

## DEVELOPMENT AND PRE-CLINICAL STUDY OF ANTI-ALLERGIC CREAM CONTAINING DEXAMETHASONE AND CHLORPHENIRAMINE

**Short title:** Pre-clinical study of dexamethasone, chlorpheniramine anti-allergic cream

### ABSTRACT

**Objectives:** In this study, we aimed to develop and optimize the anti-allergic cream containing dexamethasone and chlorpheniramine using the design of experiments (DoE) method. The optimized product was investigated the physicochemical properties and *in vivo* therapeutic effects in rabbits.

**Materials and Methods:** The creams were formulated by the simple mixing process, which was optimized by the Design Expert software. The products were then evaluated the properties such as pH, skin diffusion profile, short-term stability, qualitative, and assay, using the newly validated UV-Vis spectrophotometry quantitative method. *In vivo* efficacy test in rabbits of the best products were compared with the marketed Phenergan<sup>®</sup> (promethazine 2%).

**Results:** The UV-Vis method to simultaneously determine the amount of both dexamethasone and chlorpheniramine was successfully developed and validated. Using DoE method, it was clear that the release profile of dexamethasone was depended on the amount of sodium lauryl sulfate, propylene glycol, and DMSO. Whereas, only DMSO affected the release pattern of chlorpheniramine. Also, the best formulation was optimized by the software. The product showed acceptable parameters in pH ( $5.7 \pm 0.1$ ), short-term stability in 10 days, skin diffusion profiles of  $20.47 \pm 1.25\%$  and  $4.92 \pm 0.42\%$ , after 40 minutes, for dexamethasone and chlorpheniramine, respectively. In addition, the product demonstrated no observable inflammatory response in experimental animals. Also, it illustrated 2-fold better anti-allergic efficacy than the marketed product (i.e., 27.2 compared to 43.4 minutes in the recovery time).

**Conclusion:** We were successful in developing and optimizing the anti-allergic cream containing dexamethasone and chlorpheniramine. The best product satisfied all required parameters. Interestingly, our product showed higher efficacy than the Phenergan<sup>®</sup>. These results can be a background for further study in clinical trials.

**Keywords:** Dexamethasone, chlorpheniramine, allergic, design of experiments, *in vivo* study.

## INTRODUCTION

Allergy, a common disorder caused by an exposure to allergens, followed by an immune system response, can be classified into many types such as allergic rhinitis (i.e., respiratory system), asthma, drug allergy, food allergy, general allergy (i.e., pollens as allergens), insect allergy, and skin allergy. Among them, skin allergy is one of the most popular types, especially in children. In 2010, in the U.S., 10% of Asian children, 12% of white children, and 17% of black children had skin allergies.<sup>1</sup> Moreover, in 2012, the lifetime incidence of urticaria (i.e., hives, allergic rash) worldwide excess 20% in human.<sup>2</sup>

The general treatments for skin allergy include anti-histamines (i.e., chlorpheniramine), glucocorticoids (i.e., dexamethasone), epinephrine (adrenaline), mast cell stabilizers (i.e., cromolyn), as well as anti-leukotriene agents (i.e., montelukast). The standard oral route of these medications encounters many unwanted side effects. For example, the use of chlorpheniramine could lead to constipation, dizziness, headache, nausea, loss of appetite, or rarely dyskinesias, tremors, tachycardia, diplopia, dysuria, and even deathly agranulocytosis.<sup>3-5</sup> Hence, suitable route (i.e., local administration) should be developed. Cream formulations have been investigated intensively in recent years for skin application. Their excellent skin compatibility, high stability in normal preservative conditions, as well as ease of processability, make creams become the “formulation of choice” for scientists.

