Bull. Pharm. Sci., Assiut University, Vol. 24, Part 1, June 2001, pp. 65-71.

EFFECTS OF FORMULATIONS AND PENETRATION ENHANCERS ON THE DIFFUSIONAL PARAMETERS FOR THE PERCUTANEOUS ABSORPTION OF PROPRANOLOL HYDROCHLORIDE

K. A. Khaled[1], Y. M. El-Sayed[2] and I. S. Al-Bawardi[2]

[1]*Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt*
[2]*Department of Pharmaceutics, College of Pharmacy, King Saud University, Saudi Arabia*

فى هذا البحث تم صياغة هيدروكلوريد البروبرانولول - الصورة المتوافرة تجاريا لعقار البروبرانولول - على هيئة هلام باستخدام بلمرات السيلولوز المختلفة مثل ميثيل السيلولوز وكربوكسى ميثيل السيلولوز وايدروكسى بروبيل ميثيل السيلولوز وذلك بغرض دراسة تأثير البلمر على معدلات الاختراق الجلدى المعملى للعقار. واستخدم فى هذا الصدد خلايا انتشار فرانز المحسنة المزودة بجلد الأرنب كغشاء.

وقد اعطى هلام هيدروكسى بروبيل ميثيل السيلولوز أعلى معدل للأختراق. ولهذا فقد استخدم هذا الهلام لدراسة تاثير تركيز العقار على معدل اختراقة حيث تبين ان معدل اختراق العقاريزداد طرديا مع زيادة تركيزة فى الهلام. وبتحليل النتائج وجد ان مقاييس الانتشار التى تتحكم فى الأختراق الجلدى للعقار لم تتأثر بتغير التركيز بينما وجد أن قيم كلا من معامل النفاذ ومعامل التجزئ الظاهرين يعتمدان على تركيز العقار.

وفى محاولة للحصول على أعلى معدل ممكن للاختراق الجلدى للعقار فقد تناول البحث دراسة تأثير معززات الاختراق الجلدى مثل الأزون والكحول الديسيلى العادى و ١,٨-سينيول التى أضيفت بتركيزات مختلفة - كل على حدة - الى الهلام. وقد وجد أن لهذه المعززات تأثير حافزى كلى منخفض حيث ضاعفت معدل الاختراق الجلدى للعقار ٥,٧ ضعفا بحد أقصى. وعند تحليل هذه النتائج وجد أن لمعززات الاختراق تأثيرا مزدوجا عكسيا على كل من معامل الانتشار ومعامل التجزئ الظاهرى حيث انخفضت قيمة المعامل الأول وازدادت قيمة المعامل الأخير.

Propranolol hydrochloride, the commercial available form of propranolol, was formulated as gels using different cellulose polymers viz. methylcellulose, carboxymethylcellulose, and hydroxypropyl methylcellulose (HPMC). In vitro percutaneous absorption study of the gels was conducted using improved franz diffusion cells and rabbit skin as a membrane. The highest extent of drug penetration was obtained upon using HPMC gel formulation. Therefore, the influence of drug concentration on its percutaneous absorption was investigated utilizing HPMC gel formulation. The extent of drug percutaneous absorption was found to increase as drug concentration increases. Analysis of the diffusional parameters, govern the skin penetration process, revealed that diffusion coefficient of the drug did not change as drug concentration varies. On the other hand, the apparent permeability coefficient, and the apparent partition coefficient, showed dependency on drug concentration. The limit value of the partition coefficient was determined, graphically, and found to be 0.2411. The influence of skin penetration enhancers, such as Azone, n-decyl alcohol, and 1,8-cineol, on the percutaneous absorption of the drug was studied. An overall low enhancing effect (maximum of 5.7-fold compared to a control) was obtained using 1,8-cineol. This finding was attributed to the opposite influence of the cited enhancers on the diffusion coefficient and the partition coefficient of the drug. The enhancers increase the resistance of the skin to the penetration of drug molecules (decreasing the diffusion coefficient). Meanwhile, the enhancers facilitate the partitioning process of the drug molecules from the gel to the skin as the apparent partition coefficient increases.

