Pharmaceutical Research, Vol. 26, No. 11, November 2009 (© 2009)
DOI: 10.1007/s11095-009-9956-5

Research Paper

Utility of Nanosized Microemulsion for Transdermal Delivery of Tolterodine Tartrate: *Ex-Vivo* Permeation and *In-Vivo* Pharmacokinetic Studies

Ahmed H. Elshafeey,[1,3,4] Amany O. Kamel,[2] and Mohsen M. Fathallah[3]

Received March 30, 2009; accepted August 11, 2009; published online August 21, 2009

Purpose. The aim of this work was to investigate the feasibility of using nanosized microemulsion for transdermal delivery of tolterodine tartrate.
Methods. The effect of three microemulsions formed by Labrasol: Plurol (3:1), isopropyl myristate and water on the permeation of tolterodine through miniature pig skin was studied *in vitro* using Franz diffusion cell. For comparison purpose, the effect of different vehicles on the permeation was also studied. Drug pharmacokinetics was studied after transdermal application to human volunteers compared to the commercial oral dosage form using a newly developed LC-MS/MS assay.
Results. The vehicle PEG 400:Phosphate buffer pH 7.4 in the ratio of 1:1 significantly enhanced tolterodine permeation across pig skin. The microemulsion system (ME3) containing the highest amount of water (50%) significantly enhanced permeation with Q_{24} of 0.746 mg.cm^{-2}. In contrast to oral delivery, a sustained activity was observed over a period of 72 h after transdermal application of this microemulsion to human volunteers with significant lower C_{max} (1.06 ng/ml), delayed T_{max} (3.17 h) and higher MRT value (147.82 h) ($p<0.05$).
Conclusion. This sustained activity was due to the controlled release of drug into the systemic circulation with expected increase in the patient compliance and prevention of nocturnal enuresis.

KEY WORDS: LC/MS/MS; microemulsion; pharmacokinetics; tolterodine tartrate; transdermal.

INTRODUCTION

Overactive bladder (OAB) affects up to 20% of the world population and is characterized by urinary urgency, with or without urge incontinence, often accompanied by increased urinary frequency and nocturia (1). The overall prevalence of OAB is similar in men and women but increases with age and may become higher in men after 75 years of age (2). Benign prostatic hyperplasia and obstruction are conditions that may coexist in men and potentially complicate OAB treatment (3). OAB is known to impair health-related quality of life and to increase symptoms of depression (4). It is well-established that orally administered antimuscarinic drugs are effective in reducing symptoms and improving health-related quality of life in patients with OAB (5). However, patients often discontinue therapy with orally administered antimuscarinic medications because of systemic anticholinergic effects, such as dry mouth (6).

Tolterodine tartrate, ((*R*)-*N*,*N*-diisopropyl-3- (2-hydroxy-5-methylphenyl)-3-phenyl-propanamine l-hydrogen tartrate), is a potent and competitive muscarinic receptor antagonist used for the treatment of urge incontinence and other symptoms of unstable bladder. Tolterodine tartrate has a high affinity and specificity for muscarinic receptors *in vitro* and exhibits the selectivity for the urinary bladder over salivary glands *in vivo* (7). The usual dose of tolterodine is 4 mg/day (2 mg twice daily) (8). The absolute bioavailability of tolterodine was highly variable, ranging from 10 to 70% (9).

Transdermal route is an alternative to oral administration for antimuscarinic medication as it reduces the incidence of dry mouth and other anticholinergic adverse effects. Also, transdermal application will decrease the dose frequency and, hence, improve patient compliance.

For successful transdermal delivery, it is necessary to reversibly overcome the skin barrier. Different strategies have been proposed to overcome the skin barrier. Nanosized delivery systems, such as liposomes, nanoparticles and microemulsions, contribute to better skin delivery of drugs (10,11). These colloidal systems have attracted attention as potential delivery systems for targeting the drug, controlling its release, and increasing its availability. With the appropriate composition of nanosized carriers, enhanced permeation of drugs to deeper layers of skin or the systemic circulation has been achieved (11).

Microemulsion vehicles have a significant potential to increase penetration of hydrophilic, lipophilic, and amphiphilic substances into and through the skin compared to conventional vehicles (12–14).

The aim of the present study was to investigate the effects of microemulsion systems on the *in vitro* permeation of

[1] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.
[2] Department of Pharmaceutics, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.
[3] Genuine Research Center, Cairo, Egypt.
[4] To whom correspondence should be addressed. (e-mail: Ah_elshafeey@hotmail.com)

tolterodine tartrate across miniature pig skin. For comparison purpose, different solvents and co-solvents were studied as well. Furthermore tolterodine tartrate pharmacokinetics were studied after transdermal application to human volunteers compared to the commercial immediate release oral dosage form.

MATERIALS AND METHODS

Materials

Tolterodine tartrate, oleic acid, and ethyl acetate were obtained from Sigma, St. Louis, Mo, USA. Transcutol®, Labrasol®(PEG-8 caprylate/caprate), Plurol Di-isostearique (Polyglycerol di-isostearate), were from Gattefossé, St. Priest, France. Polyethylene glycol 400 NF (PEG 400), isopropyl myristate (IPM) were provided by Union Carbide Chemicals, Danbury, C.T, USA. Methanol and acetonitrile were from Sigma Aldrich, USA. All other chemicals were of analytical grade.