The idea of drug combination has been proposed lately in order to not only reduce the dose of the individual therapeutic agents but also increase the efficacy due to the synergism between the active substances.<sup>6-8</sup> In allergy treatment, the combination of one anti-histamine, namely chlorpheniramine, and one glucocorticoid, such as dexamethasone, is often used. However, the fact that limited skin cream products containing both of these pharmaceutical agents available in the market are undeniable. To the best of our knowledge, only one combination, namely Dexalergin<sup>®</sup>, manufactured by IVAX (Argentina) is presented in the market. Also, it is worth to notice that Dexalergin<sup>®</sup> has neomycin sulfate, an antibacterial agent, along with chlorpheniramine and dexamethasone (<http://www.medicacione.com/?c=drug&s=dexalergin%20cream>). Hence, no “pure” combination of these two drugs is available.

Chlorpheniramine or chlorphenamine (figure 1, left), commonly marketed in the form of chlorpheniramine maleate, is the first generation of alkylamine compounds for anti-

histamine purpose. It also possesses anti-depressant and anti-anxiety properties, although not generally approved.<sup>9,10</sup> Chlorpheniramine primary mechanism of action is histamine receptor H<sub>1</sub> competitive antagonist, which consequently hinder the allergic response caused by histamine. On the other hand, dexamethasone (figure 1, right) is a steroid compound, which can inhibit the formation of inflammatory and allergic mediators such as histamine, prostaglandins, as well as leukotrienes.

With all mentioned reasons, we came up with an idea of development and pre-clinical study of anti-allergic cream containing dexamethasone and chlorpheniramine. The research was conducted by the first step of experimental design and optimization to select the best formulation, followed by quantitative method development and validation, characterization of the formulas (i.e., pH, skin diffusion profile, short-term stability, qualitative, and assay), and *in vivo* efficacy test in rabbits.

## **MATERIALS AND METHODS**

### *Materials*

Standard chlorpheniramine and dexamethasone were bought from the Institute of Drug Quality Control, Ho Chi Minh City, lot number QT021050809 and QT013060909, with the purity of 99.32% and 99.43%, respectively. The chlorpheniramine and dexamethasone ingredients were imported from India and China, lot number 1010149 and 100505, purity 98.7% and 99%, respectively. Cetylstearyl alcohol, lot number 10099, was bought from Singapore; sodium lauryl sulfate, lot number 23263, was purchased from Indonesia. Dimethyl sulfoxide (DMSO), propylene glycol, glycerol, liquid paraffin, methanol, benzene, ethanol, chloroform, and hydrochloric acid were imported from China, pharmaceutical grades. Phenergan<sup>®</sup> cream (promethazine 2%, Sanofi-Aventis) was bought from Ngoc Anh drugstore, Can Tho, Vietnam. Mature rabbits were supported by the Department of Pharmacology, Can Tho University of Medicine and Pharmacy. The ethical issue was approved by the ethics committee (No. CTU 2016-5-017), based on the Animal Care and Use Committee guideline of the same university.

### *Drug quantitation*

To determine the amount of drug either in the formulations or the release medium, we developed and validated a UV-Vis spectrophotoscopic method that can measure both chlorpheniramine and dexamethasone in a same condition. The samples were dissolved in methanol, at the wavelengths of 239 nm and 262 nm for dexamethasone and chlorpheniramine, respectively. All validation values, including specificity,

linearity, precision, and accuracy were determined under the utilization of UV-Vis spectrophotometer (Hitachi U2800, Japan).

#### *Formulation*

The formulations were produced following a simple mixing process. The oil phase, which composes of cetylstearyl alcohol and liquid paraffin, was heated to approximately 70°C. Then, it was mixed with the water phase, composes of water, glycerol, sodium lauryl sulfate at the same temperature, by a high-speed homogenizer (Ultra Turrax T-25, IKA, Germany) to make the cream base. Chlorpheniramine in water and dexamethasone in propylene glycol and DMSO were then added and mixed with the cream base. The final product had the concentrations of chlorpheniramine and dexamethasone of 1% w/w and 0.1% w/w, respectively.

