In Vivo Characterization of Monolithic Matrix Type Transdermal Drug Delivery Systems of Pinacidil Monohydrate: A Technical Note

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INTRODUCTION

Transdermal drug delivery systems (TDDS) allow delivery of contained drug into the systemic circulation via permeation through skin layers at a controlled rate. These systems are easy to apply and remove as and when desired. This approach of drug delivery is more pertinent in case of chronic disorders, such as hypertension, which require long-term dosing to maintain therapeutic drug concentration. Transdermal delivery system of an antihypertensive drug, clonidine,[1] has already been marketed. Other hypotensive drugs that have been explored for their transdermal delivery potential are propranolol,[2] metoprolol,[3,4] mepindoldol,[5] captopril,[6] verapamil,[7] diltiazem,[8] nifedipine,[9] and others.

Pinacidil, an antihypertensive drug belonging to the class of potassium channel openers, has been found to be a good candidate for transdermal drug delivery.[10] The bioavailability of pinacidil from oral formulations is only 57% due to hepatic first-pass metabolism. The drug has a short biological half-life of 1.6 to 2.9 hours,[11,12] which makes frequent dosing necessary to maintain the drug within the therapeutic blood levels for long periods. The antihypertensive action requires plasma concentration in the range of 100 to 300 μg/L.

Conventional tablets of pinacidil, currently available, are limited by the variable absorption profile and the need for frequent dosing, which lead to adverse effects and poor patient compliance. Transdermal drug delivery systems present a better alternative to the limitations of oral therapy. We propose TDDS of pinacidil monohydrate for effective management of hypertension for up to 48 hours. The TDDS were previously prepared and evaluated in vitro for drug release and skin permeation. The objective of the present work was to evaluate the TDDS in vivo by monitoring the effect of the TDDS on blood pressure of methyl prednisolone acetate induced hypertensive rats.

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MATERIALS AND METHODS

Materials

Pinacidil monohydrate (PM) was a kind gift from Leo Pharmaceuticals, Ballerup, Denmark. Methyl prednisolone acetate (MPA) was purchased from Pharmacia Upjohn, Gurgaon, India. All the chemicals used were of analytical reagent grade. Rat blood pressure (BP) instrument (Digital 600 Controller) was purchased from Stoetling Inc, Rockville, MD. The animals were procured only after University Animal Ethics Committee approved the study, and the studies were conducted in accordance with standard institutional guidelines.

Preparation of TDDS

The TDDS was prepared by film casting technique on mercury substrate and evaluated in vitro prior to this work.[13] In brief, the TDDS was composed of polymers; Eudragit RL 100 and polyvinyl pyrrolidone (PVP) K-30 (in 8:2, 4:6, 2:8, and 6:4 ratios in formulations B-1, B-2, B-3, and B-4, respectively), along with 20% wt/wt of drug; pinacidil monohydrate, 5% wt/wt of plasticizer; polyethylene glycol-400 and 5% wt/wt of penetration enhancer; dimethyl sulfoxide (based on total polymer weight). All the ingredients were dissolved in isopropyl alcohol-dichloromethane (40:60) solvent system on a magnetic stirrer. The resulting solution was poured in a glass ring (6.06 cm internal diameter) placed on mercury in a Petri dish. The solvent was allowed to evaporate at ambient conditions (temperature 32°C ± 2°C and relative humidity 45% ± 5%) for 24 hours to obtain medicated polymer matrix. A backing film (aluminum foil) and a release liner (wax paper) on either side of the film were applied to complete the TDDS. The patches were cut with a circular metallic die of 2.93 cm internal diameter to form a TDDS with an area of 6.74 cm^2. The TDDS were evaluated in vitro for drug release (using paddle over disc assembly) and skin permeation (using a diffusion cell) on rat skin model.[13]

Procurement, Identification, and Housing of Animals

Thirty-six male albino rats (8 weeks old; 230-250 g) were supplied by Central Animal House facility of Hamdard University and kept under standard laboratory conditions in

12-hour light/dark cycle at 25°C ± 2°C. Animals were provided with pellet diet (Lipton, Calcutta, India) and water ad libitum. They were marked with picric acid solution for easy identification.

