ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Suppl 2, 2014

ISSN - 0974-2441

Research Article

FORMULATION AND EVALUATION OF MEMBRANE-CONTROLLED TRANSDERMAL DRUG DELIVERY OF TOLTERODINE TARTARATE

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Received: 13February 2014, Revised and Accepted: 8March 2014

ABSTRACT

Objective: The objective of the study was to formulate and evaluate membrane-controlled transdermal delivery system of Tolterodine tartrate (TT).

Methods: TT membrane controlled transdermal patches were prepared by fabricating drug reservoir in a rate controlling membrane. Drug reservoir gel was prepared by using various polymers, rate controlling membrane is prepared by solvent casting method using Eudragit RL100 and RS100 in different ratios and was evaluated. The optimized formulations were fabricated and evaluated.

Results: The membrane controlled transdermal patch of TT was optimized with HPMC K4 M gel (2.5%) and Eudragit RL100 & RS100(8:2) for rate controlling membrane. In-vitro, ex-vivo studies were conducted on rat abdominal skin and release at Q12 was 52.98±1.12µg/cm2 for F3 formulation over the control (8.85±0.74 µg/cm2). The flux was 3.574 µg/cm2/hr, lag time was 0.8 hrs, permeability coefficient was 1.068 cm/hr and permeation was enhanced by 2.33 fold for F3 formulation.

Conclusions: Out of the prepared formulations the F3 transdermal patch was optimized having a steady state transdermal flux of 3.574 µg/cm2/hr, lag time of 0.8 hrs, enhancement ratio of 2.33 with permeability coefficient of 1.068 cm/hr and was subjected to ANOVA. ANOVA results showed significant difference between control and F3 in all skin permeation parameters. The optimized formulation (F3) exhibited controlled drug release profile with zero order kinetics and Fickian diffusion mechanism.

Keywords: Membrane controlled transdermal system, Tolterodine Tartarate, Hydroxypropyl methylcellulose, Eudragit RL100, Eudragit RS100.

INTRODUCTION

Delivery of drugs through the skin has is a challenging area for research. Advances in new research are resulting in a larger number of drugs that are being delivered transdermally. Transdermal systems are desirable form of drug delivery because of the sticking advantages over other routes of drug delivery [1]. Transdermal drug delivery provides convenient and painless, self-administration and eliminates frequent dosing of the drug with a short half-life can be delivered easily. Some advantages of reduced dosage regimens for the patients are an increased compliance, reduced severity and frequency of side effects, since prolonged release formulations maintain constant blood levels and elude the fluctuations associated with the immediate release formulations administered more than once a day [2].

Tolterodine tartrate (TT) is effective in the treatment of overactive bladder. Overactive bladder is a disease that often accompanies sleeplessness, decrease in work efficiency, depression and social phobia resulted from lack of information on disease, thus greatly lowering quality of life. An optimal efficacy is obtained at an oral dosage of 1 or 2mg twice daily. The high potency and relatively short half-life makes tolterodine a suitable candidate for a patch formulation [3].

TT has a molecular weight of 475.6 and enantiomeric purity is 99%. The pKa value is 9.87 and the solubility in water is 11mg/ml. The partition coefficient (Log P) between n-octane and phosphate buffer a pH 7.32 is 1.83. The major metabolic pathway for the metabolism of TT is mediated by cytochromeP4502D6 leading to the formation of a metabolite which is equally potent as TT [4].A transdermal formulation with TT will provide an alternative route of drug delivery to the tablet formulation, thus reducing frequency of dosing and side effects [5]. The transdermal formulation avoids dose dumping which is observed with extended release oral forms and improves patient compliance. Hence it was thought that it would be beneficial to formulate a transdermal reservoir patch for the delivery of TT. The present formulation is expected to release the drug for 12hours release by maintaining constant drug level in the blood.

