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Transdermal drug delivery of paroxetine through lipid-vesicular formulation to augment its bioavailability

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ABSTRACT

Paroxetine (PAX) is the most potent serotonin reuptake blocker antidepressant clinically available. This study is aimed to reduce the side effects accompanied with the initial high plasma concentration after oral administration of PAX and fluctuations in plasma levels and also to decrease the broad metabolism of the drug in the liver by developing and optimizing liposomal transdermal formulation of PAX in order to improve its bioavailability. PAX liposomes were prepared by reverse phase evaporation technique using lecithin phosphatidylcholine (LPC), cholesterol (CHOL) and drug in different molar ratios. The prepared liposomes were characterized for size, shape, entrapment efficiency and *in vitro* drug release. The studies demonstrated successful preparation of PAX liposomes. The effect of using different molar ratios of (LPC:CHOL) on entrapment efficiency and on drug release was studied. Liposomes showed percentage entrapment efficiency (%EE) of 81.22 ± 3.08% for optimized formula (F5) which composed of (LPC:CHOL, 7:7) and 20 mg of PAX, with average vesicle size of 220.53 ± 0.757 nm. The selected formula F5 (7:7) was incorporated in gel bases of HPMC-E4M (2%, 4%, and 6%). The selected formula of PAX liposomal gel of HPMC-E4M (2% and 4%) were fabricated in the reservoir type of transdermal patches and evaluated through *in vitro* release. After that the selected formula of PAX liposomal gel transdermal patch was applied to rabbits for *in vivo* bioavailability study in comparison with oral administration of the marketed PAX tablet.

An HPLC method was developed for the determination of PAX in plasma of rabbits after transdermal patch application and oral administration of the marketed PAX tablets of 20 mg dose. The intra- and inter-day accuracy and precision were determined as relative error and relative standard deviation, respectively. The linearity was assessed in the range of 5–200 ng/ml.

Pharmacokinetic parameters were determined as the C_{max} of PAX liposomal transdermal patch was found to be 92.53 ng/ml at t_{max} of 12 h and AUC_{0-48} was 2305.656 ng h/ml and $AUC_{0-\infty}$ was 3852.726 ng h/ml, compared to the C_{max} of 172.35 ng/ml after oral administration of the marketed PAX tablet with t_{max} of 6 h and AUC_{0-24} was 1206.63 ng h/ml and $AUC_{0-\infty}$ was 1322.878 ng h/ml. These results indicate improvement of bioavailability of the PAX after liposomal transdermal patch application and sustaining of the therapeutic effects compared to oral administration.

1. Introduction

The main objective of drug delivery systems is to deliver a drug effectively, specifically to the site of action and to achieve greater efficacy and minimize the toxic effects compared to conventional drugs. Liposomal vesicles were prepared in the early years of their history from various lipid classes identical to those present in most biological membranes. Basic studies on liposomal vesicles resulted in numerous methods of their preparation and characterization (Shivhare et al., 2009). Liposomes are broadly defined as lipid bilayers surrounding an aqueous space. Multilamellar vesicles (MLV) consist of several (up to 14) lipid layers (in an onion-like arrangement) separated from one another by a layer of aqueous solution. These vesicles are over several hundred nanometers in diameter. Small unilamellar vesicles (SUV) are surrounded by a single lipid layer and are 25–50 nm (according to several authors) up till 100 nm in diameter. Large unilamellar vesicles (LUV) are, in fact, very heterogeneous group of vesicles that, like the SUVs, are surrounded by a single lipid layer. The diameter of these liposomes is very broad, from 100 nm up to cell size (Panwar et al., 2010; Woodle and Papahadjopoulos, 1989). The liposomes encapsulate a hydrophilic drug within an aqueous component, while liposomes also entrap the lipophilic drug within the lipid bilayers (Barenholz, 2003; Ying et al., 2010). Liposomes are microscopic lamellar structures can be prepared from components like soya

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lecithin, cholesterol and aqueous phase by reverse phase evaporation (Hofkens et al., 2011; Mozafari et al., 2008; Szoka and Papahadjopoulos, 1978). Liposomes have been widely evaluated for controlled and targeted drug delivery for treatment cancer, viral infections and other microbial diseases. Liposomes are found to be suitable for localization of topically applied drugs at or near the site of application, due to fact that they may act as slow releasing vesicles.

Phospholipids such as phosphatidylcholine (LPC) and cholesterol (CHOL) were selected for the formation of liposomes into which the drug was incorporated. Cholesterol was incorporated into phospholipids membranes in very high concentration up to 1:1 or 2:1 molar ratio (Gregoriadis, 2006; Maherani et al., 2011).

CHOL acts as 'fluidity buffer' since below the phase transition it tends to make membrane less ordered while above transition it tends to make membrane more ordered thus suppressing the tilts and shifts in membrane structure specifically at phase transition (Lesoin et al., 2011; Socaciu et al., 2000).

Liposomes are used as carriers to deliver the entrapped drugs into the skin, but the major limitation of using liposomes topically is the liquid nature of the preparation. Because topically applied liposomes may leak from the application site. So this challenge can be overcome by their incorporation in an adequate vehicle where original structure of vesicles is preserved and their rheological and/or mucoadhesive properties are adjusted. This can be achieved by adding gelling agents in liposomal dispersions forming liposomal hydrogels (Mourtas et al., 2008).

