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REVIEW ARTICLE

Evaluation of *in vitro* percutaneous absorption of olanzapine and fluoxetine HCl: enhancement properties of olanzapine

Marina Borovinskaya, Robert Bellantone, and Fotios M. Plakogiannis

Division of Pharmaceutical Sciences, Arnold and Marie Shwartz College of Pharmacy & Health Sciences, Long Island University, Brooklyn, NY, USA

Abstract

The diffusion characteristics of fluoxetine HCl (FLX HCl) and olanzapine (OLZ) alone and in combination with each other were studied to determine their *in vitro* permeation behavior across a series of gelling agents through a cellulose membrane and human cadaver skin. Klucel 0.5% was selected as the optimal formulation to study their diffusion through human cadaver skin. The release profiles of drugs acting alone and in combinations were identical in the case of the cellulose membrane. However, with human cadaver skin, the permeation of FLX HCl in combination with OLZ drastically increased (732 µg) compared with the release of FLX HCl alone (43.7 µg), while the release of OLZ remained the same whether alone or in combination with FLX HCl (183.7 µg). The results indicate that OLZ enhances the diffusion of FLX HCl through the cadaver skin. Follow-up studies with OLZ were conducted to further investigate this phenomenon and have shown that OLZ enhancement properties are skin reversible as well as concentration dependent. Also, a variety of experiments with different hydrophilic and lipophilic molecules were conducted, and it was found that OLZ enhances the permeation of hydrophilic compounds, while it has no influence on lipophilic compounds. Finally, a number of compounds structurally related to OLZ were investigated as enhancers, and it was determined that piperazine ring attached to the tricyclic system of OLZ is essential for enhancement of FLX HCl (1,837 µg).

Keywords: Fluoxetine HCl, olanzapine, permeation enhancers, Franz diffusion cells

Introduction

Bipolar disorder causes dramatic change in people's moods, from high feelings of euphoria or mania to sadness and hopelessness or depression, with periods of normal moods in-between. However, there are many studies that have shown that depressive symptoms last more than three times longer than manic ones. This depressive phase is difficult to treat and represents a disabling form of depression[1]. In addition, this phase is associated with high morbidity and mortality[2].

In seeking the etiology of the disorder, it was ascertained that the dysfunction of the brain monoamine systems (serotonin, norepinephrine, and dopamine) play an important role. Most antidepressants that are currently available exert their action mostly on just one monoamine system. However, the pharmacological effect on only one type of neurotransmitters is unlikely to produce sufficient and desirable changes in such a severe neurochemical dysfunction[3].

The combination therapy of the selective serotonin reuptake inhibitor fluoxetine HCl (FLX HCl) (the active ingredient of Prozac®) and the atypical antipsychotic olanzapine (OLZ) (the active ingredient of Zyprexa®) was found to be a good choice for the effective treatment of resistant patients. This combination therapy provides more significant improvements in reducing depressive symptoms, than either drug alone[3]. The combination of FLX HCl and OLZ is significantly superior in terms of effective improvements across the board on clinical signs of depression compared with OLZ monotherapy. This includes higher completion rates, lower discontinuation rates due to adverse events, quicker response time, higher rates of response, and faster remission[2].

One of the advantages associated with transdermal formulation is that it bypasses first-pass metabolism

Address of Correspondence: Dr Marina Borovinskaya, Long Island University, Pharmaceutics, 1 University plaza, Brooklyn 11201, NY, USA. E-mail: mashka.home@comcast.net

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and gastrointestinal incompatibility. In the case of OLZ, 40% of the dose metabolized before reaching systemic circulation; FLX HCl has an undesirable effect on the GI system, which causes discontinuance in 15% of the patients. Also, the transdermal route of administration can reduce the frequency of dosing and avoid peaks and troughs associated with intermittent drug administration[4]. Additionally, according to statistics, about 70% of the patients who are prescribed oral antidepressants fail to take 25–50% of their prescribed dose, which severely affects the treatment's efficacy[5]. In view of all these facts, the development of the transdermal drug delivery system offers a beneficial solution to these problems.

