International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.1, pp 863-869, Jan-Mar 2010

Development of New Topical Formulations of Diphenhydramine Hydrochloride: *In Vitro* Diffusion and *In Vivo* Preliminary Studies

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Abstract: The aim of this study was to evaluate the vehicle effect on *in vitro* diffusion of Diphenhydramine hydrochloride (DPH) from new five topical formulations: microemulsion (A), microemulsion+silica (B), Na Alginate emulgel (C), Carbopol cream (D) and hydroxyethylcellulose gel (E).Furthermore, the skin irritation potential and the formulation effect on skin reaction induced by histamine were investigated *in vivo*. The commercial cream of DPH (Allergan®) was used as reference formulation.The diffusion rate values showed the rank order E > A > B > C > D > Control, and all prepared formulations are able to improve the diffusion of drug compared with commercial cream; among the tested formulations, the hydroxyethylcellulose gel and microemulsions appear to be the most efficient vehicles in promoting the release of DPH.After *in vivo* application, the formulations do not produce skin irritation and determine a reduction of the response induced by histamine suggesting that their potential use as alternative topical dosage forms for effective local antihistaminic therapy.
Keywords: Diphenhydramine hydrochloride, diffusion studies; skin test.

INTRODUCTION

Diphenhydramine hydrochloride (DPH), a histamine H_1-receptor antagonist, is widely used as antiallergic, antiemetic and antitussive drug in many pharmaceutical preparations. It is usually administered orally and may be used by intramuscular or intravenous injection in severe allergies and applied topically for local allergic reactions [1,2].

Topical formulations are often used for relief of itching due to insect bites, mild cases of sunburn, poison ivy or oak, and other minor skin irritations [3].

Currently, DPH is available on the market in few topical dosage forms, containing 2% of drug and its percutaneous absorption was scantily investigated.

In developing novel drug preparations for topical delivery through the skin, the choice of vehicle formulations for a given drug can greatly influence the rate and extent of drug permeation across the skin[4].

The *in vitro* methodologies for the testing of skin permeability are extensively established and validated [5,6]. Skin from a wide range of animals including pigs [7], rats [8], guinea pigs, monkeys, snakes[9] and others has been suggested as a suitable replacement for human skin, since they offer similar barriers to diffusion for the penetration through human skin of molecules [10,11].

Where human or animal skin is difficult to obtain, or where a large number of experiments are to be carried out, particularly with regard to pre-formulation screening experiments, artificial membranes have been widely employed[12].

Synthetic membranes selected for use in *in vitro* diffusion experiments should usually be commercially

available; these type of membranes have little capacity to bind to the drug and also have little tendency to interact with the releasing medium thus offering the least possible diffusional resistance[13].

For design of topical formulations it is important to considerate also the physicochemical properties of the vehicles and their potential to interact with both permeant and membrane[14].

The aim of this work was to prepare novel topical formulations containing Diphenhydramine hydrochloride using five different vehicles: microemulsion (A), microemulsion+silica (B), Na Alginate emulgel (C), Carbopol cream (D) and HEC gel (E) and to compare them with a commercial available formulation (Allergan® cream).

The prepared formulations were characterized in terms of physical examination and rheological properties. In order to check the influence of vehicle compositions on the release rates of Diphenhydramine hydrochloride, *in vitro* release studies were carried out. In addition, the skin irritation potential and the effect of the formulations on cutaneous skin reactions induced by histamine were investigated by *in vivo* study.

MATERIALS AND METHODS

The following materials were used in this study: Diphenhydramine hydrochloride (DPH), glycerol, sodium alginate (Na Alginate), micronized silica, white vaselin, liquid paraffin, Polyethylene glycol 40 stearate, Carbopol 940/Acritamer 940, hydroxyethyl cellulose (HEC), from Cruciani Prodotti Crual Srl (Roma, Italy); isostearic Plurol®, Labrasol®, isostearyl isostearate, from Gattefossé (Saint-Priest, Cedex, France); stearyl alcohol, from Res Pharma (Vimodrone, Milano, Italy); sodium hydroxide solution from Merck (Darmstadt, Germany); EDTA from Carlo Erba Reagenti (Milano, Italy). Allergan® cream, marketed in Italy from Italiana Laboratori Bouty (Milano, Italy), was obtained from a local pharmacy.