Received in 27/11/2000 & Accepted in 21/2/2001

INTRODUCTION

Propranolol, a non-selective beta-adrenergic receptor antagonist has been widely used in the treatment of hypertension[1] and other cardiovascular disorders.[2] The drug has a very low and variable oral bioavailability because of extensive hepatic first-pass metabolism.[3,4] To avoid hepatic first-pass effect, the transdermal route of administration can be considered as an alternative for the oral one.[5] Propranolol possesses very suitable characteristics that a drug must have in order to be formulated as a transdermal drug delivery system (TDDS) viz, low molecular weight, high lipid solubility, relatively short half-life[6], and effective in low plasma concentration.[7]

Many drugs do not penetrate the skin at a sufficiently high rate to achieve therapeutic levels. For this reason, there have been many attempts to increase their percutaneous penetration by the use of penetration enhancers.[8-10] Penetration enhancers, accerelants or promoters are substances that combine with or dissolve in the stratum corneum which is recognized as the principal skin barrier to drug penetration. It is accepted that enhancers may modify either the postulated lipid or polar route of penetration through the skin.[11] They might enhance drug penetration by causing the stratum corneum to swell and/or leach out some of its structural components, thus reducing the diffusional resistance and increasing the permeability.[12]

The aim of this study was to formulate propranolol hydrochloride (PL), the commercial available form of propranolol, in a hydrophilic polymer matrix and to study the effect of both polymer and drug concentration of the *in-vitro* transdermal penetration of the drug through rabbit skin. Propranolol hydrochloride is a hydrophilic drug and its absorption through skin is expected to be poor. Therefore, the effect of various penetration enhancers viz. Azone, n-decyl alcohol, and 1,8-cineol on improving the *in-vitro* percutaneous absorption of the drug was also investigated. In the present study propranolol was incorporated in a gel base. Cellulose polymers were tried as gel base forming polymers because they produce neutral gels of stable viscosity, good resistance to microbial attack, high clarity, and good film strength when dried on the skin.[13] The utilized polymers include methylcellulose (MC), carboxymethylcellulose (CMC), and hydroxypropyl methylcellulose(HPMC). In all formulations, the gels were easily prepared and having acceptable consistency.

MATERIALS AND METHODS

Materials

Propranolol hydrochloride, n-decyl alcohol, and HPMC (Sigma Chemical Co., St. Louis, MO, USA); indenolol hydrochloride (Yamanouchi Pharm. Co, Ltd., Tokyo Japan)l Azone (Nelson Research, Irvine, CA, USA); and sodium metabisulfite, MC, CMC, and 1,8-cineol (BDH Chemical Ltd., Poole, UK). All other chemicals were reagent grade and solvents were HPLC grade.

Methods

Preparation of gel formulations

The cellulose polymer was dispersed in hot water containing 0.037 g methyl paraben. The dispersion was cooled to form a gel. Propranolol hydrochloride (0.25 g) was dissolved in cold water containing 0.025 g sodium metabisulfite and then mixed with the gel. Care was taken to avoid incorporation of air bubbles. The pH of the gel was adjusted to 6.5 using 1 N sodium hydroxide. The final weight of the preparation was adjusted to 25.0 g by the addition of cold water. The amounts of polymers used were 0.75 g MC, 1.0 g CMC, and 1.5 g HPMC.

Effect of permeation enhancers of the transdermal permeation of the drug

The influence of penetration enhancers on the transdermal delivery of propranolol in HPMC gel formulation was studied by preparing nine formulations containing different concentrations of the selected enhancers. Each enhancer in its proper amount was added to the formulation with the solution of the drug to

obtain final preparation containing 3.0, 6.0 and 6.0 % w/w of each of Azone, n-decyl alcohol, and 1,8-cineol.

