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EFFECT OF PENETRATION ENHANCERS ON THE PERMEABILITY OF KETOCONAZOLE GELS THROUGH RABBIT SKIN

A. H. Abd-ElGawad, F. M. Sakr, Th. M. Borg and I. I. Abu Hashim

Department of Pharmaceutics, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

تتناول الدراسة تأثير محفزات الاختراق على نفاذية عقار الكيتوكونازول خلال جلد الأرنب. تم تحضيرصياغات هلامية باستخدام هيدروكسى بروبيل ميثيل سيليلوز وكربوكسي ميثيل سيليلوز الصوديوم والكربابول ٩٣٤ وقد تم دراسة النفاذية خلال جلد الأرنب بواسطة خلية فرانز للانتشار. الصياغات الهلامية المحضرة تحتوى على محفزات نفاذية من مجموعات متباينة وتشمل هذه المجموعات أحماض دهنية مثل حامض الأوليك بتركيزات (١ ، ٥ ، ١٠%) والتربينات مثل المنثول بتركيزات (٣ ، ٥ ، ١٠%) واستزات الأحماض الدهنية مثل أيزوبروبيل ميرستات بتركـــيزات (٣ ، ٥ ، ١٠%) وقد تم تقييم النفاذية والتدفق ونسب التحفيز.

وقد أوضحت النتائج أن نفاذية عقار الكيتوكونازول من الهلاميات المحضرة قد زاد بصورة كبيرة باستخدام حامض الاوليك (١٠%) ، المنثول والأيزوبروبيل ميروستات بتركيز ٥ % لكل منهما. وقد تم التوصل الى أن نسبة التحفيز لتركيز ١٠% حامض الاوليك مساوية لـــ ١٩,٥ ، ١٦,٥ ، ١١,٩ لهلاميات كربوكسى ميثيل سيليلوز الصوديوم وهيدروكسى بروبيل ميثيل السيليلوز والكربابول على الترتيب وكانت هذه النسبة لمحفز المنثول ٥% هى ١٣,٥٠ ، ١٣,٣٠ ، ١٠,٩٠ لهلاميات هيدروكسى بروبيل ميثيل السيليلوز ، وكربوكسي ميثيل السيليلوز والكاربابول على الترتيب أما بالنسبة لمحفز الأيزوبروبيل ميرستات فكانت نسبة التحفيز هى ١١% لجميع الهلاميات. كما أوضحت الدراسة أن إنطلاق عقار الكيتوكونازول من الصياغات الهلامية المختلفة المحتوية على أنواع المحفزات المختبرة اتبعت نظام انتشار هيجوشى.

The influence of several penetration enhancers on the percutaneous penetration of ketoconazole (KC) from hydroxypropylmethyl cellulose (HPMC), sodium carboxymethyl

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cellulose (NaCMC) and carbopol 934 gel formulations was investigated. Skin permeation studies were performed using Franz-type diffusion cells and full-thickness abdominal rabbit skin. Various types of compounds such as oleic acid (OA), menthol (M) and isopropyl myristate (IPM) in various concentrations were employed as penetration enhancers. The steady-state flux, permeability coefficients, and enhancement ratios ER $_{flux}$ of KC for each formulation were calculated.

The results showed that the skin permeability of KC from gels tested was significantly increased (P< 0.05) by 10% w/w OA, 5% w/w M and 5% w/w IPM.

ER $_{flux}$ of KC gels containing 10% OA were 19.5, 16.4 & 11.9 for NaCMC, HPMC & carbopol gel respectively. ER$_{flux}$ of 5% w/w M were 13.5, 13.3 & 10.9 for HPMC, NaCMC & carbopol gel respectively. About 11 fold increase in the ER$_{flux}$ at 5% w/w IPM for all gels.

Kinetic analysis of the data indicated that the permeation of (KC) from different gel formulations obeyed Higuchi-diffusion model. In conclusion, OA, M & IPM could be considered as penetration enhancers for KC topical formulations.