Preparation of Formulations

Examined topical formulations were prepared as described and their composition are shown in Table 1.

Microemulsion (A): the oily phase was prepared by emulsifying isostearyl stearate and Plurol isostearate; then Labrasol®, water and DPH were added with gentle stirring to obtain the microemulsions.

Microemulsion added of micronized silica (B): the DPH was incorporated into microemulsion (A) and the micronized silica was added and mixed to uniformity.

Sodium Alginate emulgel (C): Sodium Alginate was dispersed in glycerol and the water was slowly added to give after 24 h the resulting gel. The DPH was incorporated in the microemulsion (A) (49.0 g) and the Sodium Alginate gel prepared (49.0 g) was added with gentle stirring to obtain the emulgel.

Carbopol cream (D): the EDTA and Polyethylene glycol stearate were dissolved in a dispersion of Carbopol 940 in purified water heated to 70 °C under stirring at a high speed; then the oily phase (vaselin, paraffin and Stearyl alcohol) previously melted at 60 °C was incorporated with continuous stirring. Sodium hydroxide was added and the mixture was stirred until cooled to room temperature. The DPH was dispersed into cream by stirring at the last stage.

Hydroxyethylcellulose (HEC) gel (E): HEC was dissolved in water under slow speed agitation to avoid air entrapment and after 3 h the DPH was incorporated.

One hundred grams of commercial Allergan® cream, used as reference formulation, contain Diphenhydramine hydrochloride 2.0 g, stearic acid 26.25 g, glycerin 6.0 g, lanolin 2.5 g, triethanolamine 1.8 g, and other additives and water to 100 g.

Characterization of Formulations

The prepared formulations were inspected visually for their homogeneity, consistency, spreadability, phase separation and pH and compared with the commercial formulation [15].

The viscosity of the different formulations was carried out using a rotational viscometer (Viscotester® VT 181, Haake, Karlsruhe, Germany) equipped with a sensor system E1000 (diameter 7.0 mm, length 17.7 mm). About 50 g of each formulation were transferred at 20 ± 1 °C in a measuring cylinder and viscosity of the formulations was performed at a rotational speed of 1 and 4. Under the same conditions, the viscosity of the control cream (Allergan®) was examined.

The average of three readings was used to calculate the viscosity.

In vitro Release Studies

The *in vitro* diffusion experiments were carried out using Franz-type modified diffusion cells apparatus with side-arm and external jacket Erweka HD/T6 (Erweka, GmbH-Heusenstamm, Germany). An exact amount of formulation (about 1.0 g) was spread out on PTFE membrane positioned between the donor and receptor chambers. The available diffusion area between cells was 0.694 cm^2. The receptor compartment was filled with phosphate buffer solution pH 7.4 (4.5 mL) and maintained at 32 ± 0.5 °C. A peristaltic pump was used to allow the complete mixing of the receiving phase during the test instead of magnetic stirring. At set time points (0.25, 0.5, 0.75, 1.0, 1.25, 1.5 h) an aliquot (1 mL) of the receptor medium was withdrawn and immediately replaced with an equal volume of the fresh buffer.

The amount of DPH diffused was determined by Hitachi-U–2001UV spectrophotometer (Hitachi Instruments, USA) at the wavelength of 258 nm [16].

Cumulative amounts of drug (□g) diffusing the surface (cm^2) were plotted against time (h). The in *vitro*

diffusion rate (μg/cm^2/h) was calculated from the slope of the linear plot of the cumulative amount diffused per unit area as a function of time, in the steady state region, according to the method described in the literature [17,18].

***In vivo* Preliminary Studies**

On the basis of the *in vitro* release results, the A, C and E formulations were selected for the *in vivo* preliminary studies.

The *in vivo* studies were carried out in accordance with the Italian law (D.L. n° 116/1992), which allows experiments on laboratory animals only after submission of a research project to the competent authorities, and in accordance to the "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985).

The experiments were performed on male albino Wistar rats weighing 150-160 g each (Harlan, Milano, Italy). The rats were maintained under controlled environmental conditions (temperature 22 ± 2 °C; humidity 50-55%; 12-h light-dark cycle). All animals received standard laboratory diet and water ad libitum.