Human skin is an effective barrier that protects the body from excessive water loss and xenobiotics ingress[6]. The main rate-controlling step of any substance through the skin is considered to be the stratum corneum that is located in the upper layer of the skin and considered to be the hardest layer to permeate[7,8]. The fundamental cell type of stratum corneum is corneocytes– flat, enucleated cells that are closely packed. They are filled with keratin and water and surrounded by protein layers– cell envelope. A lipid envelope is chemically linked to the cell envelope. This lipid layer represents an interface between the hydrophilic environment of corneocytes and the lipophilic lipid domain[9]. So, this unique model of stratum corneum severely limits the transdermal delivery of the drugs. An effective way to extend the range of drugs to penetrate this barrier is to incorporate skin penetration enhancers into formulations. Many compounds, such as azone derivatives, alcohols, fatty acids, fatty esters, glycols, pyrrolidones, sulfoxides, surfactans, and terpines, have been used to increase the permeability of the stratum corneum[10]. This study revealed a very novel finding: more particularly, it was discovered that OLZ itself acts as an enhancer for FLX HCl, increasing its penetration through cadaver skin.

The aim of this study was to evaluate the suitability of the transdermal route for the simultaneous delivery of OLZ and FLX HCl. The specific goals of the research were (i) to study the release of OLZ and FLX HCl acting separately and in combination from several polymer gel formulations through cellulose and cadaver skin membranes, (ii) to evaluate possible interactions between them, (iii) to determine possible complexations of the drugs with the chosen gelling agent, (iv) to observe any possible influence on each other through biological membrane, and (v) to calculate the physicochemical parameters of both drugs (flux and permeability coefficients).

Materials and methods

Materials

FLX HCl and OLZ were obtained from Barr Laboratories (Pomona, NY); Klucel and cellulose gum were obtained from Hercules Inc. (Wilmington, DE); methocel K 15 premium was obtained from Dow Chemical Company (Midland, Michigan); acetonitrile HPLC grade, water HPLC grade, methanol, and phosphoric acid were obtained from J.T.Baker Inc. (Phillipsburg, NJ); ethyl alcohol was obtained from Omni Solvent (Darmstand, Germany); cellulose membrane (0.006 cm), MW (1,000 cut off) was obtained from Spectrum Lab (Houston, TX); triethylamine was obtained from Sigma Chemical Co. (St. Louis, MO); PEG 400 was obtained from Spectrum (New Brunswick, NJ); and cadaver skin was obtained from The New York Firefighters skin bank (N.Y. Hospital Cornell Medical Center). All chemicals were used without any further purification.

Quantification of OLZ and FLX HCl

All samples were analyzed for drug content using HPLC according to a method developed in our laboratory. The chromatographic apparatus consisted of a programmable auto-sampler (Hitachi L–7250), a UV detector (Hitachi L–7400), and a pump (Hitachi L-7100). The analytical separation was accomplished by using a reversed CN column with 5-µm particle size (4.6 × 250 mm). The detection wavelength was 260 for both OLZ and FLX HCl with retention time 8 and 13 min, respectively. The mobile phase consisted of acetonitrile: water containing 0.5% of phosphoric acid adjusted with triethylamine to pH 7.5 (45:55 v/v) with a flow-rate of 0.8 ml/min at room temperature. All solvents were filtered (0.45-µm-pore size nylon membrane filter) and degassed prior to use. Stock solutions of OLZ, FLX HCl, and OLZ/FLX HCl in combination were prepared at concentration of 1 mg/ml of each drug. Working standards were prepared by diluting the stock standard solutions with deionized water. A five-point calibration curve was made. The equation for the calibration line of OLZ, obtained by least-square regression was $y = 430776x + 58373$. The linearity, expressed by the linear correlation coefficient r, was 0.9997. The LOQ (quantitation limit) value was 300 ng ml^{-1} and LOD (detection limit) value was 200 ng ml^{-1} with coefficient of variation (CV) of 5%. The equation for linear regression for FLX HCl was: $y = 204032x + 35761$, with linear correlation coefficient r of 0.999. The LOQ value was 450 ng ml^{-1} with CV of 7%, and LOD value was 300 ng ml^{-1} with CV of 5%. Precision was assessed by determining intra- and inter-day relative standard deviation. Values were less than 7% for intra-day relative standard deviation and around 5% for inter-day.