INTRODUCTION

Several technological advances have been made in the past couple of decades to overcome skin barrier. Classic penetration enhancement strategies include; iontophoresis,[1] electroporation,[2] phonophoresis,[3-5] compressed gas propulsion,[6] micro fabricated micro needles[7] and chemical penetration enhancers.[8&9]

Hundreds of chemical structures have been identified as penetration enhancers. These include low molecular weight alcohols,[10] alkyl methanol sulphoxides,[11] non ionic surfactants[12] and azone.[13&14] An ideal penetration enhancer should possess a unique property that it reversibly reduces the barrier resistance of the horny layer, allowing the drug to reach the living tissues at a greater rate without damaging any viable cells. Only a fraction of penetration enhancers have been tested clinically, and even fewer have been used in currently marketed transdermal formulations.[8]

It has been reported that oleic acid (OA),[15-17] menthol (M)[18-20] and isopropyl myristate (IPM)[21&22] have been used as a penetration enhancers in the transdermal delivery of several drugs.

Although KC is used topically in the treatment of tinea, cutaneous candidiasis and seborrheic dermatitis,[23] its therapeutic effects will be under question.[24]

The objective of this study was to prepare topical gel formulations containing 2% w/w KC with different

chemical penetration (OA, M and IPM). The steady-state flux, permeability coefficients, and enhancement ratios of KC for each formulation were evaluated to establish the best drug delivery systems for further clinical study.

EXPERIMENTAL

Materials

Ketoconazole (KC) was kindly supplied by Memphis Pharmaceutical Co., Cairo, Egypt. Oleic acid (OA) and triethanolamine (El Naser Chem. Co., Egypt). Menthol (M) (Sigma Chemical Co., St. Louis, Mo., USA). Isoporopyl myristate (IPM) (Merck Chemical Co., Germany). Methyl paraben and propyl paraben (Nipa Lab., Hamburg, Germany). Hydroxy-propylmethyl cellulose (HPMC) (Methocel K 100 M) (Dow Chem. Co., Midland MI), Carbopol 934P (USP 400 cps BDH, Ltd, England). All other chemicals were of analytical grade.

Apparatus

UV/VIS spectrophotometer (JASCO, V-530, Japan). Electric balance (Denver instruments Co., USA), pH-meter (Pye Unicam LTD, model 292, Cambridge, England). Centrifuge (DT. 51 Germany). Thermostatically controlled shaking water bath (Grant instrument Cambridge Ltd., Barrington Cambridge, B2, 5002, England). Modified Franz diffusion cell.

Rabbit skin

Rabbit skin samples were obtained from abdominal skin of female rabbits (2-2.5 kg body weight). The skin was excised just prior to experiments. The hair removed and the skin cleaned with saline solution (0.9% w/w) to remove all visceral debris.

Preparation of gel formulations

The ingredients of all prepared gel formulations containing 2% KC are shown in Table (1). In the preparation of each gel, the predetermined weight of each polymer (4% NaCMC, 2% HPMC, or 2% carbopol 934) was sprinkled gently with continuous stirring on the surface of distilled water previously boiled and cooled in which methyl paraben 0.2% w/w and propyl paraben 0.02% w/w preservatives were dissolved.[25] The dispersions were set-aside overnight to form a gel. The drug was levigated with propylene glycol prior to incorporation into the suggested formulation. Triethanolamine was then added and the total weight was

Table 1: Composition of 2% (w/w) (KC) gel formulations.

Ingredients	Gel I	Gel II	Gel III
(KC)	2	2	2
Sod. CMC	4	-	-
HPMC	-	2	-
Carbopol 934	-	-	2
Propylene glycol	10	10	10
Triethanolamine	0.12	0.12	0.12
Methyl paraben	0.2	0.2	0.2
Propyl paraben	0.02	0.02	0.02
Distilled water to	100	100	100

adjusted to 100 grams with distilled water. OA was incorporating to the gel formulations in a concentrations (1,5 and 10% w/w), by emulsification with Tween 80.[26] IPM and M, in a concentrations (3, 5 and 10% w/w), each one was added directly to the gel after cooling.