The rats were anesthetized by intraperitoneal injection of urethane saline solution (25%, 3.0 mL/kg) and the dorsal hair was carefully removed using electric clippers.

Selected topical formulations (500 mg) were applied to the dorsal surface of the rats and spread until complete absorption. The rats (n = 3) were divided into different groups and treated as follows: Group I received the commercialized Allergan cream, Group II the microemulsion (A formulation), Group III the emulgel (C formulation) and Group IV the HEC gel (E formulation). The groups Ia, IIa, IIIa and IVa received the corresponding base formulations without drug, while the Control Group did not received any treatment. After 1 h of application of the selected formulations, the treated skin was examined visually for erythema and edema; then 50 □l aliquots of histamine hydrochloride solution (0.1 □g/mL in a solution of water/glycerol 50/50, v/v), was injected into the upper layers of the dorsal surface, using a diabetic insulin syringe.

The effect on cutaneous skin reactions induced by histamine were visually evaluated at different time intervals like 15, 30 and 60 minutes, comparing the responses between the different groups.

Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) (Origin®, version 7.0 SR0, OriginLab Corporation) and individual differences between formulations were evaluated using a non-parametric post hoc test (Tukey's test).The differences were considered to be statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

The prepared formulations show a smooth and homogeneous appearance and were easily spreadable with acceptable bioadhesion and fair mechanical properties.

The pH values of all the prepared formulations ranged from 6.3 to 6.8, which is considered acceptable to avoid the risk of irritation upon application to the skin [19].

It is known that the viscosity of topical formulations is an important physical parameter, which affects the rate of drug release; in general, an increase of viscosity vehicles would cause a more rigid structure and decrease the drug release rate [20,21].

To investigate the possible influence of vehicle rheological properties on DPH release, the viscosity of prepared formulations was evaluated and compared to reference cream.

The recorded viscosities of the different formulations at both low and high rotational speeds are showed in Table 2.

The data related to microemulsion (formulation A) were not reported because the viscosity was not detected due to high fluidity of formulation.

At the low speed, the viscosity of reference cream (Allergan®) results significantly higher ($P<0.001$) than that of B and D formulations, while is significantly lower with respect to C formulation. Furthermore, not significant differences ($P>0.05$) were found from the comparison with E formulation.

For all the prepared formulations the viscosity decreased of about 2.0-2.7 times with increasing the rotational speed. As the speed is increased, the normally disarranged molecules of the vehicle are caused to align their long axes in the direction of flow. Such orientation reduces the internal resistance of the material and hence decreases the viscosity [22].

At the higher rotational speed, the viscosity of Allergan cream results significantly different when compared to the other vehicles, with the exception of E formulation. The Sodium Alginate emulgel possess considerably higher viscosity, while the microemulsion+silica and the Carbopol cream show the lowest values. Sodium Alginate and HEC gels form a physically bonded network by formation of the junction zones, which are responsible for the mechanical strength of the gels and determine an increase of viscosity values.

The *in vitro* diffusion experiments were carried out in order to evaluate the influence of vehicle compositions on the release rates of Diphenhydramine hydrochloride.

Diffusion of the drug from topical formulations in the donor compartment through a semipermeable membranes involves three consecutive processes: the dissolution of the dispersed drug, the diffusion of the drug across the dissolution media or swollen polymer

matrix, and finally its permeation through the membrane. All three processes make a contribution to the overall diffusion rate [23].

For the topical formulations prepared, the tests were performed for 1.5 h and the commercial Allergan® cream was used as reference formulation.

Figure 1 shows the cumulative amounts of DPH diffused from formulations through membranes.

For all formulations the diffusion profiles obtained are very similar, and a good linear relationship is observed between the amount of DPH released and the time as confirmed from the excellent correlation coefficients ranging from 0.984 to 0.998. This result suggest that the diffusion of drug through formulations is an important mechanism controlling the whole diffusional process.

Examination of the diffusion parameters of DPH across membrane of each formulation, listed in Table 3, furthermore, indicates that DPH released from the prepared topical formulations results significantly enhanced when compared to the marketed Allergan® cream and decrease in the following order: E > A > B > C > D > Allergan. Significant differences are also found from the comparison of all formulations.