Development of LC-MS/MS Assay of Tolterodine Tartrate

A sensitive, selective and accurate LC-MS/MS method was developed and validated before the study for determination of tolterodine tartrate concentrations both *in vitro* and *in vivo*. The method was validated following international guidelines (15). All chemicals and reagents were of analytical grade, solvents used were of HPLC grade. Azithromycin internal standard (IS) stock solution was prepared by dissolving 10 mg in methanol and serially diluted with mobile phase to give a final working concentration of 50 ng/ml. A shimadzu Prominence (Shimadzu, Japan) series LC system equipped with degasser (DGU-20A3), solvent delivery unit (LC-20AB) along with auto-sampler (SIL-20 AC) was used to inject 10 µl aliquots of the processed samples on a Luna C_{18} (phenomenex, USA) (50×4.6) mm, 5 µm particle size. The Guard column was phenomenex C_{18} (5×4.0) mm, 5 µm particle size. All analysis was carried out at room temperature. The isocratic mobile phase (pH 4.5) consisted of acetonitrile and (0.02 M) ammonium acetate buffer (70%, 30%, v/v) and 0.1% formic acid which was delivered at a flow rate of 0.50 ml/min into the mass spectrometer's electrospray ionization chamber. Quantitation was achieved by MS/MS detection in positive ion mode for both tolterodine tartrate and IS, using a MDS Sciex (Foster City, CA, USA) API-3200 mass spectrometer, equipped with a Turbo ionspray™ interface at 300°C. The ion spray voltage was set at 5500 V. The common parameters, viz., nebulizer gas, curtain gas, auxillary gas and collision gas were set at 14 psi, 25 psi, 30 psi and 11 psi, respectively. The compound parameters, viz., declustering potential (DP), collision energy (CE), entrance potential (EP) and collision exit potential (CXP) were 41 V, 35 V, 6 V, 4 V for tolterodine and 66 V, 53 V, 12 V, 4 V for azithromycin (IS), respectively. Detection of the ions was performed in the multiple reaction monitoring (MRM) mode, monitoring the transition of the *m*/*z* 326.24 precursor ion to the *m*/*z* 147.1 for tolterodine and *m*/*z* 749.38 precursor ion to the *m*/*z* 158.2 for IS. Quadrupoles Q1 and Q3 were set on unit resolution. The analytical data were processed by Analyst software (Version 1.4.2).

Determination of Tolterodine Tartrate Saturated Solubility in Different Solvents or Co-solvent Systems

The saturated solubility of tolterodine tartrate was determined in a variety of solvents and co-solvents. The solvents used were water, Transcutol, polyethylene glycol 400 (PEG 400), ethyl acetate, phosphate buffer pH 7.4 and oleic acid. The co-solvents used were 50% propylene glycol in isotonic phosphate buffer (pH 7.4) and oleic acid:Transcutol in 1:1 ratio. Excess amount of tolterodine was suspended in different media in tightly closed screw cap vials equilibrated in a rotating bottle for 24 h at room temperature. Then, an aliquot of the suspensions was transferred to a 1-ml micro-centrifuge filter, fitted with a 0.22 µm nylon filter (Corning Incorporated, Corning, NY, USA), and centrifuged. The filtrates were appropriately diluted with methanol and assayed by LC-MS/MS as previously described. Three replicates were done for each solvent and co-solvent.

Preparation of Different Tolterodine Tartrate Liquid Systems

Different tolterodine tartrate liquid systems were prepared by dissolving or suspending 20 mg/ml of tolterodine tartrate in different solvents viz. oleic acid, a mixture of propylene glycol in isotonic phosphate buffer of pH 7.4 (1:1) or Transcutol/oleic acid of the same ratio.

Preparation of Tolterodine Tartrate Microemulsion Systems

All microemulsion systems were prepared with double distilled water to avoid the surface active impurities. Appropriate amounts of water, isopropyl myristate (IPM) as an oily phase and Labrasol: Plurol in 3:1 w/w ratio as a surfactant/co-surfactant mixture were weighed into 10 ml teflon sealed screw-cap glass vials. At the beginning, IPM was added to the surfactant/co-surfactant mixture and stirred continuously for one minute until it became homogenous. Then, the calculated amount of water was added using a micro-syringe with continuous stirring for five minutes. Drug-loaded microemulsion systems were prepared by adding 2% w/w tolterodine tartrate to the calculated amount of water and then added to the oily phase/surfactant: co-surfactant mixtures (16). The composition of the different prepared systems is shown in Table I.

Microemulsion Evaluation

Visual Examination

The prepared microemulsions were examined for their clarity under neon light source.

Stability Study

Each prepared microemulsion of 4 ml volume was centrifuged for five minutes at 5000 rpm and then examined for any phase separation of its components.

Viscosity Measurements

The viscosity of each microemulsion containing different percentages of water content was determined by using digital

Table I. Basic Composition, Particle Size and Absolute Viscosity Of Different Microemulsion Systems

Microemulsion System	Labrasol/Plurol mix. (% w/w)	IPM (% w/w)	Water (% w/w)	Particle Size±SD (nm)	Viscosity (cp)
ME1	80	10	10	68.4±12.5	375±22
ME2	60	10	30	33.9±8.3	250±15
ME3	40	10	50	25.3±4.7	188±14

IPM isopropylmyristate

Brookfield viscometer equipped with spindle No. S4 at a speed of 30 rpm (Brookfield Viscometer, model LVT, U.S.A.).