#### *Experimental design*

Design-Expert software (version 10.0, Stat-Ease, Inc., Minnesota, U.S.A) was used to design and optimize the formulation. The response surface methodology with the linear function model was chosen for the development part. Three independent factors, including the amount (g) of sodium lauryl sulfate ( $X_1$ ), propylene glycol ( $X_2$ ), and DMSO ( $X_3$ ). The concentrations of two active compounds as well as other excipients such as cetylstearyl alcohol, liquid paraffin, and glycerol, were kept constant. Three factors were studied at five different levels ( $-\alpha$ ,  $-1$ ,  $0$ ,  $+1$ ,  $+\alpha$ ) using central composite design. The  $\alpha$  value of 1.68 was chosen to maintain the rotatability and orthogonality of the design. Two response variables were clarified, namely the percentage of *in vitro* release of dexamethasone ( $Y_1$ ) and chlorpheniramine ( $Y_2$ ) through the rat skin after 40 minutes. The predicted function can be defined as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3$$

where  $Y$  is the predicted response,  $X_1$ ,  $X_2$ ,  $X_3$  are the independent factors,  $b_0$  is the intercept, and  $b_1$ ,  $b_2$ ,  $b_3$  represent linear coefficients.

Analysis of variance (ANOVA) was used to calculate the significance of the model and p value of less than 0.05 was considered. The optimal formulation was also predicted from the software.

#### *Characterizations*

##### *Physical characteristics*

The appearance of the final product was evaluated by the naked eyes. The suitable formulation must possess a white soft creamy texture with no observable separation between the oil and the water phases. The formulation pH was determined as follows:

weigh 5 g cream and mix with 50 ml of distilled water for 5 minutes, and measure the pH by pH meter (Mettler Toledo, Switzerland) the filtrate after filtration of the mixture. The acceptable pH is in the range of 5.5 to 6.

#### *Short-term stability*

To test the stability of the formulation, acceleration study was conducted. The final product was kept at 40°C for ten days, with 2 hours of 50°C incubation each day. The criteria include the appearance, texture, color, and smell.

#### *Skin diffusion profile*

The end points of skin diffusion tests were evaluated by the Franz cell method. Briefly, 200 mg of the final product was weighted and applied onto the rat skin with an area of 3.14 cm<sup>2</sup> in the donor chamber. The acceptor chamber was filled with 18 ml of methanol and stirred with magnetic bars. The system was maintained at 37°C using a water bath. After 40 minutes, the amounts of chlorpheniramine and dexamethasone released into the acceptor chamber were measured by validated UV-Vis spectrophotometry method.

#### *Drug identification*

Thin layer chromatography (TLC) was used to identify both drugs (i.e., chlorpheniramine, dexamethasone) in the cream products. The reference (i.e., standard drugs) and the samples were dissolved and extracted, respectively, in methanol for 60 minutes. Ten µl of two samples, including the reference and the test, were applied onto the chromatography layers (Silica gel GF254, Merck, Inc., U.S.A). The mobile phase was composed of benzene – ethanol – NH<sub>4</sub>OH (85:15:1 v/v/v). The best components and ratio of the mobile phase were preliminary investigated based on the polarity of both compounds. The spots were visualized under UV light at the wavelength of 254 nm.

#### *Assay*

To determine the amount of drugs in the cream product, 1 g of the cream (equivalent to 10 mg of chlorpheniramine and 1 mg of dexamethasone) was weighed, extracted with methanol for 60 minutes, and filtered through 0.22 µm Millipore filters (Merck, Inc., U.S.A). The samples were then UV spectrophotometry measured using validated method with methanol as a blank sample. The percentage of drugs were calculated as follows:

$$\% \text{ Dexamethasone} = \frac{C_1 \times 434.5 \times 10^3}{1 \times 99\%} \times 100; \quad \% \text{ Chlorpheniramine} = \frac{C_2 \times 390.87 \times 10^3}{10 \times 98.7\%} \times 100$$

where  $C_1$ ,  $C_2$  are the concentration of dexamethasone and chlorpheniramine, respectively.