HEC hydrogel (formulation E) shows the greater release rate value that results about 5.0-fold higher than the reference cream. Furthermore, the cumulative amount of DPH released after 1.5 h resulted of 14.89±0.19 □g/cm^2 and 2.98±0.19 □g/cm^2 from hydrogel and Allergan®, respectively.

These significant variations in drug diffusion profiles may be explained based on the difference of vehicles characteristics: in fact, despite to lipophilic Allergan® cream, the hydrophilic characteristics of the HEC enhance in the diffusion of DPH through the membrane.

In the case of formulations A (microemulsion) and B (microemulsion+silica), the DPH release rate resulted about 3.5 and 2.6 fold higher compared to Allergan® cream. The higher diffusion of DPH from microemulsions is most probably due to high drug mobility in the vehicle, which promote a faster drug diffusion through the membrane [24]. This finding explains also the significant reduction ($P<0.05$) of drug release rate observed after addition of silica micronized to microemulsions. In fact, microemulsions provide all the possible requirements of a liquid system including thermodynamic stability, low viscosity with Newtonian behavior, and very small droplet size [25].

Similarly the hydrophilic properties of Na Alginate emulgel improve the drug permeation with respect to Allergan®.

The formulation D enhances up to 1.8 times the DPH release rate than control formulation, that can be explained based on the lower viscosity and higher hydrophilicity of Carbopol cream.

To investigate immediate type I hypersensitivity to allergens, skin testing is usually performed by using prick and intracutaneous techniques [26,27].

The tests consist on the introduction of allergen extract or histamine into the skin resulting in an IgE-mediated allergic response, characterized by an immediate wheal and flare reaction, due to the activation of mast cells releasing vasoactive agents, which cause both plasma extravasation and vasodilation [28,29]. Reactions are assessed by the degree of redness and swelling and the size of the wheal produced [30].

To evaluate the efficacy of tested formulations, the potential irritation and the effect of the formulations on cutaneous reactions induced by histamine injection on the dorsal surface of rats were estimated.

The visual examination of skin, 60 min after administration of developed formulations, shows that these do not produce any dermatological reaction (erythema or edema) on the site of application and therefore can be classified as a nonirritant to the skin (data not reported). Not relevant differences are observed between the Control and the various treated groups.

On the other hand, some differences were observed about the effect of the formulations on cutaneous reaction induced by histamine. The wheal and flare responses were approximately circular and so an average diameter of the response was used to calculate the area, expressed in mm^2. The observation recorded from untreated group and group treated with base formulations resulted very similar. The intensity of the wheal and flare responses is reduced by the pretreatment with Allergan®, and with prepared formulations. In fact, the wheal produced appears more superficial, with the size restricted and a degree of redness reduced than that observed in groups treated with the corresponding base formulations.

This finding might suggest that the concentration of DPH penetrated into skin from formulations is adequate to decrease the IgE-mediated response induced by histamine injection.

CONCLUSIONS

Results obtained on this study have shown that the topical delivery of DPH can be increased by appropriate vehicles. All the formulations tested are able to enhance the diffusion of drug compared to commercial cream. Among the vehicles studied, the HEC hydrophilic gel and the microemulsion demonstrated to be more effective formulations for DPH diffusion.

After topical administration, the developed formulations are well tolerated and seem to reduce the cutaneous reaction induced by histamine, indicating that can be regarded as successful vehicles for topical administration of DPH.

Table 1: Composition of the different formulations of DPH; microemulsion (A), microemulsion+Silica (B), Na Alginate emulgel (C), Carbopol cream (D) and HEC gel (E)

Ingredients (%w/w)	Formulations				
	A	**B**	**C**	**D**	**E**
Diphenhydramine hydrochloride	2	2	2	2	2
Glycerol			22		
Isostearyl isostearate	15.68	15	7.9		
Isostearic Plurol	20.09	19.3	10		
Labrasol	35.28	34	17.6		
Sodium alginate			4		
Micronized Silica		3.9			
Hydroxyethyl cellulose					2.94
White Vaseline				9.8	
Liquid paraffin				9.8	
Stearyl alcohol				7.8	
Polyethyleneglycol stearate				2.9	
Carbopol 940				0.5	
EDTA				0.1	
Sodium hydroxide solution (100 g/L)				1.15	
Purified water to	100	100	100	100	100