The HPLC analysis for the mixture of OLZ and FLX HCl rendered a similar result, indicating the absence of interferences when the two drugs were analyzed simultaneously.

Preparation of gel formulations

Three different gelling agents (Klucel®, Methocel®, and Cellulose gum®) were used to prepare the gel formulations of OLZ and FLX HCl acting alone and in combination. All formulations were prepared based on the methods, which were followed in our laboratory and published[11].

Permeation studies

In vitro diffusion studies were carried out using a modified jacketed Franz diffusion cell apparatus with a diffusional area of 1.76 cm^2.[12] These cells have a static receiver solution reservoir with a side-arm sampling port design. At predetermined time points, an aliquot of 0.5 ml of medium was removed from the receptor compartment for drug content analysis[13]. Then, the receptor compartment was immediately replaced with an equal volume of a fresh medium. The receptor compartment (13-ml volume) was filled with a degassed receiving medium to avoid air bubble formation. It contained the mixture of PEG, ethanol, and water at the ratio of 25:35:40, respectively, based on the result of solubility studies. The receptor compartment was maintained at 37°C by means of a water bath, a circulator, and a jacket surrounding the cell resulting in the skin surface temperature of 32°C[14–19]. The solution in the receiver compartment was continuously stirred by means of a coated magnetic stirrer at the constant speed of 600 rpm, to avoid diffusion layer effect. A membrane (cellulose membrane or human cadaver skin) was mounted between the donor and receiver compartments as a diffusion barrier and secured in place by means of a pinch clamp[20].

The thickness of the Spectra/Pro7-6-regenerated cellulose acetate membrane was 60–65 μm with a MW cutoff point of 1,000[21]. The cellulose membrane was soaked for 1 hour in distilled water to remove traces of the sodium azide preservative. All diffusion studies throughout these experiments were conducted in triplicate.

The release profiles of the drugs from three different gelling agents (Klucel®, Methocel®, and Cellulose gum®) were determined by placing enough quantity of formulation of OLZ or FLX HCl either separately or in combination into the donor compartment, representing 3 mg of the active ingredient of each drug. Four different concentrations of gel (0.5%, 1%, 1.5%, and 2%) with the constant amount of drugs (0.1%) were used. Samples of 500 μl were withdrawn from the receiver compartment for 48 hr and analyzed by HPLC.

To determine possible drug–polymer complexation, an equilibrium study between two compartments containing equal drug concentrations was conducted. One compartment contained the drug and the polymer, while the other contained the drug but not the polymer; systems were allowed to equilibrate for 1 week. The same study was conducted with both drugs combined. Both drugs and the polymer were placed in one compartment, while the second compartment contained both drugs again but not the polymer. Samples were analyzed by HPLC.

Human cadaver skin from the thigh of Caucasian subjects was prepared by using the experimental procedure described above. Samples were withdrawn at predetermined time intervals up to 200 hours. Klucel® (0.5%) with FLX HCl (0.1%) and OLZ (0.1%) separately and in combination were used for the cadaver skin studies. The cadaver skin was prepared by hydrating it in an isotonic phosphate buffer solution for 1 hour at room temperature before it was placed between the donor and receptor compartment as diffusion barrier[20]. The integrity of the skin was checked by visual inspection of the cumulative amount versus time plots.

Data analysis

The permeability coefficient P was calculated from the following modified Fick's diffusion equation:

$$\frac{dM_r}{dt} = AP \cdot (C_d - C_r) \quad (1)$$

where M_r denotes the mass of the drug in the receiver, and C_d and C_r denote the average drug concentrations in the donor and receiver, respectively, given by

$$C_d = \frac{M_r}{V_r} \quad C_d = \frac{M_d}{V_d} \quad (2)$$

Here, V_r and V_d are the volumes of the receiver and donor, respectively, and M_d is the amount of drug in the donor, given by

$$M_0 = M_d + M_r \quad (3)$$

where M_0 is the amount of drug initially loaded into the donor.

