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Document heading doi: 10.21276/apjhs.2019.6.2.21 **Research Article**

Anti-Hypertensive Activity of Timolol Maleate Nanoparticle Loaded Transdermal Patch on Dexamethasone Induced Hypertensive Rats

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Abstract

The rationale of the current work was to evaluate antihypertensive activity ofTimolol maleate nanoparticles loaded transdermal patch. Nanoparticles loaded transdermal patch of timolol maleate was prepared using Compritol 888, Lutrol F68, tween 80 etc. The formulated patches were subjected to skin irritation study and in–vivo antihypertensive activity. Hypertension was induced by dexamethasone in experimental rats. No any marked skin irritation observed before and after applying the patch. Both systolic and diastolic blood pressure in experimental animals was reduced significantly after 7 days treatment with Timolol maleate nanoparticles loaded transdermal patch. So, it was concluded that timolol maleate patch could be formulated into a matrix-type naoparticle loaded transdermal patch for the management of hypertension. Also, this study can be considered as a new report that focuses on the problem of the side-effects associated with the modern medicaments toward hypertension

Keywords: Hypertension, Timolol maleate, Transdermal patches.

Introduction

Hypertension is known to be one of the major risk factors for the occurrence of death from cardiovascular diseases. Although, there are involvement of various risk factors contributing to CVD death but hypertension is considered to be one of the important risk factors for all forms of cardiovascular and renal disease [1,2].According to World Health Organization-International Society of Hypertension (WHO/ISH) Guidelines for the Management of Hypertension, hypertension is defined as a systolic blood pressure (SBP) of 140 mm Hg or greater and/or a diastolic blood pressure (DBP) of 90 mmHg or greater in subjects who are not taking antihypertensive medication.

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In the epidemiological study, hypertension is commonly defined as in the same way i.e. SBP/DBP of 140 mm Hg or greater and/or 90 mmHg or greater measured on one visit. There are different factors like race/ethnicity, age, level of education, origin of birth, low family income, diabetes, obesity, disability status and insurance are the motives for the note worthy dissimilarities in the dominance of hypertension [3]. According to Warren-Find low *et al*., (2011) [4] in hypertension condition can be prevented when one should take care of his or herself very well by managing healthy and good life style. Bosworth *et al*., (2006) [5] believe that self-efficacy is especially important to hypertension self-care. It is very essential to manage and taking care of themselves to a changeable characteristic and, consequently, is open to intervention [6]. Now a day, the proper management and prevention of hypertension became the global health care challenge throughout the world. One of the best ways to manage the hypertension condition by following a healthy lifestyle and changing environment. Apart from maintaining healthy lifestyle body weight

management is also important. It is also suggested to reduce the intake of total fat and saturated fat[7].

There are large number of drugs are presently available for the treatment of hypertension disease. These anti-hypertensive drugs are classified into different groups:

1. Diuretics, where thiazide diuretics (hydrochlorothiazide, chlorothiazide, indapamide).
2. Beta-blockers (e.g. atenolol, timolol, bisoprolol, metoprolol)
3. Angiotensin converting enzyme inhibitors (e.g. enalapril, lisinopril, perindopril)
4. Angiotensin receptor blockers (e.g. telmisartan, valsartan, losartan)
5. Calcium channel blockers (e.g. nifedipine, amlodipine, nicardipine)
6. Alpha 1 Blockers (e.g. prazosin)
7. Central alpha 2 agonists (e.g. methyldopa)
8. Direct vasodilators (e.g. hydralazine, minoxidil).