The aim of present study is to formulate membrane controlled transdermal patch of TT and study in-vitro and Ex-vivo behavior of the formulation the purpose is to avoid risk of dose dumping and delivery of the drug at a controlled rate.

MATERIALS AND METHODS

TT was a gift from RA Chem.Pharma,Balanagar. HPMC K 4M from Sigma labs. Ethyl cellulose, methanol LR, acetone, chloroform, dichloromethane LR, propylene glycol, dibutyl phthalate, glycerin, Eudragit RS100/RL100 from SD Fine chemicals, Mumbai. PVA from Shinetsu,Japan. PVP K-30 from Universal laboratories, Hyderabad.

Preparation of gel reservoir system

The drug reservoir consists of accurately weighed quantity of natural gums such as xanthan gum, konjac gum and kondagogu gum and polymers such as HPMC K4M, Carbopol 940 were taken and soaked in distilled water for about 24 hrs. After swelling of the gel, the drug solution equivalent to 0.7mg was added.

Gels containing 2% and 2.5% of HPMC K4M, 1% Carbopol, 6% and 8% of sodium alginate, 1% and 1.5% of kondagogu gum and konjac gum have showed good gelation property.

Fabrication of Rate controlling Membrane

The rate controlling membranes were prepared by solvent evaporation method. Different ratios (EPT1-2:8,EPT-4:6,EPT-6:4,EPT-8:2) of the polymers (Eudragit RL100, Eudragit RS 100) were dissolved in the Dichloromethane (solvent system) and HPMC K4M, PVA(poly vinyl alcohol),PVP K30 using water as solvent. The plasticizer either dibutylpthalate(DBP) or glycerin or propylene glycol were added to the polymeric system and mixed thoroughly with the magnetic stirrer, then casted over a Teflon plate of 6cm diameter. The plate was kept in hot air oven for drying at 300C for

24hrs. An inverted funnel was placed over the plate to control the rate of drying.

The transdermal patches were prepared by filling the optimized drug reservoir (gel) within the shallow compartment and drug impermeable backing membrane and were closed by a release liner on the open side.

EVALUATION OF TRANSDERMAL MEMBRANE CONTROLLED PATCHES

Evaluation of Gel

Gels were evaluated for their clarity, pH, spreadability, viscosity, drug content, in-vitro, ex-vivo diffusion studies, skin irritation and anti-inflammatory activity [6].

Clarity: It was determined by visual inspection with white background and was graded as follows: turbid: +, clear: ++, very clear (glossy) +++.

Homogeneity: Appearance of gel was determined and presence of any aggregates.

Determination of pH: pH of formulation is determined by dissolving 1gm of gel in 100 ml of 7.4 phosphate buffer and checked with a digital pH meter at constant temperature.

Spreadability: 1g of the gel between 20x20 cm glass plates was spread. The mass of the upper plate was standardized at 150g and calculation of spreadability was done by using the formula

$S = \frac{ml}{t}$

S = spreadability, m = weight tied to the upper glass slid, l = length of the glass slide,

t = time (sec).

Determination of viscosity: Viscosity of prepared gels was determined by Brookfield programmable viscometer LVDV-II+PRO. The spindle number 64 was rotated at 10rpm. Samples of the gels were allowed to settle over 30 minutes at the temperature (25 ± 1) before taking the measurements.

Drug Content: 100 mg of gel equivalent to 0.07mg of TT was taken and dissolved in 100ml of pH 7.4phosphate buffer.The placebo gel 100 mg was dissolved in the 7.4buffer solution. Shaking of volumetric flasks was done for 15min. The solution was passed through the Whatmann filter paper no.42 for filtration. Appropriate dilutions were made and the drug content was estimated spectrophotometrically against corresponding placebo gel at 281nm.

Extrudibility: Pfizer hardness tester was used for the test.15gms of gel was filled in Aluminum tube, a plunger was adjusted to hold the tube properly and pressure ($1kg/cm^2$) was applied for 30sec. The amount of gel extruded was weighed. This procedure was repeated at three equidistance places of tube. Test was carried in triplicate.