Droplet Size

The average droplet size of microemulsions was checked by photon correlation spectroscopy instrument (Marlven, UK) at 25°C.

Ex-Vivo Permeation Study of Tolterodine Tartrate from the Prepared Liquid Formulas and Microemulsion Systems Through Miniature Pig Skin

Ex-vivo permeation studies were performed using miniature pig skin (17). Miniature pigs were sacrificed, and the full thickness abdominal and dorsal skin was excised. Any extraneous subcutaneous fat or artifacts were removed from the dorsal surface. The study performed in this section was approved by the University Protection for Animal Care and Use Committee. The skin was stored in a freezer at -20°C until utilized. Skin was slowly thawed and was cut into small circular pieces of 2.5 cm diameter. The lower surface of the skin was allowed to hydrate for 1 h at 37°C prior to experimentation.

The permeation of tolterodine tartrate from the different prepared liquid systems as well as microemulsion systems was studied through miniature pig skin using flat flange Franz diffusion cells. The membrane (full thickness pig skin) was mounted carefully onto the diffusion cell with the stratum corneum side facing the donor compartment. The donor and the receiver compartments were clamped together, and the receiver compartment was then filled with 5.3 ml of isotonic phosphate buffer of pH 7.4 and stirred with a magnetic stirrer. The receiver buffer was de-aerated before use with a sonicator fitted with vacuum pump to prevent the presence of any air bubbles that might interfere with the permeation process. Volumes of 200 µl of the tested liquid systems under investigation containing 4 mg tolterodine tartrate were placed in the donor chamber and covered with a rubber plug. The active diffusion area was 0.64 cm^2. At different time intervals (1, 2, 4, 6, 8, 12, 20, 24, 36 and 48 h), the receptor solution was completely withdrawn and replaced with equal volumes of fresh de-aerated isotonic phosphate buffer of pH 7.4 maintained at 37°C. Tolterodine tartrate concentration in the receiver fluid was determined using LC-MS/MS as previously described. Each experiment was performed in six replicates.

Data Analysis

The cumulative amounts of tolterodine tartrate penetrating per unit skin area (Q) were plotted against time. From the slope of the linear portion of the plot a steady state flux (SSF) was determined. The permeability coefficient (Kp) was calculated as the ratio of SSF and the critical concentration of tolterodine tartrate in the formula. The permeation parameters such as steady state flux (SSF) and the cumulative amount of drug permeated per unit area after 24 h (Q_{24}) obtained for various formulas were compared using a one-way ANOVA test followed by Fisher's least significant test. Differences between the treatments were assumed to be significant at $p<0.05$. All data analysis and statistical calculations were performed using Stat view Statistical Software 4.57 for Windows (CA, USA).

Pharmacokinetic Study in Healthy Human Volunteers

Study Design and Subjects

A single-dose, two-period randomized cross-over design was adopted under fasting condition. Six healthy adult male volunteers participated in this comparative study. Their mean age was 25.5±4.8 years, mean body weight 76.4±5.9 kg and mean height 164.25±8.3 cm. The purpose of the study was fully explained, and volunteers had given their written consent. The volunteers were instructed to abstain from taking any drug, including over-the-counter (OTC), for 2 weeks prior to and during the study period. The study was performed according to the revised Declaration of Helsinki for bio-medical research involving human subjects (18) and the rules of Good Clinical Practices (GCP) (19). The study protocol was reviewed and approved by the institutional review board of Genuine Research Center, Cairo, Egypt.

Drug Administration and Sample Collection

The volunteers were hospitalized at 9:00 p.m. and had a standard dinner in the clinical site. After an overnight fast (10 h), subjects were given a single oral dose of the commercial product, 2 tablets Detrusitol™ 2 mg tablets (PHAMACIA & UPJOHN), or applied 200 µl topically from the selected tolterodine formulas containing 4 mg drug to the forearm skin using HILL TOP CHAMBER® plain patch with 1 cm diameter according to a randomization plan.

Food and drink (other than water, which was allowed after 2 h) were not allowed until 4 h after dosing, and then a standard breakfast, lunch, and dinner were given to all volunteers according to a time schedule. Between studies, the subjects were domiciliary with instruction. Beverages and food containing caffeine were not permitted over the entire course of study. Volunteers sat or walked around and were prohibited from strenuous activity until the 4 hr blood collection. They were under continual medical supervision at the study site. Adverse events including abnormal labo-

ratory values were spontaneously reported or observed either by the volunteers or the resident physician and were recorded, tabulated and evaluated. Approximately 6 ml blood samples for tolterodine analysis were drawn into evacuated heparinised glass tubes through an indwelling cannula at 0.0; 0.25; 0.5; 0.75; 1.00; 1.50; 2.00; 2.5; 3.00; 4.00; 6.00; 8.00; 12.00; 24.00; 48.00 and 72.00 h after dosing. Blood samples were centrifuged at 3500 rpm for 10 min at 4°C; plasma was transferred directly into 5 ml plastic tubes and stored frozen at −20°C pending drug analysis. After a washing out period of 15 days, the study was repeated in the same manner to complete the crossover design.