Liposomes can also be entrapped in hydrogels. Liposomes entrapped in carbopol and hydroxyethylcellulose-based hydrogels can control the release of calcein and griseofulvin according to the rigidity of the liposomal membrane (Mourtas et al., 2007). Liposomes entrapped in poly (hydroxyethyl methacrylate) hydrogels mimicking contact lenses can control the release of anti-glaucoma drugs for up to 8 days (Gulsen et al., 2005).

Thereby, a drug-in-liposomes-in-gel complex formulation can be developed. The release of drug molecules from such liposomal gels depends on the stability of the liposomes (membrane integrity and mechanical stability) during their dispersion in the semisolid formulation (Mourtas et al., 2008).

The delivery of drugs into and through the skin is recognized as an effective means of therapy for local dermatologic and systemic diseases. In recent years, transdermal delivery of drugs for systemic and local effect has gained considerable attention, because they eliminate first-pass effect, provide sustained plasma levels and improve patient compliance. Gel base formulations make the drug molecules more easily remove from the system than cream and ointment ones (Pavelic et al., 2001).

Transdermal delivery is the best suited for drugs, which display high toxicity and/or narrow therapeutic widows (Chandak and Verma, 2008). Transdermal patches have the aim to transport drugs through the skin into the blood circulation. One of the proposed advantages of transdermal delivery is the possibility to attain sustained and constant drug levels (Valenta and Auner, 2004).

Paroxetine (data retrieved from website, 2011) is the most potent serotonin reuptake blocker antidepressant clinically available, but has a lower selectivity for the serotonin reuptake site than either fluvoxamine or sertraline. In addition, it blocks muscarinic acetylcholine receptors to almost the same degree as the Tricyclic Antidepressants (TCAs) imipramine or doxepin, and even more effectively than desipramine or maprotiline. PAX is efficiently absorbed from the gastrointestinal tract, but is readily metabolized during its first pass through the liver. The $t_{1/2}$ is variable, depending on both dose and duration of administration. Half-life up to 21 h after oral administration of 30 mg of paroxetine/day (Hiemke and Härtter, 2000; Knorr and Kessing, 2010).

In this study PAX was encapsulated in liposomes and evaluated for the encapsulation efficiency, particle size and zeta-potential, Differential Scanning Calorimetry analysis (DSC) and *in vitro* drug release. The selected PAX liposomal dispersion was incorporated in gel bases of HPMC-E4M (2%). The selected formula of PAX liposomal gel of HPMC-E4M (2%) was fabricated in the reservoir type of transdermal patches and evaluated through *in vitro* release. After that, the transdermal patch formula was applied to rabbits for *in vivo* bioavailability study compared to oral drug administration of the market product.

2. Materials and methods

2.1. Materials

PAX was obtained from Glaxosmithkline Beecham (England), L-α-phosphatidylcholine P3644-25G from soybean and cholesterol, from Sigma Chemical Co (St. Louis, MO, USA). Spectra/pore dialysis membrane, 12,000–14,000 molecular weight cut off (Spectrum Laboratories Inc., USA). Hydroxypropyl methylcellulose-E4M (Tama, Tokyo, Japan). All the other chemicals, reagents and solvents used like potassium dihydrogen orthophosphate, disodium hydrogen phosphate, sodium hydroxide pellets, acetone, chloroform, and methanol were of analytical reagent grade.

2.2. Preparation of PAX liposomes

PAX large unilamellar and oligolamellar liposomes were prepared using the reverse-phase evaporation (Hofkens et al., 2011) technique according to Szoka and Papahadjopoulos (Mozafari et al., 2008; Szoka and Papahadjopoulos, 1978) as follows: the liposomal components (LPC:CHOL), equivalent to 200 mg, were weighed into 100 ml round bottomed flask and dissolved in 10 ml of chloroform. The organic solvent system was slowly evaporated under reduced pressure, using rotary evaporator (Heidolph-Laborota 4000, D-91126, Germany), at 40 °C and at 60 rpm for 15 min, such that a thin film of dry lipid was formed on the inner wall of the rotating flask.

The lipid film was re-dissolved in 5 ml of diethyl ether, and the drug solution (20 mg of PAX in 5 ml of acetone) and 10 ml of phosphate buffered saline (PBS), pH 7.4, was added at this point. The resulting two-phase system was sonicated for 2 min in bath-type sonicator. The mixture was then placed on the rotary evaporator and the organic solvents were removed under reduced pressure at 40 °C and 60 rpm for 15 min. The liposomes were allowed to equilibrate at room temperature, and the liposomal suspension was kept in the refrigerator (4 °C) to mature over night (Kallinteri et al., 2002; Manosroi et al., 2004; Rossi-Bergmann et al., 2011).

2.3. Evaluation of PAX liposomes

2.3.1. Determination of PAX entrapment efficiency percent (%EE) in liposomes

The trapped PAX containing liposomes were separated from unentrapped PAX by cooling centrifugation (Megafuge 1.0/1.0R, Kendro Laboratory Products (Fussex, UK)) of a known aliquot (1 ml) of the prepared liposomal suspension at 15,000 rpm for 20 min at 4 °C (Fang et al., 2004; Tabandeh et al., 2010). The supernatant was separated from the liposomal precipitate. The precipitated liposomes was washed by 1 ml of PBS, pH 7.4, and recentrifuged for 20 min to remove excess unentrapped PAX, then the combined supernatant was diluted to 10 ml by PBS (pH 7.4). The concentration of unentrapped paroxetine was determined spectrophotometrically (Model 6705-Jenway, Multicell Changer, BibbyScientific Ltd., UK) by measuring the U.V. absorbance at λ 294.3 nm.