Evaluation of rate controlling membrane [7]

Thickness Variation Test: digital screw gauge was used to measure the thickness of the films at six different points of the patch.

Weight Variation Test: The formulated films were prepared in triplicate. Three films from each batch were weighed one after the other and the average weight is noted.

Folding Endurance: Folding endurance of patches was determined by folding a small strip of film (2cmx2cm) at the same place repeatedly till it breaks. The number of times of folding at the same place without breaking was the value of folding endurance.

Moisture Uptake: The films (n=3) were weighed accurately and placed in a desiccator chamber. The humidity condition of 75%RH was maintained by using saturated solution of sodium chloride. The films were taken out after 3 days and reweighed the percentage of moisture uptake was calculated.

$$\%\ Moistureuptake = \frac{Finalwt - Initialwt}{Initialwt} X\ 100$$

Moisture Content: The films (n=3) were weighed and placed in a desiccator containing calcium chloride for 24 hr. at 40oC.When there was no further change in the weight of individual patch the final weight was noted. The percentage of moisture content was calculated.

$$\%\ Moisturecontent = \frac{Initialwt - Finalwt}{Finalwt} X\ 100$$

Water Vapor Transmission Rate (WVTR) Studies: Glass vials of uniform diameter were used as transmission cells. The transmission cells were washed and dried in oven at 100oC for 5 mins. About 1gm anhydrous calcium chloride was placed in the cells and respective polymer film (1sqcm) was tied over brim. The cell were weighed accurately and kept in a closed desiccator containing saturated solution of potassium chloride (200ml), thus relative humidity of 84% is maintained. The cells were taken out and weighed. The water vapor transmitted was found using following formula.

WVT=WL/S

W = water vapor transmitted in gm, L = thickness of the film in cm, S = exposed surface area in square cm.

It is represented as the number of grams of moisture gained/hr.sqcm.

In-vitro diffusion studies: Diffusion study of the patch was performed using Franz diffusion cell. The cell was locally fabricated and volume of receptor compartment was 25ml. The dialysis membrane was fixed between the donor and receptor compartments. Patch formulation (2cmx2cm) was applied uniformly on the dialysis membrane. Phosphate buffer saline pH 7.4 was poured in the receptor compartment and stirring is carried out with a magnetic bead to maintain the hydrodynamics. 1ml of sample was withdrawn and same amount of buffer was replaced at pre-determined time intervals. Analysis of the samples was done after making appropriate dilution for drug content spectrophotometrically [8].

EVALUATION OF OPTMIZED TRANSDERMAL PATCHES

EX-VIVO SKIN PERMEATION STUDIES

Preparation of rat abdominal skin: Fresh, full thickness and hairless skin obtained from Wistar Rats (150-180g) was used as a permeation barrier. The animal was sacrificed and the hair was removed using an electrical hair clipper. Abdominal sections were excised using surgical scissors. Then the skin surface was observed under microscope for existence of cuts and wounds. The full thickness skin thus prepared was soaked in distilled water at 60oC for 60 sec, followed with careful removal of the epidermis with the intact stratum cornea. The epidermis was washed with distilled water and used [9].

Calculation of skin permeability parameters: calculation of the parameters in the study to compare the drug transfer and permeation properties among the tested formulae. A description of parameters includes steady state flux, permeability coefficient, and enhancement ratio, lag time, and cumulative amount permeated through skin [10].

Steady state flux ($\mu g/cm^2$/h): Flux was calculated when drug permeation reaches the study state, using

The following equation:

Steady state flux (Jss) = $\frac{dM}{S} \cdot dt$

Where dM - Amount of drug permeated, S - Unit cross-section area, t -time(t).

From the above equation, the slope of the steady state portion of the permeation curve is known by plotting the cumulative amount of drug permeated (µg) versus time in hours.