Permeability studies

The in-vitro permeation of (KC) from different topical gel formulations was done using a specially designed Franz diffusion upright glass cell. The donor half cell has inside diameter of 3 cm. the total barrier surface available for diffusion was 7.07 cm^2. the cell was positioned in the middle of 250 ml beaker serving as receptor compartment. The whole assembly was sheked (25 strokes per minute) in a thermostatically controlled skaking water bath.

The skin barrier was mounted on the mouth of the donor cell having a diameter of 3 cm between the cell flange and faceplate, five grams of each formulation were accurately weighed and thoroughly spread on the skin barrier to occupy 3 cm diameter circle. The donor cells were then immersed upside-down in 250 ml beaker containing 100 ml phosphate buffer pH (7.4) which was maintained at 37±1° in a constant temperature shaking water bath. The donor cells height was adjusted so that the skin barrier was just below the surface of the release medium. Two milliliter sample was withdrawn from the receiver compartment at time intervals and replaced by equal volumes of fresh phosphate buffer pH (7.4). KC concentration was determined spectrophotometrically at predetermined λ_{max} (225 nm). The total permeability coefficient (*P*) is calculated at steady state under sink condition. Experiment is run long enough so that the steady-state portions are typically arround 3 to 5 times longer than the lag times. The method reported by Yoneto *et al.*,[27] was used to determine the free diffusion coefficients and analysis of permeation data. The permeability coefficient (*P*) and Steady-state Fluxes (*J*) were calculated from the slopes of the best–fit regression line between the treatments. Enhancement ratios of flux (ER_{flux}) is also calculated.[28]

Triplicate experiments were carried out for each study and the results were treated statistically according to ANOVA test followed by Tukey-Kramer multiple comparisons test using SPSS computer program. All the permeation data were analyzed according to zero-order, first order[29] and Higuchi model[30] to determine the mechanism of drug release. The best linear relation was based on the correlation coefficient (r) for the parameter studied.

RESULTS AND DISCUSSION

Passage of drug molecules through the skin could be an important and rather troublesome stage in percutaneous drug delivery. Among the various methods for

improving the passage through the skin is the use of penetration enhancers. In this study the effect of incorporation of various penetration enhancers in different gel forms on the absorption of KC through rabbit abdominal skin was evaluated.

Permeability of (KC) from different gel formulations containing OA

In order to incorporate OA into an aqueous gel base, a surfactant, Tween 80, was added to form an emulsion gel.[26] The effect of OA in amount of 1, 5 and 10% w/w within various KC gels on the permeability rate of drug through rabbit skin is shown in Figures (1-3). OA is a popular penetration enhancer and penetrates the stratum corneum and decompresses this layer and reduce its resistance to drug penetration.[31] The fluidizing effect of OA could be attributed to the presence of *cis-double* bond in its structure.This leads to a kink of the molecule which may cause a decrease in the ordered structure of the lipid Lamellae and hence increase the penetration ability.[32]

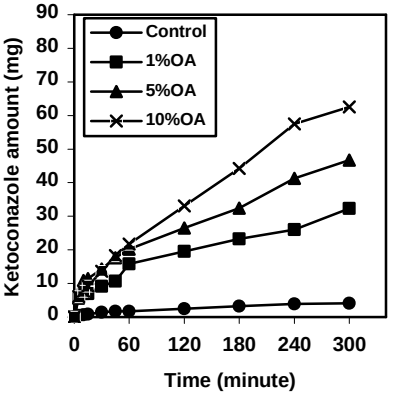

Fig. 1: Permeation of KC from NaCMC gel containing different concentrations of OA.