In Eq. 1, the value of the release rate dM_r/dt was taken as the average slope of the plot of M_r versus time, from the earliest times at which the drug began to accumulate in the receiver until the end of the experiment.

The average flux (J_{ave}) was calculated from the penetration profile using the equation:

$$J_{ave} = \frac{1}{A}\frac{dM}{dt} \quad (4)$$

Where $\frac{dM}{dt}$ is the average slope of a straight line obtained by a linear regression of the M versus t profile over the experimental timeframe, and A is the diffusional area[11].

Estimates for all these parameters are presented in Table 1.

Results and discussion

Diffusion experiments using cellulose membrane

The permeation of solutes through biological membranes is a very complicated phenomenon. The human membrane is greatly heterogeneous. The permeation of

Table 1. Effect of the presence of OLZ on FLX HCL penetration through the human cadaver skin; Physicochemical parameters of OLZ itself.

Drug molecules	J_{ave} (mg/cm^2sec)	P (cm/hour)	T lag (hour)
FLX HCl alone	0.4 (0.3)	0.55×10^{-3} (0.2)	24 (0.4)
FLX HCl in the presence of OLZ	6.9 (0.4)	2.3×10^{-3} (0.2)	8 (0.5)
OLZ	1.94 (0.2)	1.2×10^{-4} (0.3)	14 (0.3)

Standard deviations are shown in parenthesis.

solutes depends also on the physicochemical parameters of the formulation such as density and viscosity that affect the rate of diffusion of the solute through the skin into the blood stream[22]. Therefore, a highly permeable artificial membrane of a known physicochemical structure was initially used to determine any interaction between the drugs and the formulation system.

Determination of drug interaction and release characteristics of gels

In this phase of the research, gel-like formulations and cellulose membrane were used. The mechanism of diffusion that takes place in this type of formulations is called interstitial mechanism of diffusion; the molecules naturally dissolve in a host lattice by occupying interstitial positions. The interstitial molecules take up regular positions in certain energetically favored interstices[23]. The drugs that were released separately and in combination were evaluated by using three different gelling agents: Klucel®, Methocel®, and Cellulose gum® at four different concentrations (0.5%, 1%, 1.5%, and 2%). The cumulative amount of the drug versus time was plotted, and the percentage release of each drug through each gelling agent was calculated. The results have shown that the release profiles of the drugs acting alone or in combination are identical and this is consistent for all gelling agents. Therefore, their diffusion properties remain the same whether or not they are together in the formulation. Also, it was found that the release profiles of these drugs were independent of the presence of each other, which indicates that there was no interaction between the two drugs. Also, the data revealed that the best system with the best release was Klucel® gel 0.5% compared with other hydrogels. In fact, 87% of FLX HCl and 72% of OLZ were released. It also appeared that by increasing the polymer concentration, the percentage of release of the drugs decreased. This pattern is common for all three gelling agents investigated. This may be due to the fact that gels form a polymeric network that resists flow by entrapment and immobilization of solvent molecules. This polymeric network is made of polymeric chains with a variety of structures; one such structure is called "random coil," which is the least ordered and occurs most frequently with synthetic polymers, such as resin and cellulose derivatives[24]. So, the random coils represent the main diffusion barrier. Therefore, the higher the concentration of polymer in the gel, the more crowded the random coils become, and the more difficult the drug release from the gel[25].

Determination of possible complexation of the drugs with Klucel®

To evaluate possible complexation of the drugs with Klucel® (0.5%), as was mentioned above, two compartments, one containing the drug and the polymer and the other containing the drug in solution, were equilibrated for 1 week. After 1 week, the concentration of the drug in the solution compartment did not change (Table 2), indicating the absence of drug–polymer complexation.

Diffusion experiments using human cadaver skin

The evaluation of the diffusion of the drugs acting alone and in combination through human cadaver skin is shown in Figure 1. As can be seen, only 43.7 μg (1.33%) of the FLX HCl released after 220 hr, when alone in the formulation. However, in the presence of OLZ, the release of FLX HCl was increased to 732 μg (24.3%) after 220 hr. In contrast, 183.7 μg (6.6%) of OLZ released after 220 hr either alone or in combination with FLX HCl. So, as can be seen, the release profile of FLX HCl in the presence of OLZ through human cadaver skin significantly increases,

Table 2. Equilibrium data for evaluation of any possible complexation between the polymer and each drug incorporated in the polymer matrix separately or together.