Permeability coefficient (cm/hr):

The permeability coefficient (Kp) was calculated with the following equation:

$Kp=\frac{JSS}{CV}$

CV is the total donor concentration of the formulation

Enhancement ratio

The effect of permeation enhancer on diffusion and permeation of selected drug molecules is evaluated by

$$ER = \frac{\text{Permeability coefficient of drug with enhancer}}{\text{Permeation coefficient of drug alone}}$$

Lag Time (hrs): Lag time is the time required for the drug to get released from the reservoir. It is calculated by plotting cumulative amount of drug permeated vs time. The x-intercept value gives the lag time.

Skin irritation studies: A protocol for the study was prepared. After approval from Institutional Ethics Committee ID no. GPRCP /IAEC/11/13/3/PCE/AE-8, the study was conducted as per protocol. Skin irritation test was conducted to test the different formulations of patch upon the rabbit skin. The rabbits were divided into two groups having 6 animals each. The hair on the ventral surface was removed using a depilator. The test formulations were applied on the depilated area of the animal and kept under observation for 3days. Symptoms of Flushing (Redness of skin), prepare the drug reservoir.

Papules, wheals and erythema, and marked edema were observed [11].

Stability Studies: The purpose of stability study is to provide evidence on the quality of drug substance or drug product which varies with time under the influence of a variety of environment factors such as temperature, humidity and light. One formulation was selected for the stability studies on the basis of physiochemical characteristics, ex-vivo drug permeation of the formulations. The formulation was subjected to accelerated stability studies as per ICH guidelines. The most satisfactory formulation was sealed in an aluminum foil and stored at room temperature for one month. Patches were periodically removed and evaluated for for drug content and ex vivo drug release [12].

RESULTS AND DISCUSSION

Characterization of TT: TT was identified and characterized as per official compendia. The drug purity was determined by IR spectra and melting point was found to be 206°C. The drug was found to be freely soluble in water. The partition coefficient of TT in n-octanol: pH 7.4 phosphate buffer was found to be 1.62±0.16.

Various natural gums such as kondagogu gum, Konjac gum, sodium alginate and polymers Carbopol 940 and HPMC K4M were used to

Table 1: Evaluation of TT Reservoir system (gel):

Clarity	pH±SD	Spreadability (gm.cm/sec) ±SD	Extru-Dibility	Formulation code	Homo-geniety	Drug content (mg) ±SD	Viscosity measurement (cps) ±SD	CPR (%) ±SD	Release rate (µg/cm2/hr1/2)
+++	6.9±0.53	27.07±0.28	+++	CGT 1%	Good	0.69±0.32	39820 ± 1.12	35.7 ± 0.67	5.99 ± 0.97
+	7.0±0.16	14.96±0.23	++	SGT 6%	Good	0.68±0.22	1530±1.23	96.5± 0.31	29.53 ± 1.09
+	7.1±0.21	17.52±0.12	++	SGT 8%	Good	0.68±0.12	2330±1.22	92.8 ±0.39	28.36 ± 0.56
++	6.9±0.75	15.84±0.21	+	KDGT 1%	Good	0.69±0.13	18670±0.68	29.8± 0.76	1.38 ± 0.67
++	7.1±0.34	18.44±0.11	+	KDGT 1.5%	Good	0.69±0.13	18930±1.12	19.8±0.15	1.56 ± 0.89
++	6.7±0.43	16.82±0.17	+	KJGT 1%	Good	0.69±0.03	25380±1.22	35.4 ± 0.17	5.14 ± 1.15
++	7.0±0.11	18.73±0.16	+	KJGT 1.5%	Good	0.69±0.06	25830±1.11	41.6 ± 0.13	4.72 ± 0.98
+++	6.9±0.41	18.07±0.32	+++	HGT 2%	Good	0.69±0.12	2570±0.87	46.8 ± 0.21	6.62 ± 0.65
+++	6.3±0.23	25.45±0.21	+++	HGT 2.5%	Good	0.69±0.02	4340±0.84	42.8 ±0.39	5.97 ± 0.92

Note: + Satisfactory, ++ Good, +++ Excellent, CGT – Carbopol gel, HGT – HPMC K4M gel, SGT – Sodium alginate gel, KDGT – Kondagogu gum gel, KJGT – Konjac gum gel, CPR – cumulative percentage release; All values are expressed as n=3.