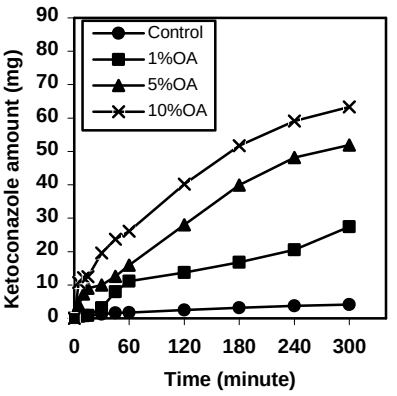

Fig. 2: Permeation of KC from HPMC gel containing different concentrations of OA.

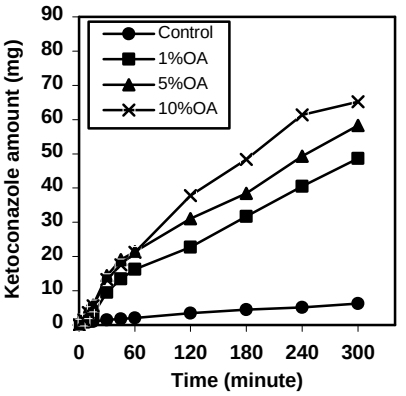

Fig. 3: Permeation of KC from carbopol gel containing different concentrations of OA.

The steady state flux (*Jss*) ($\mu g/min.cm^2$), permeability coefficient (*P*) ($g/cm^2.minx10^{-3}$) and

enhancement ratio (ER_{flux}) of various (KC) gel formulations using different concentrations of OA are presented in Table (2).

It was found that increasing enhancer concentration was associated with a significant increase ($P< 0.05$) in the flux and permeability coefficient of different (KC) gels. The flux values of the drug from NaCMC were 15.50, 16.0 & 27.9 ($\mu g/min.cm^2$) at 1, 5 & 10% w/w OA respectively.

The same pattern was observed for HPMC and carbopol 934 gels. The presence of 10% w/w OA seems to have the greatest effect on the permeation of (KC) through rabbit skin in various gel formulations. The (ER_{flux}) of 10% w/w OA were 19.5, 16.4 & 11.9 for NaCMC, HPMC & carbopol 934 gels respectively. These results were found to be in agreement with those reported by Soni *et al.*,[32] and Santoyo *et al.*[33]

The permeability of (KC) from different gel formulations containing M

Terpene compounds are derived from plant essential oils and combine good penetration enhancing abilities with low skin irritancy and low systemic toxicity.[34,35] They have been reported as clinically acceptable enhancers for use with lipophilic drugs.[36]

Twenty percent ethanol was incorporated into the formulation to provide solubilization and percutaneous penetration enhancing actions.[19] The steady state flux (J_{ss}) ($\mu g/min.cm^2$), permeability coefficient (P) ($g/cm^2.minx10^{-3}$) and enhancement ratio (ER_{flux}) of various (KC) gel formulations using different concentrations of M are illustrated in Table (3). The results revealed that increasing enhancer concentration was associated with a significant increase in the flux of (KC) from

Table 2: The Flux, permeability coefficient and enhancement ratio of (KC) from gel formulations containing different concentrations of OA.

Gel Type	Mean Flux (J_{ss}) (μg / $min.cm^2$)				Mean Permeability Coefficient (P) (g / $cm^2.minx10^{-3}$)				Enhancement Ratio (ER_{flux})		
	Concentration of OA (% w/w)				Concentration of OA (% w/w)				Concentration of OA (% w/w)		
	0	1	5	10	0	1	5	10	1	5	10
NaCMC	1.43 ±0.03	15.50 ±0.02	16.00 ±0.03	27.90 ±0.04	0.72 ±0.05	0.75 ±0.06	0.80 ±0.09	1.40 ±0.03	10.5	11.2	19.5
HPMC	1.59 ±0.03	16.66 ±0.04	25.6 ±0.04	26.1 ±0.02	0.79 ±0.07	0.80 ±0.03	1.28 ±0.01	1.31 ±0.05	10.4	16.1	16.4
Carbopol	2.39 ±0.02	24.42 ±0.06	26.40 ±0.03	28.6 ±0.05	1.19 ±0.02	1.20 ±0.04	1.32 ±0.02	1.43 ±0.04	10.1	11.0	11.9

Table 3: The Flux, Permeability coefficient and enhancement ratio of (KC) from gel formulations containing different concentrations of Menthol.