Conditioning/Training of Animals

For conducting the BP measurement studies, the animals were required to be kept in a restrainer (rat holder). It had only one side open for entry/exit of the animal with proper ventilation at all other sides. As the rats were unaccustomed to remain in the restrainer in a calm and nonaggressive manner for as long as 48 hours, they were trained for their stay in the restrainer because slight movement and aggression by the animal would have led to variation in BP reading. For this reason, a rat was inserted in the restrainer headlong until the whole body was conveniently accommodated inside. The restrainer was locked by screwing the open side of the apparatus leaving the tail outside. The exercise was repeated several times until the animals learned to stay in the restrainer without aggression and were familiar with the conditions.

Measurement of Initial Blood Pressure of Rats

The initial BP of all the rats was recorded using blood pressure measuring instrument (Digital 600 Controller, Stoetling). The restrainer carrying the rat was placed in the BP instrument with tail protruding out. The tail was gently placed in contact with a transducer membrane, which was connected to the digital BP display panel. The instrument was then turned on and allowed to stabilize until steady pulse rate was observed. Once the "pulse level ready" signal appeared, the BP recording button was pressed and the mean arterial BP was recorded.

Induction of Hypertension in Normotensive Rats

After recording the initial BP of rats, the animals were divided into 6 groups of 6 animals each. Group 1 was taken as control. Hypertension was induced in the remaining 5 groups (Groups 2 to 6) by subcutaneous injection of methyl prednisolone acetate (20 mg/kg/wk) for 2 weeks as per method reported by Krakoff et al.[14]

Post-TDDS Treatment Blood Pressure Assessment of MPA-induced Hypertensive Rats

After MPA treatment, groups 3, 4, 5, and 6 were subjected to TDDS (formulations B-1, B-2, B-3, and B-4, respectively). Group 2 served as toxic control and received no further treatment. The TDDS was applied to the previously shaven abdominal area of rat skin with the entire release surface in intimate contact with the stratum corneum. A microporous adhesive tape (Johnson & Johnson, New Brunswick, NJ) was then rolled over to keep the patch secured at the site of application. The rat was then placed in the restrainer and the BP was recorded up to 48 hours as described under Measurement of Initial Blood Pressure of Rats.

Statistical Analysis

The statistical analysis was performed using GRAPHPAD INSTAT 3 software (GraphPad Software Inc, San Diego, CA). The results were expressed as arithmetic mean ± SEM. The pre- and posttreatment values within a group were compared using paired *t* test. The comparison between various groups was performed by one-way analysis of variance (ANOVA), and the effect in treatment groups (3, 4, 5, 6) and toxic control (2) was compared with that in control (1) group by Dunnet multiple comparison test. The percentage reduction in BP for all the treatment groups was also calculated and compared.

Results and Discussion

Hypertension was successfully induced in the normotensive rats by MPA administration as highly significant difference (paired *t* test, $P < .001$) was found in the pre- and posttreatment values (Group 2, Table 1). This was authenticated by Dunnet test, which showed significant difference ($P < .01$) in BP values of control (1) and toxic control (2) groups corroborating the reports that excessive production[15,16] or administration[17,18] of glucocorticoid is associated with systemic hypertension. The rats remained hypertensive (with a minimum mean BP of 150 mmHg) for 3 days. Thus, post-TDDS treatment BP studies could be performed up to 48 hours.