Sample Preparation

All frozen human plasma samples were thawed at ambient temperature. Human plasma samples (0.5 ml) were placed in 7 ml glass tubes, and 100 µl of IS solution was added to each and vortexed for 1 min. Four ml Methyl-*t*-butyl-ether (MTBE) was then added, and samples were then vortexed for 2 min. The tubes were then centrifuged for 10 min at 4000 rpm (1790 g). The upper organic phases were then transferred to clean glass tubes and evaporated to dryness using centrifugal vacuum concentrator Vacufuge® 5301 (Eppendorf, Germany) at 40°C. Dry residues were then dissolved in 200 µl of mobile phase and vortexed for 1 min to reconstitute residues, and 20 µl was injected using the autosampler.

Pharmacokinetic and Statistical Analysis

Plasma concentration-time data of tolterodine tartrate was analyzed for each subject by non-compartmental pharmacokinetic models using kinetica® software (version 4.4.1). The peak plasma concentrations (C_{max}) and the time of their occurrence (T_{max}) were directly obtained from the concentration-time data. The area under the plasma concentration-time curve (AUC) from time zero to last measured concentration (AUC_{0-t}) was calculated according to the linear trapezoidal rule. The terminal elimination rate constant (λz) was estimated by linear regression of the terminal portion of the ln (concentration)-time curve, and the elimination half life was calculated.

Two-way analysis of variance (ANOVA GLM procedure; Kinetica ™ 2000 Computer program for a crossover design) was used to assess the effect of formulation, period, and subjects on C_{max} and AUC_{0-t}. Differences between two related parameters were considered statistically significant for *p*-value equal to or less than 0.05.

RESULTS & DISCUSSIONS

Tolterodine Tartrate Solubility in Different Solvents and Co-solvents

Table II shows that tolterodine tartarate solubility was limited in oleic acid and ethyl acetate with values of 5.80 and 6.69 mg/ml, respectively. Solvents having moderate polarity like Transcutol and PEG 400 gave drug solubility of 10.26 and 12.59 mg/ml, respectively. It was also noted that tolterodine tartrate solubility was highly affected by pH changes; its solubility significantly increased from 11.93 mg/ml in distilled water (pH≈5.8) to 25.95 mg/ml in phosphate buffer pH 7.4 ($P<0.05$). Furthermore, phosphate buffer pH 7.4 significantly increased tolterodine tartrate solubility from 12.59 mg/ml in PEG 400 to 21.30 mg/ml in co-solvent system consisting of 1:1 PEG 400 with phosphate buffer pH 7.4 ($P < 0.05$). In addition, Transcutol enhanced the drug solubility in oleic acid from 5.8 to 8.75 mg/ml in oleic acid/Transcutol co-solvent system; however, this difference was not significant. The variability in drug solubility would depend upon the dielectric constant of the tried solvents or co-solvents.

Table II. Solubility Study of Tolterodine Tartrate in Different Solvents and Co-solvents

Solvent Type	Solubility mg/ml ± S.D
Phosphate buffer pH 7.4	25.95±3.65
PEG 400	12.59±2.45
Oleic acid	5.80±1.36
PEG-Buffer (1:1)	21.30±3.89
Transcutol	10.26±0.78
Ethyl acetate	6.69±2.74
Water	11.93±4.14
Oleic acid: Transcutol (1:1)	8.75±2.14

Microemulsion Systems Evaluation

The three microemulsion systems prepared were shown to be very clear and slightly viscous. They did not exhibit any phase separation after centrifugation at 5000 rpm for five minutes. It is evident from Table I that the viscosity of the prepared microemulsions decreased linearly with the increase in water content from ME1 to ME3. The average size of all microemulsion vehicles ranged from 25.3 to 68.4 nm.

Ex-Vivo Permeation Study

Liquid Formulas

Skin permeation rate of drug depends on both the solubility and the diffusion of the drug in the vehicle. The skin permeation studies were carried out with three vehicles. The first was oleic acid because of its well-known property of disrupting the skin lipids (20). The second was oleic acid with 50% Transcutol, because Transcutol can modify skin permeability by shifting solubility parameter of the skin in the direction of that of the permeation (21). Furthermore, the combination of lipophilic and hydrophilic vehicles can improve the permeation rate compared to that obtained from each one individually (22). In order to investigate whether the mechanism of permeation is due to the thermodynamic activity or to the concentration gradient, the unsaturated drug vehicle PEG 400: phosphate buffer (1:1) was also chosen as the third vehicle.

The permeation profile of cumulative amounts of tolterodine tartrate permeated through miniature pig skin from different liquid formulas is shown in Fig. 1. It is obvious that the highest drug permeation observed after 24 h was reached with the system composed of PEG 400: phosphate buffer (1:1) compared to each of oleic acid or oleic acid: Transcutol mixture (1:1). Furthermore, Table III shows that

