

In Vitro and *In Vivo* Evaluation of a Rosiglitazone Maleate-loaded HPMC-PVA Blend Patch

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Abstract

A rosiglitazone maleate (RM)-loaded transdermal HPMC-PVA patch was prepared as matrix-dispersion system and drug delivery patterns from the patch was evaluated *in-vitro* as well as *in-vivo*. It has been found that 81% of the drug is released *in-vitro* in the period of 12 hours and the release pattern was a zero-order process. The system was assembled like a hand watch belt so one can easily wear according to one's need. Ex-vivo skin permeation studies in alloxan-induced diabetic rats showed significant improvement on day-to-day diabetic control. Treatment with the patch produced hypoglycemia in normal rats whereas in diabetic rats blood glucose level returned to normal level within 16 hours. These findings suggest that delivery of this drug through TDDS may by-pass the first-pass effect when administered orally and thus gives better glycemic control in diabetic patients.

Keywords: Rosiglitazone maleate, TDDS, HPMC, PVA, Diabetes.

Introduction

Transdermal drug delivery systems (TDDS), also known as "patches," are dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin. These systems are easy to apply and remove as and when desired (Jain, 2001). TDDS offers many advantages over conventional injection and oral methods. It reduces the load that the oral route commonly places on the digestive tract and liver. It enhances patient compliance and minimizes harmful side effects of a drug caused from temporary overdose. Another advantage is convenience, especially notable in patches that require only once weekly application (Robinson *et al.*, 1987)

Rosiglitazone is a weak base with a molecular weight of 357.44 Da. As a maleate salt, 2 to 8mg of it is administered in tablet form twice daily, with or without a combination of metformin. Rosiglitazone, a member of the thiazolidinedione class of antidiabetic agents, improves glycemic control by improving insulin sensitivity.

However, rosiglitazone is metabolized extensively by *n-demethylation* and hydroxylation, followed

by conjugation with *sulfate and glucuronic acid* in the liver (Cox *et al.*, 2000). Therefore, alternative routes such as transdermal delivery may be a good choice to deliver the drug directly into systemic circulation through intact skin by by-passing the hepatic first-pass effect to reduce dose frequency by maintaining a prolonged therapeutic blood level of rosiglitazone. This article describes herein the formulation and *in-vitro* and *in vivo* evaluation of a transdermal preparation of rosiglitazone maleate.

Materials and Methods

Materials

Rosiglitazone maleate was collected from Square Pharmaceuticals, Pabna, Bangladesh. Hydroxy Propyl Methyl Cellulose (HPMC), Polyvinyl Alcohol (PVA), Disodium Hydrogen Phosphate, Potassium Dihydrogen Phosphate and Sodium chloride were purchased from Fluka, Switzerland.

Preparation of HPMC-PVA based TDS patch and drug loading

TDS patch were formulated with the help of polymers - HPMC and PVA. After keeping PVA

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(50mg) in a beaker which contained 4ml distilled water was heated in a hot plate. HPMC (150mg) was added in the melted PVA and mingled properly with a glass rod. Next the weighed drug (2mg) was added and placed separately in film boxes which act as a backing layer. The patches obtained in this way were cooled and introduced separately in respective film box. Then the film boxes with HPMC-PVA based TDS-patches were subjected to three successive freezing (-20°C) for 16 hours followed by thawing for 8 hours (room temperature). In this way, three successive cycles were performed to get the perfect cross-linked hydrogel patch with good mechanical resistance, white and opaque, which proves heterogeneous structure (Szilagyí et al., 2005).

***In-vitro* Dissolution Studies**

The dissolution studies of Rosiglitazone maleate in TDS-patch containing of HPMC and PVA in the formulation was carried out in an Electrolab Tablet Dissolution Tester (USP XXI TDT – 06). Dissolution studies of RM-loaded MC formulations were carried out in USP XXI dissolution apparatus type II. The RM-loaded patch was suspended in 900ml of phosphate buffer of pH 5.4 as dissolution medium stirring at 50rpm and maintained at constant temperature (32±2°C). At predetermined time intervals, 5ml aliquots were withdrawn and replaced by an equal volume of fresh pre-warmed dissolution medium. After suitable dilution, the samples were analyzed at 229nm (λ_{\max} of RM) using UV-Vis spectrophotometer taking phosphate buffer pH 5.4 as blank. The concentration of the RM released at different time intervals was determined.