***In-vitro* diffusion study**

The dermal side of rabbit full-thickness skin was soaked in an isotonic 0.1 M phosphate buffer pH 7.2 for 2 h at 5°. The skin was tightly secured between the receptor and donor compartment of improved franz diffusion cell (Crown Glass Co., Somerville, NJ, USA). The receptor compartment was filled with 15 ml of the isotonic phosphate buffer. The cell cap was open to the air allowing quick application of the gel. The temperature of the liquid bathing the skin was maintained at 37°±0.5 A dose equivalent to 10 mg of the drug (1 g of the gel) from each formulation was applied to the epidermal surface in the donor compartment, which was sufficient to cover the exposed surface area of the skin (3.14 cm^2). One cell was used as a reference where drug-free formulation was applied. One ml samples were withdrawn from the receptor phase, from each cell, at different time intervals, to be assayed for drug content. An equal volume of fresh buffer immediately replaced each collected sample. Care was taken to avoid air bubbles formation under the skin. Concentration of the drug in the collected sample were determined using a high performance liquid chromatography method of assay.[14]

Analyses of data

The *in-vitro* diffusional parameters were calculated from the permeation data by using the following equations.[15]

$$J_{ss} = D K_m C / h = K_p C$$
$$D = h^2/6\tau$$
$$K_m = K_p h / D$$

Where J_{ss}, the *in vitro* steady-state drug flux, is the amount of drug that diffuses across the area, A, in time, t. J_{ss} was calculated using the linear portion of the amount penetrated of the drug versus time plot employing least-squares linear regression. K_m, is the skin-gel formulation apparent partition coefficient of drug, D, is the diffusion coefficient within the skin, τ, is the lag time, h, is the thickness of the skin (0.0875 cm) as measured with a micrometer, K_p, is the apparent permeability coefficient through the skin, and C is the drug concentration in the donor compartment.

RESULTS AND DISCUSSION

Propranolol hydrochloride (PL) was formulated as hydrophilic gels. Gels are cosmetically accepted and commonly preferred. Moreover, the concentration of the water soluble drug can be varied over a wide range upon using gels. The cumulative amounts of PL penetrated the skin during 24 h of the experiment from MC, CMC and HPMC gel formulations were shown in Figure 1. It is clear that the highest amount of PL delivered through rabbit skin was obtained upon using HPMC gel formulation. This formulation delivered the drug 4.2-fold and 1.6-fold more than CMC and MC formulations, respectively. The possibility of forming a complex between PL and CMC, via the interaction between the basic nitrogen of the drug and the carboxylic group of the polymer, may offer an explanation for the low overall percutaneous absorption of the drug from CMC gel formulation. Upon forming the complex, the thermodynamic activity of the drug in the gel will be decreased and so the drug transdermal permeation.

It was in the interest of the study to investigate the effect of drug concentration on its permeation through the skin. This investigation may allow a more reliable calculation for the diffusion parameters which govern the drug permeation process through the skin. Therefore, HPMC gel formulations containing 5.0, 10.0, 20.0, 40.0 and 50.0 mg of PL per gram of gel were prepared. The permeating profiles of the drug from these gels are depicted in Figure 2 which shows a continuous increase in the cumulative amounts of the drug that permeated the skin as drug concentration increases.

Preliminary work has shown that the solubility of PL in HPMC gel is about 35 mg/g of the gel and its solubility in the buffer is about 55 mg/ml. The cumulative amount of the drug penetrated skin during 24 h of the experiment was found to range from 348 μg for the 5 mg PL gel formulation to 5030 μg for the 50 mg

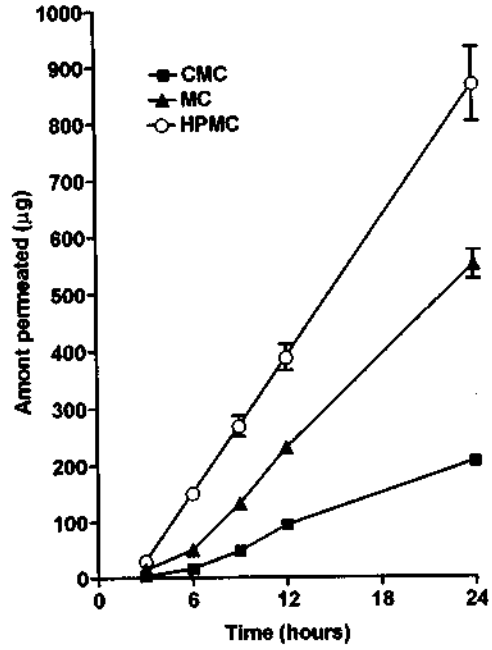

Fig. 1: Cumulative amount of propranolol hydrochloride permeated across rabbit skin following the application of 10 mg drug as gel formulations prepared using different cellulose polymers.