	Concentration before 1 week		Concentration after 1 week	
Drug	Drugs alone	Drugs in combination	Drugs alone	Drugs in combination
OLZ	0.499 mg/ml	0.496 mg/ml	0.497 mg/ml	0.494mg/ml
FLX HCL	0.484 mg/ml	0.479 mg/ml	0.478 mg/ml	0.477mg/ml

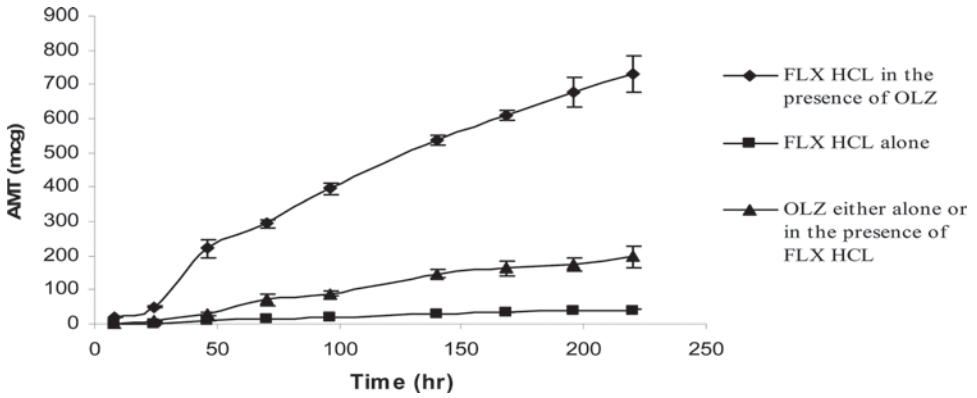


Figure 1. Release profiles of OLZ (0.1%) alone or in the presence of FLX HCl (0.1%), and FLX HCl (0.1%) alone or in the presence of OLZ.

while the release through cellulose membrane clearly showed identical release profiles whether or not FLX HCl was alone or in combination with OLZ. This finding suggests that some unique enhancing properties of lipophilic OLZ has been uncovered.

Therefore, a series of experiments were designed to further investigate this phenomenon.

1. FLX HCl gel formulation was placed in the donor compartment for 48 hr. At the end of 48 hr, the samples of FLX HCl were analyzed, and the amount of FLX HCl appeared to be negligible. Then, an equivalent quantity of OLZ was added, and as we can see in Figure 2, the release profile of FLX HCl started to change drastically and reached 650 μg (20%) at the end of 220 hr. In addition, as previously noted, the release profile of OLZ remained constant. Controlled cells with OLZ alone and FLX HCl alone were run in the same pattern. So, it is apparent that OLZ has an influence on the transdermal delivery of FLX HCl.
2. In this experiment, the skin was pretreated with OLZ gel formulation for 48 hr; then, the gel was removed and the cadaver skin was washed out using an isotonic phosphate-buffer solution, and the receiving media was replaced by a fresh one and the donor compartment was loaded with FLX HCl gel formulation. The amount of FLX HCl released after the skin was treated with OLZ for 48 hr was 85 μg (2.9%) after 220 hr compared with the control sample of FLX HCl, which was 43 μg (1.33%) after 220 hr (Figure 3). So, this indicates that the enhancement property of OLZ in the cadaver skin is reversible. Once OLZ is taken away from the skin surface, its influence on the skin barrier function vanishes. It is actually one feature, which ideal enhancers should possess. The mechanism of reversibility could be attributed to the insertion of the enhancer within the stratum corneum intercellular lipid lamella. This disruption of the intercellular lipid matrix eases the permeation of the drugs through the tortuous pathway. When the enhancer is removed, bonds between the lipids would start reform and the depletion of the enhancer would allow the packing of the lipids to revert back to its original alignment[26]. A control sample of FLX HCl alone (0.1%) was run, as well for comparison purposes. So, we can conclude that OLZ enhances the release of FLX HCl when released together, and OLZ has a reversible action on the skin.
3. Concentration-dependency studies: Four different concentrations (0.05%, 0.1%, 1.5%, and 2%) of OLZ