From the table no:1, gels containing 2% and 2.5% of HPMC K4M, 1% Carbopol have showed good gelation property and release rate, these formulations have been selected for the further studies.

Preparation of rate controlling membrane: The patches prepared using various combinations of HPMC K15M, PVA, konjac gum and PVP K 30.Among the patches prepared using various combinations of Eudragits have shown the best film forming property. The percentage moisture uptake of all the rate controlling membranes falls in the range of 0.91 to 1.47. The low moisture uptake of the rate controlling membranes was observed due to the hydrophobicity of the polymers [13]. The moisture uptake was observed to fall in the range of 6.652 to 7.531 gm/cm2/hr, due to the hydrophilic and lipophilic nature of the polymers[14]. Eudragit RL100 and Eudragit RS100 with ratio 8:2 and 6:4 were optimized. Being a freely permeable polymer, Eudragit RL100 has the major influence on drug release and permeation [15].

The optimized gel preparations were fabricated with these membranes and further drug release studies were conducted.The cumulative drug release of the formulations for 12hrs (2%HPMC K4M, 2.5% HPMC K4 M, Carbopol 1% respectively) was 12.76%,11.91%, 12.71% with 3.09,2.601,

3.051µg/cm2/hr1/2release rate respectively. Iso-propyl myristate 5% has been added as penetration enhancer. The drug release studies were performed and drug release for Carbopol 1% and HPMC K4M 2.5% with EPT3 and EPT4 were enhanced (F1 - HPMC K4M 2. 5%+ EPT3, F2 - Carbopol 1% + EPT3, F3 - HPMC K4M 2.5% + EPT4, F4 - Carbopol 1% + EPT4).

Table 3: Model dependent kinetics of the optimized formulations

Formulation code	Zero order	First order	Higuchi	Korsemeyer-Peppas equation		Diffusion mechanism	Q12
	R^2	R^2	R^2	R^2	n		
F1	0.984	0.965	0.969	0.979	0.453	Fickian diffusion	63.42±0.95
F2	0.994	0.980	0.981	0.985	0.421	Fickian diffusion	60.13±1.99
F3	0.996	0.977	0.983	0.992	0.428	Fickian diffusion	81.98±0.97
F4	0.998	0.991	0.993	0.995	0.448	Fickian diffusion	75.42±0.97

Table no.3 reveals that the prepared formulations of transdermal patch follows zero order kinetics and Fickian diffusion mechanism of drug release.

EX- VIVO SKIN PERMEATION STUDIES

Ex-vivo skin permeation studies were conducted for optimized formulations, the rate and extent of drug release was estimated. The drug release studies were conducted for about 12hrs. The effect of stratum corneum on the skin permeability of TT was evaluated by studying the skin permeation through a depilated rat abdominal skin using Franz diffusion cell [16].

Table4: Ex vivo skin permeation parameters

Formulation code	Q12 (µg/cm2) ±SD	SSTF (µg/hr/cm2) ±SD	Enhancement ratio	Lag time (hrs) ±SD	Permeability coefficient(cm/hr) ±SD
Control	8.85±0.032	0.788±0.03	1	3±0.11	0.485±0.14
F1	48.42±0.021	3.738±0.01	2.32	1.6±0.25	1.063±0.23
F2	43.13±0.17	3.074±0.012	1.91	1.2±0.16	0.875±0.12
F3	52.98±0.12	3.754±0.021	2.33	0.8±0.74	1.068±0.06
F4	50.42±0.10	3.756±0.023	2.33	1±0.14	1.067±0.21

[Note: Q12 - Cumulative amount permeated; SSTF- Steady state transdermal flux; all values are expressed as n=3]

From table no.4 depicts all the skin permeation parameters of the formulations in comparison with the control. The cumulative amount permeated was maximum for F3 (52.98µg/cm2) and minimum for F2 (43.13µg/cm2). The maximum amount permeated might be due to the hydrophilic nature of the gel reservoir which helps to permeate faster through the hydrophobic rate controlling membrane [17].