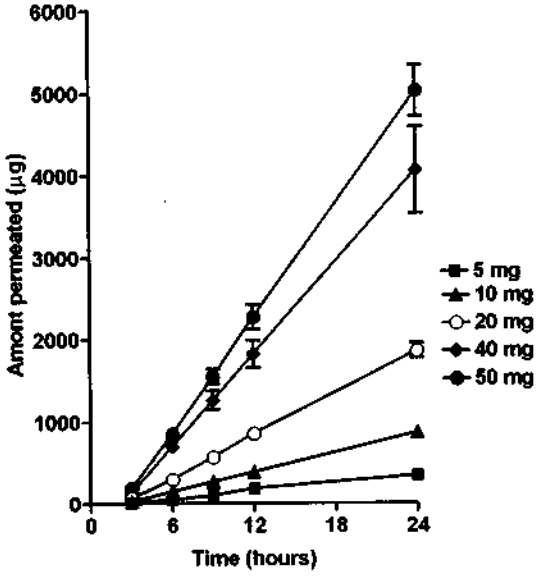

Fig. 2: Cumulative amount of propranolol hydrochloride permeated across rabbit skin following the application of HPMC gel formulations containing different drug concentrations.

one. Taking in consideration that the receptor compartment contains 15 ml of the buffer, simple calculation can show that the highest concentration of the drug in the receptor compartment is less than 1% of its solubility. This means the maintaining of sink conditions in the receptor compartment during the experiment. In addition, the drug concentration in the donor compartment did not change appreciable during the experiment as less than 10% of the drug permeated the skin. This finding indicates that an almost constant thermodynamic activity of the drug was maintained during the study. Maintaining both constant thermodynamic activity in the donor compartment and sink conditions in the receptor one allow the calculation of the diffusional parameters for the drug assuming the establishment of the steady state and applying a zero-order kinetics (Table 1). The data listed in the table shows the lag time, for all experiments, was almost unchanged as its value (average ± SD) was 8227 sec ± 160 with a C.V., of 1.94%. Consequently, the value of the drug diffusion coefficient was almost unchanged as its average value ± SD was 1.55 cm^2 / sec ± 0.03. This finding indicates that the integrity of the skin did not change or vary from one experiment to another. On the other hand, the permeability coefficient, and consequently the partition coefficient, of the drug were found to be drug concentration dependent. To find out the empirical relationship between drug concentration, C, and the apparent partition coefficient, K_p, the plot of K_p versus the reciprocal of C (1/C) was constructed (Figure 3). It is obvious that a linear relationship is established between K_p and 1/C (r= 0.997). The relationship may be described using the following straight line equation.

$$K_p = 0.2411 - 0.00041\ (1/C)$$

The limit value of K_p when C is sufficiently high is 0.2411. This limit value is quite useful in designing a TDDS of the drug that is supposed to deliver drug at a rate which is independent of drug concentration.

Table 1: Diffusional parameters for the percutaneous absorption of propranolol hydrochloride from HPMC gels containing different drug concentrations.

Drug conc. (mg/g of gel)	Lag time (sec)	$j_{ss} \times 10^{-3}$ ($\mu g/cm^2/sec$)	$D \times 10^{-7}$ cm^2/sec	$K_p \times 10^{-7}$ cm/sec	K_m
5	8158	1.44	1.56	2.87	0.161
10	8081	3.54	1.58	3.54	0.196
20	8486	7.64	1.50	3.82	0.223
40	8140	16.65	1.57	4.14	0.231
50	8270	20.50	1.54	4.10	0.233

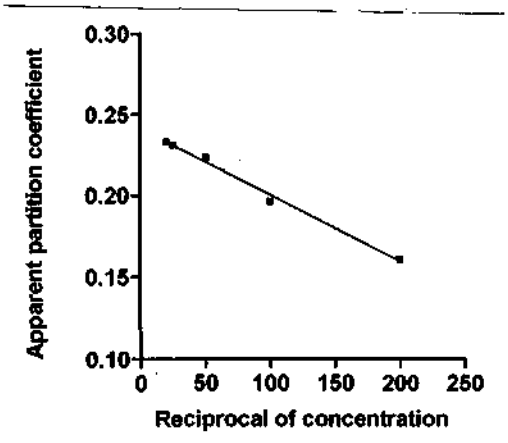

Fig. 3: Apparent partition coefficient as a function of the reciprocal of drug concentration.