On treating experimental hypertensive rats with pinacidil TDDS, a significant fall in BP ($P < .01$) was observed in the treatment groups 3, 4, and 5 (Table 1). The effect in group 6 (B-4 TDDS) was even more pronounced ($P < .001$). This was confirmed by Dunnet test, which revealed that there was significant difference ($P < .01$) in after-treatment values of control (1) and treatment groups 3, 4, and 5. However, posttreatment BP values in control and treatment group 6 were comparable and not significant ($P > .01$). On comparing the effects of all the systems, the percentage reduction in mean rat BP by formulations B-1, B-2, B-3, and B-4 was 20.35%, 17.80%, 11.77%, and 37.96%, respectively (Table 1). Therefore, formulation B-4 was found to be superior to the other 3 TDDS. Although there was significant fall in BP with B-1, B-2, and B-3 TDDS, these formulations failed to restore the normotensive BP values. Formulation B-4, instead was successful in returning the rat BP to normal values. The results are in

Table 1. Effect of Transdermal Drug Delivery Systems (B-1, B-2, B-3, and B-4) of Pinacidil Monohydrate on Mean Blood Pressure in Control and Methyl Prednisolone Acetate Induced Hypertensive Rats*

Group	Treatment	*Mean BP (mmHg) ± SEM			% Reduction in BP	Amount of Drug Permeated (mg/cm^2)	Permeability Coefficient (cm/h)
		Before Treatment	After MPA Treatment	After TDDS Treatment			
1	Control†	95.2 ± 6.5	-	94.3 ± 7.4 ns	-	-	-
2	MPA + placebo TDDS‡	93.9 ± 5.4	165.3 ± 4.7 s	166.7 ± 5.6 ns	-	-	-
3	MPA + B-1 TDDS§	90.8 ± 4.7	157.5 ± 6.3 s	125.4 ± 5.4 s	20.3	4.68	0.0119
4	MPA + B-2 TDDS§	94.9 ± 5.1	167.3 ± 4.6 s	137.5 ± 3.8 s	17.8	4.21	0.0118
5	MPA + B-3 TDDS§	91.4 ± 4.8	152.6 ± 7.4 s	134.7 ± 3.7 s	11.8	3.51	0.0095
6	MPA + B-4 TDDS§	92.6 ± 3.9	160.3 ± 4.9 s	99.4 ± 4.5 s	37.9	6.72	0.0183
F 27.12s							

*BP indicates blood pressure; MPA, methyl prednisolone acetate; TDDS, transdermal drug delivery systems; ns, not significant; and s, significant ($P < .01$).

†Control Group: received no treatment. After treatment value represents final pressure at 48 hours.

‡Toxic Control Group: received methyl prednisolone acetate sc 20 mg/kg/wk for 2 weeks followed by placebo TDDS for 48 hours.

§Treatment Groups: received methyl prednisolone acetate sc 20 mg/kg/wk for 2 weeks followed by B-1, B-2, B-3, and B-4 in that order for 48 hours.

conformity with in vitro drug release and skin permeation data.[13] The amount of drug released in 48 hours from formulations B-1, B-2, B-3, and B-4 was found to be 63.96%, 55.95%, 52.26%, and 92.18%, respectively. The extent of drug permeated and rate of skin permeation (permeability coefficient) for the above formulations was in the same order with the highest value observed for formulation B-4 (Table 1). The results indicate that increasing the quantity of Eudragit RL 100 (a freely permeable polymer) up to 60% wt/wt leads to an increment in the rate and extent of drug absorbed and higher percentage reduction in BP. The addition of Eudragit RL 100 in the drug-polymer matrix was also driven by the fact that Eudragits produce crystallization-free polymeric films leading to higher drug