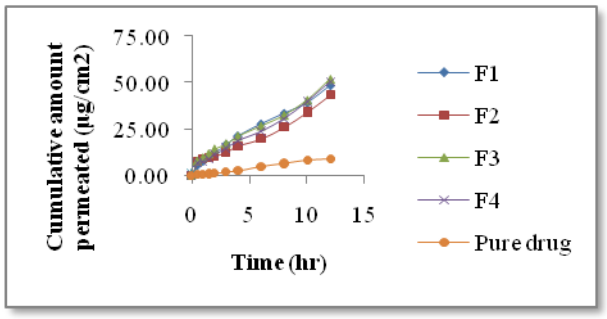

Fig.1: Ex-vivo drug release from TT from transdermal patch

Statistical analysis of skin permeation parameters of control with each formulation using one way ANOVA (Tukey's multiple comparison test)) showed that there was a significant difference between the Q12(µg/cm2), Steady state transdermal flux (µg hr/cm2), Enhancement ratio, Lag time (hr), Permeability coefficient (cm/hr)of the formulations when compared with the control (P<0.001).

Table no.6 indicates that there was significant difference in the cumulative amount permeated of the formulations when compared within the formulations (P<0.001), there was a no significant difference in the steady state transdermal flux between the formulation F1 with F3 and F4 and between F3 and F4 (P>0.05), there was a no significant difference in the permeability coefficient (P>0.05), there was no significant difference in the lag time between the formulation F1 with F2 and between F2 and F3, F2 and F4 and F3 and F4 (P>0.05), there was a significant difference between F1 and F3 (P<0.005) and F1 and F4 (P<0.01).

Skin irritation study: The skin irritation studies were conducted on depilated rabbit, the studies confirmed that there was no sign of erythema or edema.

Stability studies: The stability studies were conducted for one month at room temperature and ambient humidity. There was no leakage of the patch and drug content was uniform and there were no major changes in drug release.

Table 6: Statistical analysis of skin permeation parameters within the formulations using one way ANOVA (Tukey's multiple comparison test)

Parameter	F1 vs F2	F1 vs F3	F1 vs F4	F2 vs F3	F2 vs F4	F3 vs F4	F Value
Cumulative amount permeated (Q12)(µg/cm2)	***	***	***	***	***	***	428406
Steady state transdermal flux (µg hr/cm2)	***	Ns	Ns	***	***	Ns	493.6
Enhancement ratio	***	Ns	Ns	***	***	Ns	776.9
Lag time (hr)	Ns	*	**	Ns	ns	Ns	57.90
Permeability coefficient (cm/hr)	Ns	Ns	Ns	Ns	Ns	Ns	19.11

Note: (n=3); One way ANOVA (Tukey's multiple comparison test); *** (P<0.001);** (P<0.01); * (P<0.005); ns-Non-significant (P>0.05)

CONCLUSION

The membrane controlled transdermal patch of TT was formulated. Based on the physicochemical parameters, in- vitro and ex-vivo release studies, it was found that formulation containing HPMC K4 M 2.5% and EPT4 with 5% Isopropyl myristate was a better formulation. Results of the present study encouraged that the TT reservoir type transdermal patch can be used as controlled drug delivery system and frequency of administration can be minimized. Further in-vivo studies are to be carried out to predict the pharmacokinetics of the drug through membrane-controlled transdermal system.

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