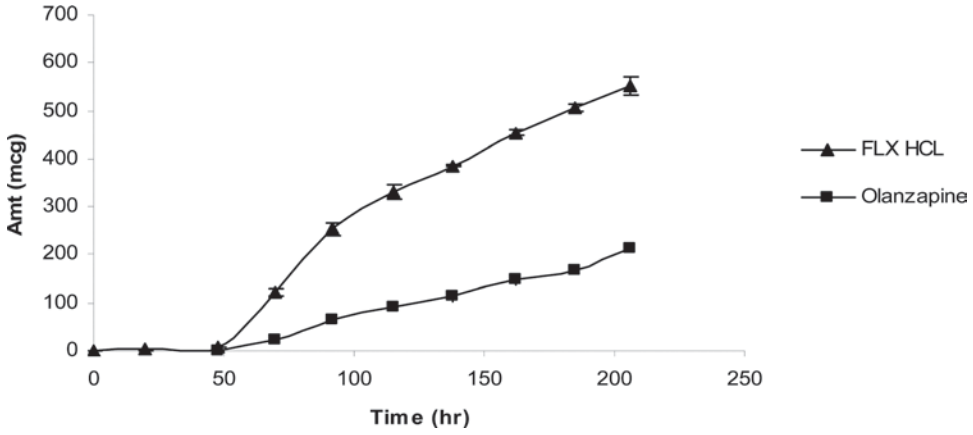


Figure 2. Release of FLX HCl (0.1%) from Klucel® gel formulation (0.5%) through cadaver skin, while OLZ was added at 48 hours.

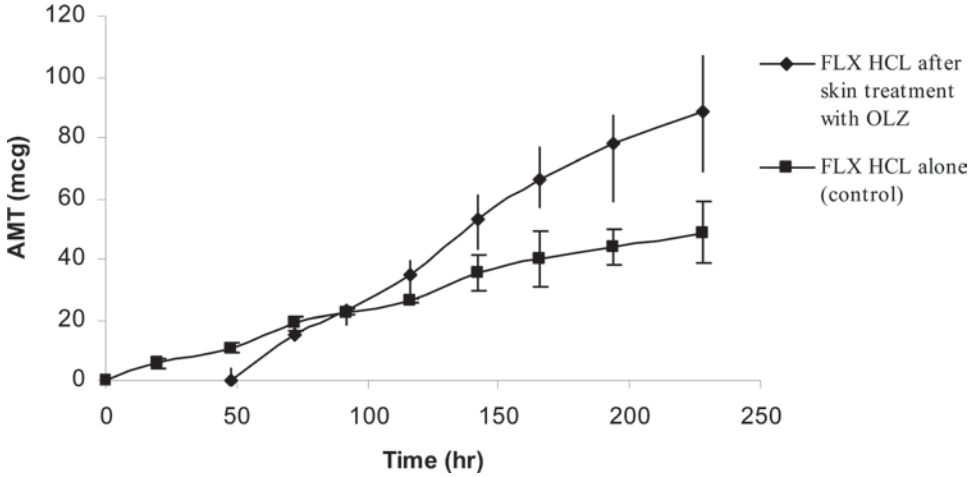


Figure 3. Release of FLX HCl (0.1%) from Klucel® (0.5%) through cadaver skin, previously treated with OLZ for 48 hours.