Gel Type	Mean Flux (J_{ss}) (µg / min.cm^2)				Mean Permeability Coefficient (P) (g / cm^2.minx10^{-3})				Enhancement Ratio (ER_{flux})		
	Concentration of M (% w/w)				Concentration of M (% w/w)				Concentration of M (% w/w)		
	0	3	5	10	0	1	5	10	3	5	10
NaCMC	1.39 ±0.04	14.5 ±0.04	18.5 ±0.04	29.0 ±0.03	0.072 ±0.05	0.75 ±0.06	0.80 ±0.09	1.40 ±0.03	10.4	13.3	20.9
HPMC	2.79 ±0.04	36.5 ±0.05	37.7 ±0.04	42.5 ±0.04	0.079 ±0.07	0.80 ±0.03	1.28 ±0.01	1.31 ±0.05	12.8	13.5	15.2
Carbopol	2.05 ±0.06	21.7 ±0.05	22.4 ±0.03	23.0 ±0.04	0.119 ±0.02	1.20 ±0.04	1.32 ±0.02	1.43 ±0.04	10.6	10.9	11.2

different gels. Actually, the presence of 10% w/w M resulted in the greatest (KC) permeation from different gels. However, it was found that at this concentration M was separated from the gel base later on resulting in heterogeneous gel. So, 5% w/w M is the most suitable concentration for studying the percutaneous penetration of (KC). (ER_{flux})of 5% w/w M were 13.5, 13.3 & 10.9 for HPMC, NaCMC & carbopol gel respectively.

The results of this study are in good agreement with other investigations carried out on the effect of M on the percutaneous penetration of many drugs. Tamoxifen,[15] ketoprofen[37] and midazolam.[19] Menthol when incorporated with lipophilic drugs could be attributed to the improvement in the partitioning of the drug to the stratum corneum. Furthermore, terpenes in combination with ethanol could be used to enhance the percutaneous absorption of the highly lipophilic drugs.[15]

The permeability of (KC) from different gel formulations containing IPM

The third penetration enhancer used in this study was IPM. It is an aliphatic ester, which is widely used as a safe penetration enhancer in topical formulations.[22] This agent was incorporated within the different gels in concentrations of 3, 5 and 10% w/w. These results are shown in Figures (4-6). The permeated amounts of (KC) without IPM were 9.51, 6.20 & 4.96 mg from NaCMC, HPMC and carbopol 934 gel respectively. Increasing the concentration of IPM in the gels from 3 to 5% w/w showed marked increase in the permeated amounts of the drug during the designated time. However, at concentration 10% w/w IPM, the permeated drug amount was even less than that in the control. The presence of large amount of this fatty acid ester could slow down the partitioning of (KC) out of the gel base and stratum corneum leading to a decrease in the

permeability rate of the drug. ER_{flux} of 5% w/w IPM 11, 10.8 & 10.7 for NaCMC, HPMC & carbopol respectively. Statistical analysis revealed that there is insignificant difference between ER_{flux} of all tested gels. IPM showed penetration enhancement of many drugs; Naproxen[38] and diclofenac sodium.[21] Its mechanism of action is not precisely understood, but it seems that it penetrates between the lipid bilayers of stratum cornium and due to its chain structure, disturbs the order of lipid and hence improve drug penetration.[34] Also, differential scanning calorimetry indicated that IPM decreases the lipid phase transition temperature.[34] It should also be noted that IPM has a synergistic effect in the presence of propylene glycol[22] and its permeation enhancement gets magnified.