The above maintained drug groups are being largely tested in many clinical trials. Data and results of the clinical trial showed that drugs of above maintained groups are successfully and effectively reduce blood pressure and CVD events such as coronary heart diseases, strokes, and heart failure [8]. Still there is lots of adverse or side effects such as drug metabolism by first pass effect in the liver, poor patient compliance, or rejection of an invasive medication often hamper the success and efficacy of therapeutic treatment. To overcome these problems many drug carriers were developed but some carrier such as liposomes, niosomes, or micro emulsions has problem that they remain mostly confined to the skin surface and therefore do not transport drugs efficiently through the skin [9]. To overcome the problem of the stratum corneum barrier, various approaches can be adopted. These include augmentation of skin permeability using penetration enhancers, use of forces which are not dependent on concentration gradient (Iontophoresis, electroporation, phonophoresis, microneedles, jet injectors, etc.,) and more recently the drug carrier systems like vesicles[10]. Drug delivery systems using vesicular carriers such as nanoparticles loaded transdermal patch have soft, flexible, self-regulating and self-optimising vesicular characteristics. Flexibility of transdermal patches membrane is achieved by mixing suitable surface-active components in the proper ratios[11]. These properties allow them to penetrate easily into deeper layers of the skin and circulation.

Nanoparticles Loaded Transdermal Patch

Nanotechnology is broadly defined as the study and use of structures in range between 1 to 1000 nanometers in size. Nanotech-enabled products discovery and its applicability in practically everything we touch on a day-to-day basis, such as medicine, pharmaceuticals, chemicals, and information technology[12]. In particular, the pharmaceutical industry has been energized with breakthroughs in nano-engineering, especially in the fields of drug delivery and formulation development. Over the last few periods, there has been an outburst of research at both academic and industrial levels - pertaining to nanoformulations: nanoparticles, liposomes, [13], nano-emulsions [14]and dendrimers [15].

Transdermal patches are dosageforms that are placed on the skin to deliver a therapeutically effective amountofmedication or drug to the skin and into the systemic circulation. Several system designs have been used in development and formulationof transdermal patches.

Drug permeation through skin and permeation enhancement methods

The skin is the biggest organ of the body, representing over 10% of weight. Broad investigations have demonstrated that the skin is more unpredictable than simply a boundary and this becomes apparent when agents are applied to the skin either intentionally or incidentally. The degree of assimilation through the epidermis, dermis and systemically becomes important when we consider that medicament is applied to the skin (figure 1). A few speculations have been proposed for drug entry through the stratum corneum into the viable epidermis and dermis, including the "bricks and mortar" hypothesis, representing to keratinocytes held together by a lipid bilayer. In any case, contrasts in skin thickness, density of skin appendages (hair follicles and organs), vascularity and metabolic enzymes imply that diverse areas of skin in a similar individual presentation distinctive pharmacokinetics of percutaneous drug penetration.

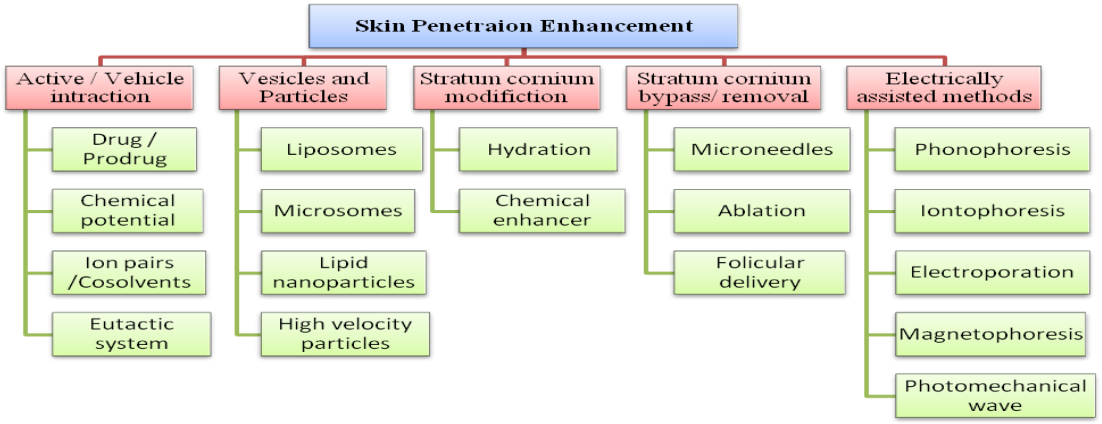

Figure 1: Different approaches for skin permeation enhancement

Material and methods

Chemicals and reagents used

Timolol Maleate (TM) was generously gifted by Gangwal Pharmaceuticals, Mumbai, India. Tween 20 and PEG 400 was purchased from SD Fine Chemicals, Compritol 888 ATO from Gettefosse (India) Pvt. Ltd; Mumbai, Lutrol F68 from BASF, India. HPMCK 100M was gifted by Colorcon Asia Pvt. Ltd; Goa, Analytical grades of orthophosphoric acid and Nylon membrane filters (0.45µm) were purchased from Fisher Scientific (Mumbai, India). All aqueous solutions were prepared using Milli Q/Elix water (Millipore, Moscheim Cedex, France). All other chemicals used were of analytical grade.