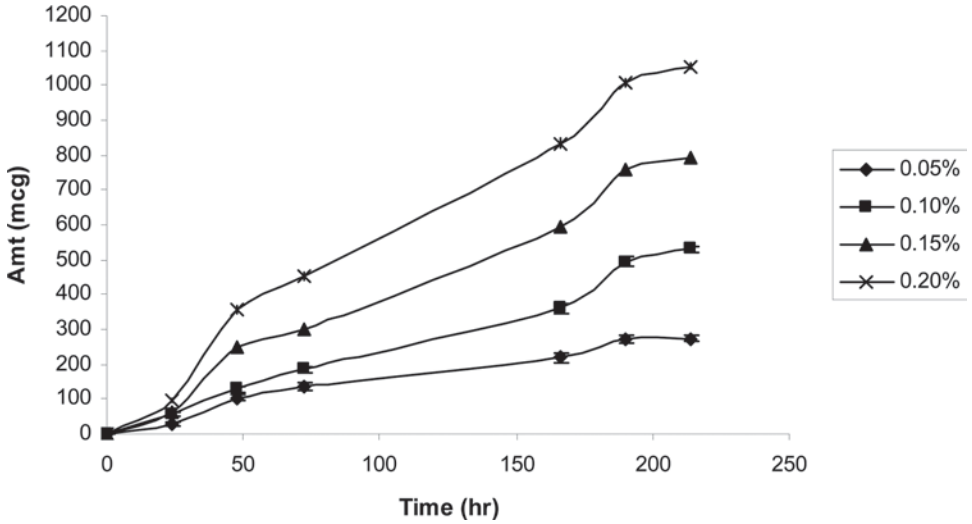


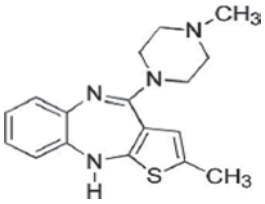
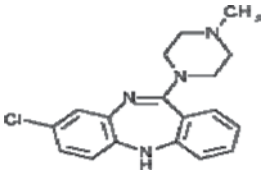
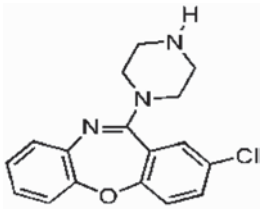
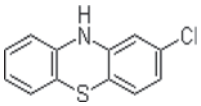
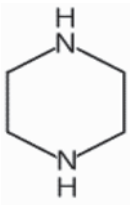
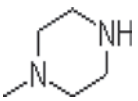
Figure 4. Permeation of FLX HCl (0.1%) in the presence of different concentrations of OLZ (0.05%, 0.1%, and 0.2%).

with the constant amount of 0.1% of FLX HCl were prepared, and the release studies were conducted as previously described. The results are shown in Figure 4. As can be seen on this graph, the release of FLX HCL is proportional to the concentration of OLZ. So, the enhancement of the properties of OLZ is concentration dependant.

Further studies were carried out to investigate if this unique enhancing property of OLZ is related only to FLX HCl, so other hydrophilic and lipophilic drugs were studied. Therefore, doxepine HCl was chosen as a hydrophilic drug, and simvastatin represented the lypophilic compound. It was found that OLZ effectively increased the permeation of doxapine HCl from 29 μg (0.9%) after 220 hr when released alone versus to the 630 μg (20.5%) after 220 hr when released in combination with OLZ. However, OLZ had no effect on the lypophilic drug simvastatin.

Therefore, the next logic step was to investigate if the whole molecule or some particular group of OLZ is responsible for its enhancement properties. A variety of different compounds structurally related to OLZ were investigated. All of them resemble more or less of either the whole molecule of OLZ or some part thereof. These compounds included clozapine, amoxapine, 2-chlorophenothiazine, piperazine, and 1-methylpiperazine. All these compounds were run together with FLX HCl as previously described in Table 3. All these four compounds OLZ, clozapine, amoxapine, and 2-chlorophenothyazine belong to diarylazepine derivatives. They all have a tricyclic ring, which is differently modified. OLZ's tricyclic ring has a thiophene where methyl group is introduced in position 2. Amoxapine's and 2-chlorophenothiazine's tricyclic rings differ by a replacement of the central heteroatom NH by classical isomeric substitution (O.S). Also, their tricyclic rings differ structurally from OLZ on account of the substitution of the thiophene ring by a benzene ring. Despite the fact that OLZ, clozapine and amoxapine have

Table 3. Percent release of FLX HCl in the combination of OLZ and OLZ structurally related compounds.