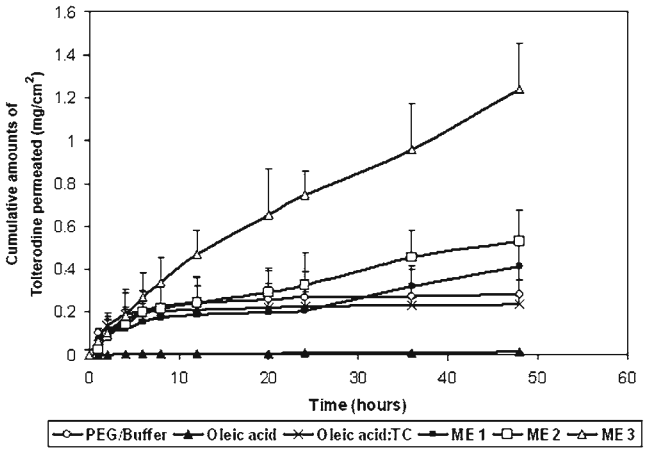

Fig. 1. Permeation profile of cumulative amounts of tolterodine tartrate permeated through pig skin after 48 h from different formulas.

the liquid formulas could be arranged in an ascending order in relation to the cumulative amount of drug permeated after 24 h (Q_{24}) as follows: oleic acid (0.007 mg.cm^{-2})<oleic acid: Transcutol mixture (1:1) (0.223 mg.cm^{-2})<PEG 400: phosphate buffer (1:1) (0.266 mg.cm^{-2}). Relevant to the steady state flux (SSF) of tolterodine tartrate across animal skin, it was observed from Table III that PEG 400:buffer pH 7.4 system had the most significant and highest SSF value (0.019 mg.cm^{-2}.hr^{-1}) at $p<0.05$, while oleic acid and its blend with Transcutol showed nearly the same values. In relation to the permeability coefficients of tolterodine tartrate in the tested liquid preparations shown in Table III, it was obvious that the highest-reached permeability coefficient of tolterodine tartrate was shown by PEG 400:buffer pH 7.4 (1:1) that amounted to be 0.0005 cm.hr^{-1}. However, oleic acid and Transcutol/oleic acid mixture in 1:1 ratio had lower permeability coefficient values of 0.0002 and 0.0001 cm.hr^{-1}, respectively. The latter finding would indicate the superiority of drug diffusivity when placed in PEG 400:buffer pH 7.4 mixture of 1:1 ratio as measured by Q_{24}, SSF and permeability coefficient.

It is worth noting that the cumulative amount of tolterodine tartrate permeated was parallel to its solubility in different vehicles. The higher the drug solubility, the higher the drug permeation through animal skin. Also, the addition of Transcutol to oleic acid significantly increased Q_{24} from 0.007 in oleic acid alone to 0.223 mg.cm^{-2} in the blend between them.

It is clear that the use of different solvents for tolterodine tartrate permeability across pig skin resulted in different permeation profiles. The transport of tolterodine tartrate through the skin includes the following two processes: first, the partition into the stratum corneum (SC) from the vehicle, and second, the diffusion in the SC and partitioning from the SC into viable tissue. The partitioning into the SC from the vehicle is affected by the lipophilic properties of tolterodine tartrate in the vehicle. Partitioning from the SC into viable tissue is influenced by the hydrophilicity of tolterodine tartrate in solution. Therefore, transdermal agents should have appropriate hydrophilicity and lipophilicity (23).

The diffusion in the SC is influenced by the diffusion coefficient and the concentration gradient within the skin in accordance with Fick's law. The concentration gradient of drug within the skin is also influenced by the ability of the solution to partition into the SC and its ability to partition from the SC into the viable tissues (24). The drug solubility in the vehicle is also an important factor for drug penetration across the skin or artificial membranes: the solubility of the drug in the vehicle will influence both the drug concentration gradient in the solution and partition coefficient between the vehicle and the membrane (25). The comparison between tolterodine tartrate solubility in different solvents or co-solvents and its permeation across the animal skin suggests that the main driving-force for tolterodine tartrate permeation is the concentration gradient of the dissolved drug rather than the thermodynamic activity. The amount released is high when drug solubility is high, indicating that the dissolved fraction drives the release. Also, a higher percentage of water in the case of PEG 400/buffer pH 7.4 opens up the compact structure of the horney layer (26). Moreover, the diffusion coefficient can be increased by partitioning of solvents into the SC rapidly to aid drug diffusion and disrupt the ordered intercellular lipids with solvents. PEG as well as Transcutol have these features and therefore satisfy the diffusion coefficient (27).

Microemulsion Systems

Figure 1 shows that ME2 and ME3 caused an increase in the permeation of tolterodine tartrate across the pig skin after 48 h compared to the liquid formulas. ME1 caused an increase in the permeation of tolterodine; it reached a platform between 8 h and 30 h. However, this increase was lower than that of PEG:buffer 1:1 and oleic acid:Transcutol 1:1 liquid formulas. After 30 h, ME1 caused another accelerated linear increase in the permeation of tolterodine.