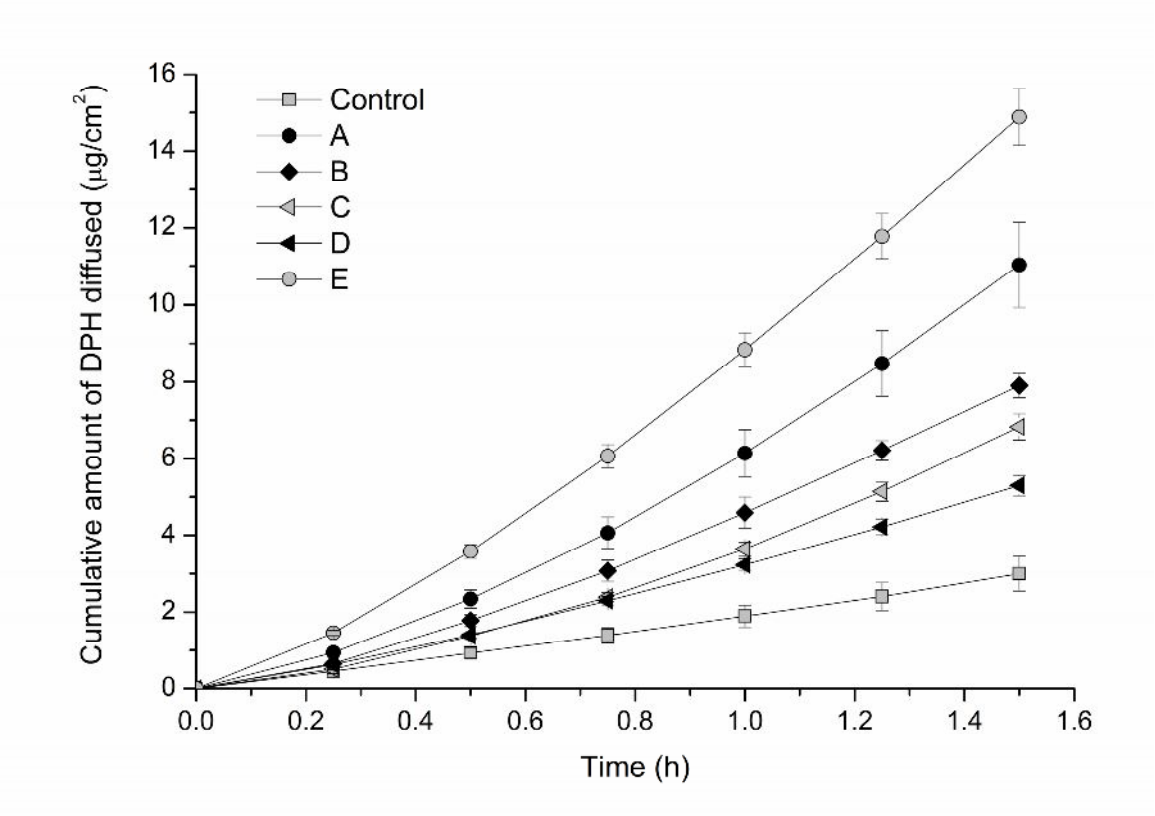
Table 2: Viscosity (in mPa*s) of DPH formulations measured at low and high rotation speeds (mean ± SD, n = 3)

Formulation	Viscosity (mPa*s)	
	Low speed	**High speed**
Control	7320±119	3670±200
B	3660±199	1370±198
C	10980±207	4130±207
D	1830±173	915±173
E	7322±305	3673±154

Table 3: Flux and cumulated amount of DPH released from different formulations (mean ± SD, n = 3)

Formulation	Flux ($mg/cm^2/h$)	Cumulative amount of DPH permeated after 1.5 h (mg/cm^2)
Control	2.02±0.12	2.98±0.19
A	8.11±0.13	11.03±0.26
B	5.84±0.14	7.91±0.19
C	5.09±0.24	6.83±0.39
D	3.74±0.12	5.30±0.40
E	10.81±0.33	14.89±0.19

Fig. 1. *In vitro* diffusion profiles of DPH from various formulations (mean ± SD; n = 3).



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