Drugs used in combination with FLX HCL	% of FLX HCL
Fluoxetine HCl control	1.33%
Olanzapine 	23.4%
Clozapine 	20.6%
Amoxapine 	37.5%
2-Chlorophe 	No effect
Piperazine 	55.9%
1-Methylpiperazine 	21.3%

modified tricyclic rings, they all possess enhancement properties. However, 2-chlorophenothiazine did not exhibit any enhancement activity. This difference could be related to the piperazine ring that is attached to all three drugs but is absent in 2-chlorophenothiazine. Therefore, piperazine and 1-methylpiperazine were tested as well and were found to possess the enhancement properties. In fact, piperazine has the highest enhancement effect among all tested compounds. Apparently, the absence of methyl group from piperazine ring is associated with the increscent of enhancement activity. Also, amoxapine with the attached piperazine ring has a higher enhancement effect than OLZ and clozapine, which have the methyl group in piperazine moiety.

From the above findings, it appears that the piperazine ring attached to the tricyclic system is essential for its enhancement activity of FLX HCl. Furthermore, it appears that the presence of methyl group decreases the enhancing activities of the piperazine moiety. Diffusion of the molecule through the stratum corneum is known to be the slowest step due to intercellular lipids that are arranged in bilayer. Ceramides, which are abundantly present in stratum corneum, are tightly packed in the bilayer due to high degree of hydrogen bonding (through amide groups at the head of ceramides). This hydrogen bonding provides strength and stability to lipid bilayer. One of the ways an enhancer modulator can work is by insertion into the bilayers with disruption of bilayer structure[27]. In our studies, the enhancement effect of OLZ and its structure-related molecules might be due to incorporation itself between ceramides hydrogen bonds and as a result loosening bilayer network.

The higher enhancement properties of piperazine ring versus 1-methylpiperazine ring might be due to reduction in negative partial charge. This reduction is less on the 1-methylpiperazine and this probably does not provide enough interaction with the–OH group of the ceramides to insert itself in the polar head group region. Also, piperazine ring is a good hydrogen bond acceptor[28], and NH group is more likely to participate in hydrogen bonding versus NCH_3 group. Another possibility of higher enhancement properties of piperazine ring versus 1-methylpiperazine ring would be the ability of piperazine ring to adopt a chair conformation[29], which energetically is more favorable than 1-methylpiperazine molecule due to higher bulkiness. So, piperazine molecule more easily incorporates inside the lipid lamella and easily disrupts its structure, letting the drug molecules to penetrate more easily.

Conclusions

The release of the selective serotonin reuptake inhibitor, FLX HCl, and the atypical antipsychotic, OLZ, acting alone and in combination was investigated through several membranes. Using cellulose membranes and a variety of concentrations of Klucel®, Methocel®, and Cellulose gum® donor systems, the system with the fastest release of the drugs was chosen for further studies. A final formulation of Klucel® 0.5% was selected. Studies with cellulose membrane showed that there is no interaction between these two drugs and their release profiles are independent of each other. Studies with human cadaver skin yielded very interesting results: OLZ significantly enhanced the release of FLX HCl. FLX HCl permeation appeared to be 17 times higher when it was run in combination with OLZ compared with the release of FLX HCl alone. Also, the follow-up studies indicated that the enhancement effect of OLZ on the skin is reversible and concentration dependant. In addition, studies with hydrophilic drug doxepine HCl and lypophilic simvastatin were conducted. It was found that OLZ has an enhancement effect on hydrophilic compounds, but it did not show any enhancement activity on their lipophilic counterparts. Furthermore, studies with a variety of compounds that structurally resemble OLZ molecule showed that the piperazine ring attached to the tricyclic ring of OLZ is responsible for the enhancement activity of OLZ and all other diarylazepine derivatives tested. We hypothesized that the way OLZ might work is by insertion of its molecule into the bilayers and disrupt its structure allowing the drug molecule to penetrate more easily.

To further elucidate the underlying mechanism of enhancement properties of OLZ and of all its structurally related analogous, studies are currently in process and results will be reported in the next article.

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Declaration of interest

The authors report no declaration of interest.

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