Microemulsions, in fact, have a very low interfacial tension that improves the contact between vehicle and membrane surface; moreover, the high loading capacity of

Table III. Permeation Parameters of Different Tolterodine Tartrate Transdermal Formulas Through Pig Skin

Liquid Preparations	Q_{24} (mg.cm^{-2}) ± S.D	SSF (mg.cm^{-2}.hr^{-1}) ± S.D	Permeability Coefficient (cm^{-2}.hr^{-1})	r^2
Oleic acid	0.007 ± 0.005	0.0006 ± 0.0002	0.0002	0.911
Oleic acid : Transcutol	0.223 ± 0.083	0.0005 ± 0.0001	0.0001	0.993
PEG 400/Buffer	0.266±0.124	0.019 ± 0.01	0.0005	0.999
ME1	0.206±0.121	0.0018±0.0008	0.0005	0.939
ME2	0.325±0.154	0.0066±0.0011	0.0017	0.992
ME3	0.746 ± 0.112	0.020 ± 0.014	0.0052	0.997

Q_{24} Cumulative amount of tolterodine permeated in 24 h, *SSF* Steady state flux, *r^2* Determination coefficient of linear part of plots

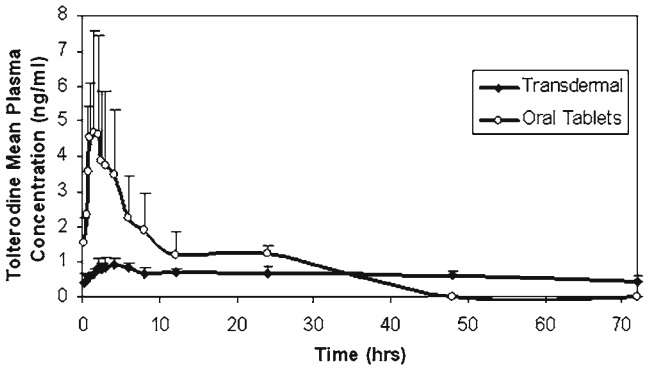

Fig. 2. Tolterodine tartrate mean plasma concentration (ng/ml) after oral administration of Detrusitol™ tablets (PHAMACIA & UPJOHN) and transdermal application of microemulsion system to human volunteers.

microemulsions ensures a concentration gradient suitable to improve permeation of the drug (28).

The microemulsion system composed of Labrasol: Plurol as a surfactant: co-surfactant mixture can be used for transdermal delivery of tolterodine tartrate across pig skin. An important characteristic of this system is that it employs non-irritant surfactant and co-surfactant, suitable for topical application (16). A topically applied microemulsion is expected to penetrate the stratum corneum and exist intact in the whole horney layer as previously suggested in the double labeling studies (29) and the freeze fracture-electron microscopic studies (30). Once it enters into the stratum corneum, microemulsions may simultaneously alter both the lipid and the polar pathways. The lipophilic domain of the microemulsion can interact with the stratum corneum in many ways. Tolterodine tartrate dissolved in the lipid domain of a microemulsion can directly partition into the lipids of the stratum corneum, or the lipid vesicles themselves can intercalate between the lipid chains of the stratum corneum, thereby destabilizing its bilayer structure. These interactions will lead to an increase in the permeability of the lipid pathway to tolterodine tartrate. As a result of the hydrophilic domain of the microemulsion, it can hydrate the stratum corneum to a greater extent; when the aqueous fluid of the microemulsion enters the polar pathways, it will increase the interlamellar volume of stratum corneum lipid bilayers, resulting in a disruption of the interfacial structure. Since some lipid chains are covalently attached to corneocytes, hydration of these proteins will also lead to the disorder of lipid bilayers. Similarly, swelling of the intercellular proteins may also disturb the lipid bilayers, so tolterodine tartrate can then permeate more easily through the lipid pathway of the stratum corneum. The greater drug penetration enhancing activity of microemulsions may be attributed to the combined effects of both the lipophilic and hydrophilic domains of microemulsions (31).

Surprisingly, unlike ME2 and ME3, ME1 did not enhance drug permeation compared to PEG:buffer 1:1 liquid formula until 30 h (Fig. 1). This might be attributed to its higher viscosity (375 cps) that might have slowed permeation. Furthermore, the increase in droplet size of ME1 compared to the other two emulsions caused a decrease in permeation through the pig skin.

It is obvious from Fig. 1 that ME3 showed a remarkable increase in drug permeation compared to ME1 and ME2. Furthermore, as can be observed from Table III, the cumulative amount of drug permeated across the pig skins in 24 h can be arranged in ascending order as follows ME1 ($0.206\ mg.cm^{-2}$)<ME2 ($0.325\ mg.cm^{-2}$) < ME3($0.746\ mg.cm^{-2}$). The fluxes of ME1, ME2 and ME3 were 0.0018, 0.0066 and $0.02\ mg.cm^{-2}.hr^{-1}$ with permeability coefficient of 0.0005. 0.0017 and $0.0052\ cm^{-2}.hr^{-1}$, respectively. As can be seen the Q_{24}, flux and permeability coefficient went parallel with the increase in the amount of water in microemulsions. This might be related to diminution of skin hydration with decrease in water content and consequently lower drug permeation. This diminution in skin hydration could also be attributed to the increase in microemulsion viscosity as shown in Table I, where ME1 containing the lowest water content showed the highest viscosity (375 cp), while ME3 containing the highest water content showed the lowest viscosity (188 cp) (32). ME3 (Labrasol: Plurol of 3:1) comprising 50% water seemed to be superior in relation to enhancing tolterodine tartrate permeability determined by Q_{24} in comparison with ME2 (containing 30% water) and ME1 (10% water).

Pharmacokinetic Study in Healthy Human Volunteers

All volunteers fully completed the study. No adverse reactions were reported by any of the subjects.

