0174	0.09594 ± 0.0017
F4	polyurethane membrane	3.5238 ± 0.4427	1.1570 ± 0.1240	0.1157 ± 0.0037
F4	rat skin	1.5047 ± 0.2786	0.1338 ± 0.0234	0.0134 ± 0.0042
F4	human skin	0.6915 ± 0.1561	0.0736 ± 0.0094	0.0074 ± 0.0019

J_{ss}, steady-state flux; pK_a, permeability coefficient; SD, standard deviation.

Table 4
Kinetic parameters of the active substance calculated from the in vitro data by using cellulose dialysis membrane

Code of Formulation	1st-order kinetic			0-order kinetic			Q vt kinetic		
	k_0	r^2	SWSD	k_r	r^2	SWSD	K	r^2	SWSD
F1	0.4432	0.9820	0.2029	5.1193	0.9851	0.1718	5.3224	0.9828	0.1431
F2	0.5141	0.9802	0.2664	6.0955	0.9818	0.2168	0.0713	0.9780	0.2266
F3	0.4996	0.9827	0.3223	5.9130	0.9893	0.2729	7.3067	0.9932	0.9423
F4	0.5592	0.9615	0.2928	6.3524	0.9738	0.2375	8.2190	0.9951	0.1807
F5	0.3590	0.9823	0.1519	3.9402	0.9781	0.1112	2.1509	0.9444	0.3624
F6	0.3523	0.9866	0.1049	3.9210	0.9905	0.9155	3.1616	0.9931	0.8356
F7	0.4750	0.9631	0.3061	5.5496	0.9737	0.2661	0.0666	0.9973	0.7260
F8	0.4918	0.9730	0.2654	5.8316	0.9809	0.3252	7.5132	0.9871	0.2195
F9	0.5332	0.9358	0.3050	6.2868	0.9509	0.2572	7.6877	0.9866	0.2271

SWSD ,sum of weight of squared deviation; r^2, determination coefficient; k_0, first-order rate constant; k_r, zero-order rate constant; K, Qvt kinetic rate constant

Pharmaceutical product Polaramin Crema®, which contains CPM as a ratio of 1 was studied for drug release characteristic by Ceschel at all using porcine skin throughout 24 h [2]. The amount released of CPM was found to be as 0.0131 mg at the end of the experiment. This value is less than our datas obtained with human and rat skin (Table 3). At the same study the solution formulations of CPM was prepared at the same ratio and subjected to drug release. For different solution formulations CPM released amount were found as 2.149, 2.782 and 1.764 mg. In another study CPM solution as 11.7 mg per ml (aproximately 1%) was subjected drug release by using hairless mouse skin [27]. The amount of CPM released at the end of 24 h was found to be 1.87 mg. These values are higher than our data (Table 3). When we looked at these experiment results we can conclude that the release of CPM from the formulations increases as follows: cream < gel < solution. This can be attributed that solution formulations make the active substance move more easily from the cream and gel ones. By other means low viscosity value of the formulation increases drug release [4,20].

In order to develop an ideal kinetic model to interpret diffusion rate data in terms of meaningful parameters, various kinetic models were applied to obtain the best fit of the data. The diffusion rate data were treated with first-order, zero-order and Qvt kinetic fashions. It has been found that generally the releases have been realized in accordance with Qvt kinetic (Table 4). Besides the fact that it is emitted in in accordance with first and zero-order kinetics shows that the agent is distributed homogenously in the formulations. According to model developed by Higuchi this harmony among kinetics is seen in case of homogenous distribution of the active agent in the formulation [28].

In conclusion, percutaneous absorption of CPM across the skin appears to be achievable through topical application with the gel bases of cellulose derivatives. Thus some side affects especially seen in the use of oral applications can be easily eliminated and desired therapeutic concentration at the application site achieved. The type and concentration of the polymer in the formulation have remarkable effect on the permeation of CPM. By working with the same polymer at different viscosity derivatives the use of low polymer concentration in spite of its higher viscosity value gives the maximum drug release profile. No correlation was found between transfer through the synthetic membranes and the natural ones. The synthetic membranes can be used for assessing product performance in quality assurance but give little indication of its performance ex vivo. Natural membranes such as rat skin must be used to obtain healthy results compared to human one. Chlorpheniramine maleate seems a suitable drug entity for developing diadermatic dosage forms.

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