The cumulative amount of PL penetrated the skin during the 24 h of the experiment, at different time intervals, from HPMC gel formulation containing 10 mg of the drug per gram of the gel and different concentrations of enhancers are listed in Table 2. It can be seen from the obtained data that Azone increased the amount of PL penetrated the skin from all formulations. The maximum enhancing effect (1.17-fold) was achieved using the formulation containing 6% Azone. This weak enhancing effect of Azone may be attributed to the fact that Azone is effective with simple formulation such as solution rather than with other formulations.[16] The lower overall enhancing effect of 9% Azone gel relative to 6% one could be due to a dual action of the enhancer on both animal skin and the physical properties of the gel as similar results were reported.[16] Upon using n-decyl alcohol as an enhancer, it was found an increase in the cumulative amount of drug penetrated skin in all formulations. The enhancing effect of the alcohol increases as the concentration of the alcohol in the gel increases. The maximum enhancing effect (3.79-fold) was achieved upon using the formulation containing 9% of the alcohol. Finally, the Table shows the enhancing effect of 1,8-cineol on the cumulative amount of the drug penetrated the skin. It is clear that the enhancing effect of 1.8-cineol is more pronounced than the effects of the other two enhancers. The maximum enhancing effect was obtained upon using gel formulation containing 9% of 1.8-cineol and was about 5.71-fold as compared to the enhancer-free formulation. Based on the collected data, it appears that, among the tested penetration emhancers, 1,8-cineol in a concentration of 9% was the most effective agent for enhancing the percutaneous absorption of PL.

The diffusional parameters for gels containing enhancers were calculated utilizing the data of the first 12 h of the experiment as 10% or less of the loaded drug permeated the skin during this period. No calculations were made for gels containing 9% of either n-decyl alcohol or 1,8-cineol as thermodynamic activities of the drug in the donor compartment were decreased appreciably during the first 12 h of the experiment (more than 10% of the drug has been penetrated). In all cases, a minimum of 4

Table 2: Effect of enhancers on the cumulative amounts* of propranolol (μg) penetrated the skin from HPMC gel formulations.

Time (hrs)	Enhancer	Concentration of the enhancer			
		0%	3%	6%	9%
3	Azone	30.5±1.7	20.4±1.3	66.9±3.0	29.2±1.8
6		151.3±5.4	116.1±6.3	235.1±9.6	133.9±8.5
9		268.8±18.0	256.9±11.9	644.3±20.8	327.4±12.6
12		389.1±0.1	445.9±26.5	729.6±32.2	588.3±26.4
24		869.6±66.4	1025.3±62.9	1646.5±93.5	1423.1±85.6
3	n-decyl alc.	30.5±1.7	23.4±1.6	32.3±1.8	54.3±3.4
6		151.3±5.4	132.0±9.9	198.8±10.2	390.9±27.1
9		268.8±18.0	270.1±11.9	471.7±20.1	795.7±29.8
12		389.1±0.1	402.7±26.5	839.1±33.4	1390.9±75.7
24		869.6±66.4	1044.0±74.4	1975.9±98.3	3214.1±168.0
3	1,8-cineol	30.5±1.7	39.8±1.2	63.7±4.0	124.2±5.1
6		151.3±5.4	323.3±17.2	334.7±15.3	640.1±24.7
9		268.8±18.0	666.1±27.4	847.9±25.8	1271.4±52.1
12		389.1±0.1	1008.6±35.5	1428.5±47.4	2093.0±99.3
24		869.6±66.4	2299.9±145.3	3124.6±137.2	4833.5±298.0

*Average of six cells ± SD.