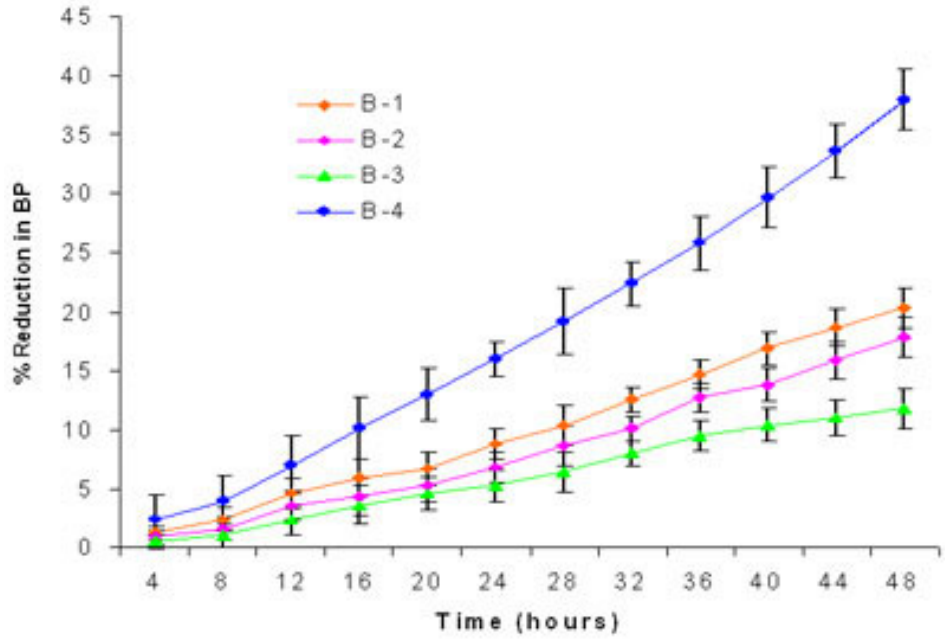

Figure 1. Overtime BP reduction profile of pinacidil transdermal formulations B-1, B-2, B-3, and B-4 (treatment groups 3, 4, 5, and 6, respectively).

release and skin permeation[19] Initially there was rapid drug release (burst effect) from the TDDS, which might be due to rapid dissolution of the surface drug.[20,21] The burst release can be useful for dermal penetration of drugs.[22] Moreover, rapid leaching out of the PVP content results in formation of pores and decreased mean diffusional path length of the drug molecules to the reach the dissolution medium leading to higher release rates.[21] Initial rapid release would maintain sufficient concentration gradient of the drug required for diffusion across the skin. Also, PVP has antinucleating effect that converts crystalline drug into higher energy amorphous state with improved solubility. The enhancement in solubility of drug increases thermodynamic activity that facilitates permeation of drug across the skin. Pyrrolidones are also reported to fluidize the lipids in the stratum corneum and decreasing barrier resistance.[23,24] Thus, liberation of the active from the TDDS is regulated by the physicochemical characteristics of the drug and delivery device as well as the physiological behavior of the biological surface. These factors essentially influence bioavailability of the active from the TDDS, which dictates the therapeutic performance of the latter. The in vitro drug release and absorption pattern was markedly reflected in the in vivo pharmacodynamic behavior of the above TDDS. Over time TDDS treatment profile revealed that the BP values declined at a constant rate up to 48 hours (Figure 1), which manifested the controlled release behavior of the TDDS. It is anticipated that the investigated TDDS would minimize the variable absorption and the adverse effects associated with oral formulations of PM with concomitant improvement in bioavailability and better management of hypertension on long-term basis. The same could be established by extensive pharmacokinetic and pharmacodynamic studies on human volunteers and hypertensive patients.

Summary and Conclusions

The present work aimed to characterize transdermal drug delivery systems of pinacidil monohydrate in vivo by monitoring the effect of the TDDS on blood pressure of methyl prednisolone acetate induced hypertensive rats. The blood pressure of rats was measured using a noninvasive rat BP instrument based on cuff tail technique. A significant fall in rat BP ($P < .01$) was observed in treatment of hypertensive rats with all the formulations, which was maintained for 48 hours. Interformulation comparison revealed that formulation B-4 was the most effective with 37.96% reduction in BP (160.33 ± 4.96 vs 99.44 ± 4.46 mmHg). It was concluded that a single patch application of pinacidil TDDS (B-4) can effectively control hypertension in rats for 2 days. The system holds promise for clinical studies.

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