The LC-MS/MS assay has been validated and has a good linearity from 0.1–60 ng/ml with acceptable within-and

Table IV. Pharmacokinetic Parameters of Tolterodine Tartrate After Oral Administration Of 4 mg Immediate Release Tablets and Transdermal Application of 4 mg in Microemulsion System to Healthy Volunteers

Pharmacokinetic parameter	Commercial oral tablet	Transdermal (ME3)
C_{max} (ng/ ml)±SD	5.69±2.32	1.06±0.214
T_{max} (hour)±SD	1.54±0.56	3.17±1.47
$AUC_{(0-72)}$ (ng.hr/ ml)±SD	39.43±15.48	44.51±9.53
$t_{1/2}$ (hour)±SD	5.44±3.78	102.04±48.83
K ($hour^{-1}$)±SD	0.17±0.07	0.008±0.004
MRT (h)±SD	9.35±5.16	147.82±70.68
Relative bioavailability (%)	–	112.90

C_{max} (ng/ml) (Oral), (Transdermal), *T_{max}(hr)* (Oral), (Transdermal), *$AUC_{(0-72)}$ (ng/ml.hr)* (Oral, Transdermal), *$t_{1/2}$(hr)* (Oral), (Transdermal), *K($hour^{-1}$)* (Oral), (Transdermal), *MRT (hr)* (Oral), (Transdermal)
All formulas between the same brackets are not significantly different from each other but differ significantly from those included in other

between day reproducibility. The lower limit of tolterodine tartrate quantification in plasma was 0.1 ng/ml.

The tolterodine tartrate mean plasma concentration-time profiles following single oral dose administration of 2 immediate release tablets of Detrusitol™ 2 mg (PHAMACIA & UPJOHN) and a single percutaneous application of ME3 to six healthy human volunteers are shown in Fig. 2. Corresponding pharmacokinetic parameters are summarized in Table IV.

After oral administration, tolterodine tartrate was rapidly absorbed and reached a C_{max} of 5.69±2.32 ng/ml at a T_{max} of 1.54±0.56 h. After the application of transdermal ME3 tolterodine tartrate, its concentration rose much more slowly and reached a plateau between 8 and 72 h (T_{max}=3.17±1.47 h) with a significant lower C_{max} of 1.06±0.21 ng/ml (P<0.05). The mean AUC_{0-72} values after oral and transdermal treatment were 39.43±15.48 ng.hr/ml and 44.51±9.53 ng.hr/mL respectively. However, there was no significant difference (p<0.001) in the extent of drug absorbed from the two dosage forms. The calculated relative bioavailability of tolterodine tartrate after the transdermal application compared to the oral administration of immediate release tablet was approximately 112.90%. It was reported previously (33,34) that the single dose relative bioavailability of the ER (extended release) tolterodine tablet formulation 4 mg compared with the IR (immediate release) tablet formulation lies within the 90% Cl of 80–125% for bioequivalence accepted by the US FDA.

Regarding the elimination half life ($t_{1/2}$), transdermal tolterodine tartrate showed about 20 times longer elimination half life compared to the oral route, which confirmed the sustaining effect of transdermal permeation in comparison to the immediate release oral route. The reported t ½ for extended release tablet formulation (34) was 7.9 h (4–13 h), which was almost one-tenth the calculated t ½ after transdermal application, which means that the transdermal application has much more superiority in drug sustainment compared to the immediate-release or even the extended-release tablet formulation. The same conclusion was reached by looking at the mean residence time results (MRT) in which the transdermal route showed MRT of 147.82±70.68 h, while the oral administration showed only 9.35±5.16 h.

The low T_{max} and high C_{max} values following oral administration are due to rapid absorption from the gastrointestinal tract. In contrast, the low C_{max} and prolonged T_{max} after transdermal administration of ME3 are due to the barrier properties of the skin which led to an early accumulation of drug in the skin followed by its sustained release into the systemic circulation. The higher MRT values following transdermal delivery compared with the oral route may be due to the continuous replenishment of drug in the systemic circulation by constant and controlled delivery of drug from the transdermal microemulsion system.

CONCLUSION

The results of this study indicated that the transdermal delivery of tolterodine tartrate from microemulsion has comparable potential to the oral route with more sustaining and less frequent dosing using the same dosing level. Therefore, the transdermal formula will increase the patient compliance and prevent the nocturnal enuresis.