Table 3: Diffusional parameters for the percutaneous absorption of propranolol hydrochloride from HPMC gels containing different enhancers.

Enhancer (conc)	Lag time (sec)	$j_{ss} \times 10^{-3}$ (μg/cm²/sec)	$D \times 10^{-7}$ cm²/sec	$K_p \times 10^{-7}$ cm/sec	K_m
3% Azone	11160	4.3	1.44	4.27	0.33
6% Azone	8774	6.5	1.45	6.54	0.39
9% Azone	11431	5.5	1.12	5.52	0.43
3% decyl alc.	11273	4.1	1.13	4.07	0.32
6% decyl alc.	11543	8.0	1.11	7.94	0.63
3% cineol	11414	10.1	1.12	10.11	0.79
6% cineol	11326	13.6	1.13	13.59	1.05

data points was used for linear regression. The calculated parameters are listed in Table 3. It is noticeable from the cited data, with the exception of 6% Azone formulation, that the lag times, and consequently the diffusion coefficients, for the rest formulations are almost the same. Statistical analysis of the data reflects that the average value of the diffusion coefficient (average ± SD) was $1.18 \times 10^{-7} \pm 0.13$ cm^2/sec and C.V., % was 11.1%. The calculated diffusion coefficients are lower than that of the control gel (enhancer free gel). This indicates that the enhancers have increased the resistance of the skin to drug penetration by the same degree regardless of the nature of enhancer or its concentration. On the other hand, the calculated partition coefficients showed dependency on the concentration of the enhancer as well as its nature. As enhancer concentration increases the partition coefficient increases. This increase varies from one enhancer to another. The highest influence was observed with 1,8-cineol. These findings may indicate the effect of enhancers on the percutaneous absorption of PL is making drug molecules experience a little more resistance, in their diffusion through the skin barrier, but greater partitioning to the skin. These opposite effects may explain the overall weak enhancing influence of the tested enhancers.

REFERENCES

1- R. Tarazi and H. P. Dustan, Am. J. Cardiol., 29, 633 (1972).
2- A. J. Boakes and B. N. C. Prichard, Br. J. Pharmacol., 47, 673 (1973).
3- T. Walle, U. K. Walle, M. J., Wilson, T. C. Fagan, and T. E. Gaffiney, Br. J. Clin. Pharmacol., 18, 741 (1984).
4- E. Cid, F. Mella, L. Luchini, M. Carcamo and J. Monasterio, J. Biopharm. Drug Dispos., 7, 559 (1986).
5- S. F. Chang, L. Moore and Y. W. Chien, Pharm. Ress., 5, 718 (1988).
6- L. Shargel and A. B. C. Yu, Applied Biopharmaceutics and Pharmacokinetics, 3rd Edn, Printice-Hall International., 595 (1995).
7- R. Krihsna and J. K. Pandit, Drug Develop. Ind. Pharm., 20, 2459 (1994).
8- M. Okumura, K. Sugibayshi and Y. Morimoto, Chem. Pharm. Bull., 37, 1375 (1989).
9- K. Brain, J. Hadgraft, D. Lewis and G. Allan, Int. J. Pharm., 71. R9 (1991).
10- T. Turunen, S. Buyuktimkin, N. Buyuktimkin, A. Utti, P. Paronen and H. Pytting, ibid., 92, 85 (1993).
11- B. W. Barry and S. L. Bennet, J. Pharm. Pharmcol., 39, 535 (1987).
12- J. Hadgraft, Pharm. Ind,. 5, 252 (1984).
13- D. M. Collett, In D. M. Collett and M. E. Aulton, Eds. Pharmaceutical Practice. 1st Edn., Longman Group, U.K., 125 (1990).
14- A. A. Al-Angry, Y. M. El-Sayed, M. A. Al-Meshal, M. M. Al-Dardiri and G. M. Mahrous, J. Clin. Pharm. Ther., 16, 93 (1991).
15- D. Chow, I. Kaka and T. L. Warng, J. Pharm. Sci., 73, 1794 (1984).
16- R. Stroughton and W. McClure, Drug Develop. Ind. Pharm., 9, 725 (1983).