To prepare a standard curve for RM in phosphate buffer of pH 5.4, dilute solution were made and UV light absorption was checked at λ_{\max} of 229nm. Then standard curve was prepared by plotting absorbance data against drug concentration. Since the value of R^2 is 0.9879, so there is an acceptable linearity between absorbance and drug concentration (Figure 1) and the slope value would be valid one to calculate the drug concentration during cumulative dissolution.

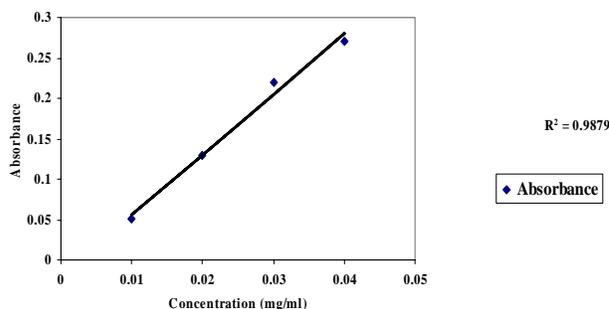


Figure 1: Working curve preparation for Rosiglitazone maleate dissolution studies (pH 5.4)

***In Vivo* Studies**

The animals used for *in vivo* experiments were adult albino rats (4-6 weeks old) of either sex, weighing 120-140g, from the Department of Pharmacy, Jahangir Nagar Univeisty, Savar, Dhaka. The animals were housed in polypropylene cages, four per cage, with free access to standard laboratory diet and water. They were kept under standard environmental conditions. Diabetes was induced by intraperitoneal injection of 120 mg/kg of alloxan monohydrate in sterile saline water. Animal were fasted for 16h prior to drug administration allowing access only to water and were deprived of food during the experiment. Fasting blood glucose levels were measured on hour 72 and when the condition of diabetes were established in animals with a blood glucose level of 200mg/dl or more, they were separated and used for the study.

Hypoglycemic Activity

For evaluating the hypoglycemic activity of RM-loaded patch, healthy rats were divided into four groups ($n=5$). The hair on the backside of the rats was removed with an electric hair clipper on the previous day of the experiment. All the rats were kept fasting before 24 h of the experiment, and then, the rats of Group II and Group IV were treated with RM-loaded TDS-patch.

- Group I — Group I: Normal (Control)
- Group II : Normal + TDS-patch of RM
- Group III: Diabetic Control
- Group IV: Diabetic + TDS patch of RM

Blood samples were collected from orbital sinuses using heparinized capillaries, and the blood

glucose levels were determined by placing one drop of the fresh blood on One touch™ Basic™ plus Blood glucose meter (Life scan, Incorporation 2000, USA) (Figure 2).

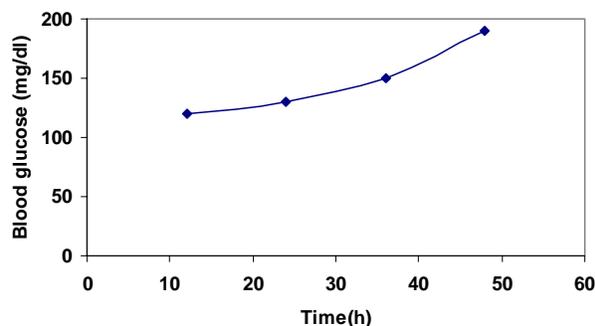


Figure 2: Alloxan induced diabetes mellitus

Blood glucose concentrations were measured at 0, 4, 8, and 16h after dosing, respectively. Results were shown as percentage reduction of blood glucose level (\pm SD) of five animals. The mean blood glucose levels determined in samples collected before RM administration were taken as the baseline levels. Using these data, the percentage of glucose reduction at each time after dosing was calculated and plotted against time.

Statistical Analysis

Statistical analysis of the results was performed using Student's *t* test; $p < 0.05$ was considered as significant. All values were reported as mean \pm SD.