#### *In vivo tests*

##### *Inflammatory response*

Inflammatory responses of the cream products were evaluated using rabbits. Mature rabbits (weight  $\geq 2$  kg) were cultivated in normal conditions 5 days prior testing. For the duration of 4 hours, the cream samples (0.5 g) and the control (i.e., cream base) were applied onto the hair-free areas in the rabbits back, with an area of approximately 10 cm x 15 cm, at  $25 \pm 3^\circ\text{C}$ , 30-70% humidity, and light on-off interval of 12 hours. Then, the remaining creams were washed off, and the skin inflammation was observed at 1, 24, 48, and 72 hours afterward. A score, which range from 0-0.5 for no significant inflammatory response, to 3.5-4.0 for serious inflammatory response (i.e., redness, swelling, animal pain) was given for each corresponding reaction, compared with the control and the blank area (i.e., no treatment). The experiment was done in triplicate, and the degree of inflammatory reaction of the product was collected and averaged.

##### *Efficacy test*

The efficacy of the cream, in comparison with the marketed Phenergan<sup>®</sup> (promethazine 2%) was done similarly to the inflammatory response test. The chloroform-induced allergy model was used. Briefly, 1 ml of chloroform was applied, using soft tissues, onto the hair-free areas (6 squares, 2.5 cm x 2.5 cm) for 40 seconds. Then, samples include (a) negative control; (b) cream, 0.05 g; (c) cream, 0.1 g; (d) positive control; (e) Phenergan<sup>®</sup>, 0.05 g; (f) Phenergan<sup>®</sup>, 0.1 g, were treated (figure 2). The efficacy was observed for one hour and the time until the rabbit skin returned to normal (i.e., the recovery time), compared to the negative control, was recorded. The experiment was repeated ten times, with ten different rabbits, and the time average values were reported. The student's T-test was used to compare the significant differences between our products and the marketed Phenergan<sup>®</sup>.

## **RESULTS AND DISCUSSION**

#### *Drug quantitation*

The concentrations of both active substances were measured simultaneously in the same samples, by two different wavelengths, 239 nm for dexamethasone and 262 nm for chlorpheniramine. Due to the fact that these two substances have absorbance values at both wavelengths, and the range of two wavelengths is more than 10 nm (i.e., 23 nm), we calculated the concentrations based on the following equations:

$$A_{(\lambda_1)} = \varepsilon_{\lambda_1}^1 C_1 + \varepsilon_{\lambda_1}^2 C_2$$

$$A_{(\lambda_2)} = \varepsilon_{\lambda_2}^1 C_1 + \varepsilon_{\lambda_2}^2 C_2$$

where  $A_1$ ,  $A_2$  are the absorbance values at 239 nm and 262 nm;  $\varepsilon_{\lambda_1}^1, \varepsilon_{\lambda_1}^2, \varepsilon_{\lambda_2}^1, \varepsilon_{\lambda_2}^2$  are the molar absorptivity of the chlorpheniramine and dexamethasone at 239 nm and 262 nm, respectively;  $C_1$ ,  $C_2$  are the concentrations of chlorpheniramine and dexamethasone.

By calculating from the standard samples, the  $\varepsilon_{\lambda_1}^1, \varepsilon_{\lambda_1}^2, \varepsilon_{\lambda_2}^1, \varepsilon_{\lambda_2}^2$  values were determined at 4336.0, 16260.8, 5351.6, and 8608.7, respectively.

The specificity was confirmed by the perfect overlay of the standard and the test samples spectra (data not shown). Moreover, the ratio of absorbance values between the blank (i.e., methanol) and the test samples was less than 1% (i.e., 0.57% at 239 nm and 0.51% at 262 nm), indicating the sensitivity of the method.