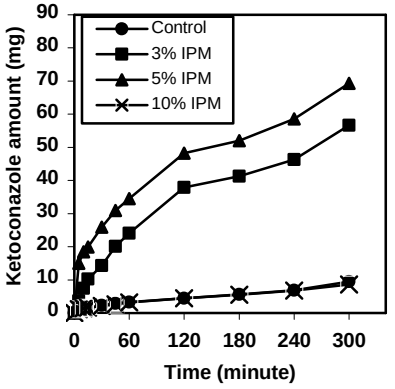

Fig. 4: Permeation of KC from NaCMC gel containing different concentrations of IPM.

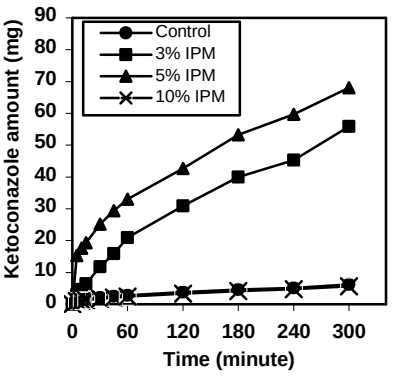

Fig. 5: Permeation of KC from HPMC gel containing different concentrations of IPM.

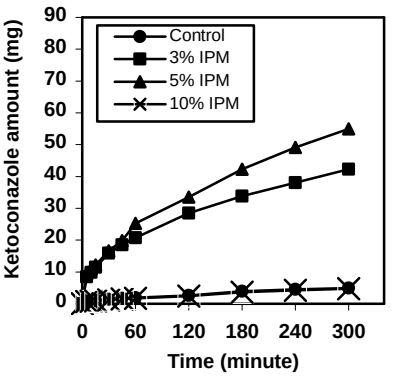

Fig. 6: Permeation of KC from carbopol gel containing different concentrations of IPM.

Kinetic analysis of the data

Table (4) illustrates the kinetic evaluation of (KC) permeation through rabbit skin from different gel formulations in the presence of various concentrations of OA as an example of enhancers.

Table 4: Kinetic modeling of KC permeation from different gel formulations containing various concentrations of OA.

Correlation Coefficient (r)				
Gel formulations (%w/w)	OA concentration (%w/w)	Model Employed		
		Zero-Order	First-Order	Higuchi-Model
Na CMC	1%	0.9646	0.9663	0.9962
	5%	0.9823	0.9834	0.9877
	10%	0.9875	0.9866	0.9888
HPMC	1%	0.9796	0.9810	0.9933
	5%	0.9616	0.9641	0.9966
	10%	0.9667	0.9688	0.9930
Carbopol	1%	0.9749	0.9771	0.9958
	5%	0.9893	0.9874	0.9906
	10%	0.9786	0.9811	0.9963

The obtained results revealed that the best line fitting for all gel formulations in the presence of OA obeyed Higuchi diffusion model. The correlation coefficient (r) is not less than 0.9877. For M, the correlation coefficient (r) is not less than 0.9835. While for IPM the correlation coefficient (r) is not less than 0.9853. Also, the presence of enhancers did not affect the nature of the matrix and the mobility of the drug from it. These results are in agreement with many authors. Verma & Murthy,[39] reported that the release of flurbiprofen from different concentrations and grades of HPMC follows Higuchi kinetics, Shaker,[40] found that the release kinetics of phenylephrine HCl from carbopol hydrogels was obtained with diffusion equation.

Conclusion

It is possible to enhance the permeation of (KC) through rabbit skin by chemical enhancers. The type and concentration of penetration enhancer for incorporation into a specific formulation containing drug should be selected carefully and following extensive initial studies, in order to achieve a formulation with desirable permeability rate and efficacy. For further clinical study the best formulations for KC permeation are NaCMC gel conaining 10% w/w of OA, HPMC gel containing 5% w/w M & NaCMC gel containing 5% w/w of IPM. Kinetic analysis of the data indicated that the permeation of (KC) from different gel formulations obeyed Higuchi-diffusion model.

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