Results and Discussion

In vitro dissolution study of RM-loaded transdermal patch is depicted in Figure 3. RM-loaded TDS-patch exhibited 81% cumulative percentage of drug release in 12 hours exhibiting a significant control on the release of drug ($p < 0.01$). The study was performed using phosphate buffer with pH 5.4 which simulated the skin pH. The shape of the curve indicated the release of RM from the patch followed zero-order process.

To study *in vivo* hypoglycemic activity of the RM-loaded TDS-patch, diabetes was induced in normal rat by the application of alloxan. Alloxan is used in medical research to produce experimental diabetes, since it is selectively destroys the cells of the pancreas that secrete insulin (Rerup, 1990).

Rosiglitazone improves glycemic control by increasing insulin hypersensitivity. The mechanism of action of Rosiglitazone is by activation of the intracellular receptor class of the peroxisome proliferator-activated receptors (PPARs), specifically PPAR γ . Rosiglitazone is a selective ligand of PPAR γ , has no PPAR α -binding action.

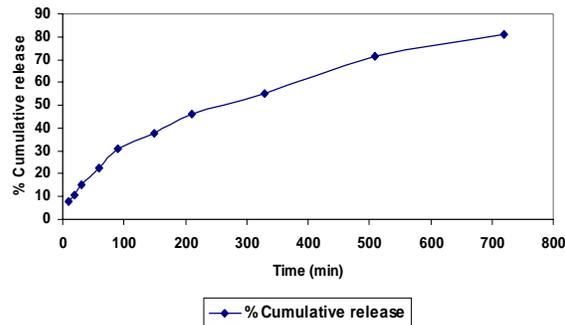


Figure 3: Rosiglitazone maleate release from HPMC-PVA based TDS-patch

The *in vivo* study (Figure 4 and 5) indicated that the transdermal system of RM produced significant hypoglycemic effect in the diabetic rats ($37.93 \pm 5.81\%$) after 16 hrs of treatment. Compared with diabetic control group, rosiglitazone produced significant decline in the blood-glucose concentration ($p < 0.005$) in the diabetic rats. It took 16 hrs to bring the blood glucose level of the hyperglycemic rats to normal. RM-loaded patch produced hypoglycemia in the normal rats in the same treatment duration.

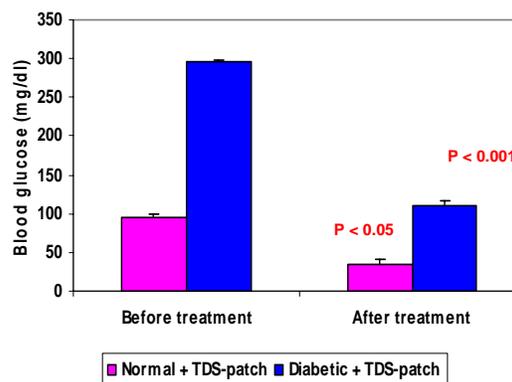


Figure 4: Effect of Transdermal drug delivery systems of Rosiglitazone maleate on blood glucose level in alloxan induced diabetic rats after 16 hours

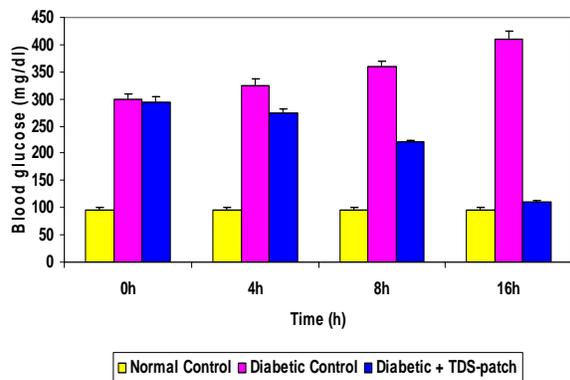


Figure 5: Effect of Transdermal drug delivery systems of Rosiglitazone maleate on blood glucose level in alloxan induced diabetic rats at different time interval

Conclusion

On treating with TDDS of Rosiglitazone maleate in normal rats hypoglycemia was observed (35mg/dl) and in diabetic rats blood glucose level returned to normal level (110mg/dl) within 16 hours. This study predicts that Rosiglitazone maleate from a HPMC-PVA blend patch easily crosses across the skin to the systemic circulation.

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