The linearity of the method was also specified (figure 3). These two substances showed linearity in the range of 0-70 ppm for chlorpheniramine and 0-7 ppm for dexamethasone with coefficients of determination ( $R^2$ ) at their corresponding maximal absorption wavelengths of 0.9993 and 0.9981, respectively. The linearity of these two was also seen in the other wavelength (i.e., 239 nm for chlorpheniramine) (data not shown).

In the precision test, six independent samples contained both chlorpheniramine and dexamethasone were prepared and measured. The relative standard deviations (RSD) of 0.37% for chlorpheniramine and 1.59% for dexamethasone, which less than 2%, indicated the precision of the method. In addition, the recovery values of these two substances at the concentrations of 80%, 100%, and 120% compared to the standard ones, were in the range of 98-102%, which demonstrated the method accuracy. In summary, the quantitative method was validated and satisfied all required parameters.

#### *Experimental design*

From the Design-Expert software, a total of 20 formulations was proposed, formulated, and evaluated in term of the *in vitro* release (%) of dexamethasone ( $Y_1$ ) and chlorpheniramine ( $Y_2$ ) through the rat skin after 40 minutes. The optimal correlations are shown as follows:

$$Y_1 = 3.9325 + 0.3375X_1 + 0.5025X_2 + 0.71X_3 \quad (1)$$

$$Y_2 = 3.9325 + 0.30125X_3 \quad (2)$$

where  $X_1$ ,  $X_2$ ,  $X_3$  are the amount (g) of sodium lauryl sulfate, propylene glycol, and DMSO, respectively.

The regression model was tested by ANOVA. The high F values of 215.05 and 145.63 for equation (1) and (2), respectively, as well as the low p values of less than 0.0001 for both equations, indicating the statistical significance of the model. Moreover, the insignificances of the lack of fit values with  $p = 0.473$  and  $0.359$  clearly demonstrate that the model fitted well. These can confirm the reliability of this model.

From the equations, it is likely that the release profile of dexamethasone ( $Y_1$ ) was positive linearly depended on the amount of sodium lauryl sulfate, propylene glycol, and DMSO. Whereas, only DMSO affected the release pattern of chlorpheniramine ( $Y_2$ ) in a similar manner. This can be explained by the extremely low solubility of dexamethasone in water (0.1 mg/ml), which led to the help of surfactant (i.e., sodium lauryl sulfate) and organic solvents in its dissolution and penetration through the rat skin.<sup>11</sup> On the other hand, chlorpheniramine has a water solubility at 160 mg/ml, which is 1600 times higher than that of dexamethasone, needed only DMSO to pass through the same skin (<http://www.hmdb.ca/metabolites/HMDB01944>).

Optimal conditions were determined based on the correlations between these factors, with the desired limits as to maximize the percentage of drug penetrated through the rat skin after 40 minutes, as well as to keep the concentrations of surfactant and organic solvents at acceptable values. Based on the literature, the concentrations of sodium lauryl sulfate, propylene glycol, and DMSO in the skin cream should be less than 1%, 30%, and 50%, respectively, in order to prevent dermatitis and other kinds of skin damage.<sup>12-16</sup> The optimal formulation was then prepared and further studied.

### *Characterizations*

#### *Physical characteristics*

The appearance of the optimized product possessed a white soft creamy texture with no observable separation between the oil and the water phases. The formulation pH was  $5.7 \pm 0.1$ , which was within the acceptable pH range of 5.5 to 6.

#### *Short-term stability*

The acceleration test was conducted with the final products in 10 days. After this duration, all formulations exhibited excellent stability without any significant changes in term of appearance, texture, color, and smell.