REFERENCES

1. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, *et al*. The standardisation of terminology in lower urinary tract function: Report from the standardisation subcommittee of the International Continence Society. Urology. 2003;61:37–49.
2. Stewart WF, Van Rooyen JB, Cundiff GW. Prevalence and burden of overactive bladder in the United States. World J Urol. 2003;20:327–36.
3. Berry SJ, Coffey DS, Walsh PC, Ewing LL. The development of human benign prostatic hyperplasia with age. J Urol. 1984;132:474–9.
4. Irwin DE, Milsom I, Kopp Z. Impact of overactive bladder symptoms on employment, social interactions and emotional wellbeing in six European countries. BJU Int. 2006;97:96–100.
5. Kaplan SA, Roehrborn CG, Dmochowski R. Tolterodine extended release improves overactive bladder symptoms in men with overactive bladder and nocturia. Urology. 2006;68:328–32.
6. Yu YF, Nichol MB, Yu AP, Ahn J. Persistence and adherence of medications for chronic overactive bladder/urinary incontinence in the California Medicaid Program. Value Health. 2005;8:495–505.
7. Malone-Lee J, Shaffu B, Anand C, Powell C. Tolterodine superior tolerability and comparable efficacy to oxybutynin in individuals 50 years old or older with overactive bladder: a randomized controlled trial. J Urol. 2001;165:1452–6.
8. Serels SR, Appell RA. Tolterodine: a new antimuscarinic agent for the treatment of the overactive bladder. Expert Opin Investig Drugs. 1999;8(7):1073–8.
9. Brynne N, Stahl MM, Hallen B, Edlund PO, Palmer L, Hoglund P, *et al*. Pharmacokinetics and pharmacodynamics of tolterodine in man: a new drug for the treatment of urinary bladder overactivity. Int J Clin Pharmacol Ther. 1997;35(7):287–95.
10. Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM. Lipid vesicles for skin delivery of drugs: reviewing three decades of research. Int. J. Pharm. 2007;332:1–16.
11. Kogan A, Garti N. Microemulsions as transdermal drug delivery vehicles. Adv. Colloid Interface Sci. 2006;123–126:369–385.
12. Kreilgaard M, Kemme MJ, Burggraaf J, Schoemaker RC, Cohen AF. Influence of a microemulsion vehicle on cutaneous bioequivalence of a lipophilic model drug assessed by microdialysis and pharmacodynamics. Pharm Res. 2001;18(5):593–9.
13. Sintov AC, Botner S. Transdermal drug delivery using microemulsion and aqueous systems: influence of skin storage conditions on the *in vitro* permeability of diclofenac from aqueous vehicle systems. Int J Pharm. 2006;311(1–2):55–62.
14. Spiclin P, Homar M, Zupancic-Valant A, Gasperlin M. Sodium ascorbyl phosphate in topical microemulsions. Int J Pharm. 2003;256(1–2):65–73.
15. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, *et al*. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. Eur J Drug Metab Pharmacokinet. 1991;16(4):249–55.
16. Alvarez-Figueroa MJ, Blanco-Mendez J. Transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions. Int. J. Pharm. 2001;215(1–2):57–65.
17. Godin B, Touitou E. Transdermal skin delivery: predictions for humans from *in vivo*, *ex vivo* and animal models. Adv Drug Deliv Rev. 2007;59(11):1152–61.
18. ''Declaration of Helsinki.'' As amended by the 52nd World Medical Assembly (WMA). World Medical Association, Edinburgh, Scotland, October 2000.
19. International conference of harmonization of technical requirements for registration of pharmaceuticals for human use. ICH harmonized tripartite guideline. Guidelines for good clinical practice. May 1996.
20. Ongpipattanakul B, Burnette RR, Potts RO, Francoeur ML. Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm. Res. 1991;8:350–354.
21. Hadgraft J. Passive enhancement strategies in topical and transdermal drug delivery Int. J. of Pharm. 1999;184:1–6.
22. Mayorga P, Puisieux F, Couarraze G. Formulation study of a transdermal delivery system of primaquine. Int. J. Pharm. 1996;132:71–79.
23. Zheng XS, Duan CZ, Xiao ZD, Yao BA. Transdermal delivery of praziquantel: effects of solvents on permeation across rabbit skin. Biol Pharm Bull. 2008;31(5):1045–8.

24. Thomas BJ, Finnin BC. The transdermal revolution. Drug Discov. Today. 2004;9(16):697–703.
25. Fini A, Bergamante V, Ceschel GC, Ronchi C, De Moraes CA. Control of transdermal permeation of hydrocortisone acetate from hydrophilic and lipophilic formulations. AAPS PharmSciTech. 2008;9(3):762–8.
26. Menon G.K. New insights into skin structure: scratching the surface. Adv Drug Deliv Rev. 54 (1): S3–17(2002).
27. Klamerus K, Lee G. Effects of some hydrophilic permeation enhancers on the absorption of bepridil through excised human skin. Drug Dev. and Ind. Pharmacy. 1992;18(13):1411–1422.
28. M. Kreilgaard, E.J. Pedersen, J.W. Jaroszewski. NMR. characterisation and transdermal drug delivery potential of microemulsion systems. J. Control Rel. 69:421–433 (2000).
29. H. E. J. Hofland, J. A. Bouwstra, F. Spies, G. Gooris, H. E. Junginger. Interaction between liposomes and human skin *in vitro*: Poster presentation. Conference: Liposomes in Drug Delivery 21 years On, London, 12–15 (1990).
30. N. Weiner, K. Egbaria. Topical application of liposomal systems: Poster presentation. Conference: Liposomes in Drug Delivery 21 years On, London, 12–15 (1990).
31. Kweon JH, Chi SC, Park Transdermal ES. delivery of diclofenac using microemulsions. Arch Pharm Res. 2004;27(3):351–6.
32. Delgado-Charro MB, Iglesias-Vilas G, Blanco-Mendez J, Lopez-Quintela MA, Marty JP, Guy RH. Delivery of a hydrophilic solute through the skin from novel microemulsion systems. Eur. J. Pharm. Biopharm. 1997;43:37–42.
33. Olsson B, Szamosi J. Multiple dose pharmacokinetics of a new once daily extended release tolterodine formulation *versus* immediate release tolterodine. Clin Pharmacokinet. 2001;40(3):227–35.
34. Guay D.R.P. Clinical Pharmacokinetics of Drugs Used to Treat Urge Incontinence Clin Pharmacokinet. 42(14): 1243–1285 (2003).