#### *Skin diffusion profile*



Franz cells method was used to evaluate the diffusion profile of the product. Rat skin was used due to its reproducibility property. The optimal release profiles of dexamethasone and chlorpheniramine were  $20.47 \pm 1.25\%$  and  $4.92 \pm 0.42\%$  (mean  $\pm$  SD, n = 3), respectively, after 40 minutes of application. The relationship between the formulation ingredients and the skin diffusion (i.e., release) profiles of these two substances were optimized in the experimental design part.

#### *Drug identification*

TLC pictures of the two active substances, using benzene – ethanol –  $\text{NH}_4\text{OH}$  (85:15:1 v/v/v) as a mobile phase, are illustrated in figure 4. Clearly, the optimal cream product demonstrates a suitable separation of dexamethasone and chlorpheniramine, with no observable interference.

#### *Assay*

Following the procedure in the method section, the concentrations of dexamethasone and chlorpheniramine in the optimal product was  $100.6 \pm 0.84\%$  and  $99.9 \pm 0.37\%$  (mean  $\pm$  SD, n = 3), respectively. These results indicated that the final product satisfies the quantitative requirement for both substances.

#### *In vivo tests*

##### *Inflammatory response*

No significant inflammatory response was encountered under the experimental conditions stated in the method section. The final product demonstrated good compatibility with the rabbit skin, without any redness, swelling, or observable reactions, compared to the control groups. The average score for three independent tests was 0, which is in the range of “no significant inflammatory response” (i.e., 0-0.5). Hence, we could conclude that our product shows no irritation on the skin.

##### *Efficacy test*

Preliminary studies about the induce dose and time of chloroform to get the allergic response were conducted. We found out that 1 ml of chloroform in 40 seconds was the best option. The same dose at 60 seconds could induce irreversible ulceration, which is not a good choice for anti-allergic study. Moreover, the effects of chloroform were insignificantly different amongst the rabbit skin area (figure 5).

The efficacy of our cream, in comparison with the marketed Phenergan<sup>®</sup> (promethazine 2%) in reducing the allergic response in rabbits was evaluated, in term of the recovery time (i.e., the necessary time required for the rabbit skin to recover after chloroform exposure). The results are demonstrated in figure 6. In brief, similar

to Phenergan<sup>®</sup>, our cream was a dose-dependent product, with a significant 2-fold lower of the recovery time in the 0.1 g dose ( $27.2 \pm 1.42$  min) compared to the 0.05 g dose ( $55.8 \pm 1.54$  min) ( $p < 0.05$ ,  $n = 10$ ). Interestingly, our optimal product showed meaningful higher therapeutic effects (i.e., lower recovery time), than the marketed product Phenergan<sup>®</sup>, in both doses ( $p < 0.05$ ,  $n = 10$ ). This could be due to the synergistic effect of dexamethasone and chlorpheniramine. Last but not least, the use of combination product (i.e., more than 1 active substance in a product) might reduce the dose of each individual substance, and hence, hinder the unwanted effects.

## **CONCLUSION**

In summary, anti-allergic cream containing dexamethasone and chlorpheniramine was successfully prepared, optimized, characterized, as well as *in vitro* and *in vivo* evaluated. The optimal formulation was considered a novel formula, which balanced the efficacy and the toxicity of the product, regarding organic solvents such as propylene glycol and DMSO. Additionally, our product satisfied all of the evaluation parameters (i.e., physical properties, stability, skin diffusion profiles, drug identification, assay, toxicity, and efficacy). This product, which showed a potentially better therapeutic effect in comparison with the marketed Phenergan<sup>®</sup>, has been further developing and undergoing clinical trials in our laboratory. Our ideas and research works could be applied in other pharmaceutical products to enhance the development of inexpensive medicines for the developing countries.

## **ACKNOWLEDGEMENTS**

DT Pham wants to sincerely thank the Department of Pharmacology, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy, for supporting the animals and necessary help. The authors also appreciate the cooperation of senior pharmacy students.

## **CONFLICT OF INTEREST**

The authors declare no conflict of interest.