Formation of transdermal patch

In order to prepare Solid Lipid Nanoparticles (SLN) of TM, various ingredients*viz.* Compritol 888, Lutrol F68, tween 80 etc. were selected on the basis of literature reports.[16]. These ingredients were tried in different concentration to develop suitable SLN's of TM.

Solid Lipid Nanoparticlespreparations

To purified water Lutrol F68 and tween 80 are added under stirring to get a clear solution and the temperature maintained at 85-90ºC (5% overages of purified water taken to compensate loss on heating). Compritol 888 ATO is heated to melt to this Timolol maleate is added under stirring to get a clear solution. Above mixture added and mixed using ultratrax stirrer at 13000 rpm for 15 min by maintaining the temp at 85-90ºC. After homogenization the dispersion is kept aside to come to room temperature. [16]

Preparation of SLNs Transdermal Patches

Transdermal patches containing Timolol Maleate were prepared by solvent casting technique employing mercury as substrate. Different formulations were formulated using different grades of HPMC i.e. HPMC K100M, HPMC 50 cps and ethyl acetate, different percentage of glycerine and 100mg of optimized nanoparticles. The prepared solution was poured into glass petri dishes of 25 cm^2 area and dried at room temperature [16]. After 12 h, the patches were cut in 5 cm^2 area and packed in aluminium foil.

Experimental animals

Forty-five Wistar albino rats in adult size of either sex in weight ranging from 150-200 g were used for the study and were kept in groups and each group consists of nine animals. Animal obtained from the Institute of Biomedical and industrial research, Jaipur. Under standard environmental conditions, the animals were allowed to acclimatize. The animals were kept in the animal house. Each group was kept in clean poly-acrylic cages lined with husk, renewed every 48 h. It was maintained for 12 hrs. day and light cycles at an average of ambient temperature of25± 2º C and 60±10% relative humidity (RH). All the animals had free access to standard pellet diet and water during the exposure of treatment. The experiment was carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on Animal (CPCSEA). The experimental protocol was approved by the Institutional Animal Ethics Committee, Institute of Biomedical and industrial research, Jaipur wide protocol approval number IAEC/2018-07/04.

Experimental design

The animals were divided in to 5 main groups, each group contain 9 animals. Group I received normal saline, Group II received dexamethasone subcutaneous injection plus normal saline (35 µg/ rat)for 7 days, Group III received nicorandil (6mg/kg, oral), Group IV received transdermal patch with penetration enhancer of timolol maleate, Group V received solid lipid nanoparticle loaded transdermal patch of timolol maleate[17, 18].

Skin Irritation Study

The selected formulation was tested for its potential to cause skin irritation/sensitization in rats. Skin irritation studies designed to detect irritation under conditions of maximal stress and during the assessment of transdermal drug products. Study performed on Wister rats for 7 days. Skin irritation study performed as the group assigned. Patches are applied on the backside of hairless skin of rats for 23 ± 1 hr up to 14 days to the same skin site. After 24 hrs if any type of irritation found then patch should be applied on other site. Each day skin was examined for any type of major and minor skin reactions as mention below scale of 0 to 7 numbers, as per the literature.

Table:1The ranking of irritation in animals

Ranking and Criteria
0- No evidence of irritation
1- Minimal erythema, barely perceptible
2- Definite erythema, readily visible; minimal edema or minimal popular response
3- Erythema and papules
4- Definite edemal
5- Erythema, edema, and papules
6- Vesicular eruption
7- Strong reaction spreading beyond test site

Evaluation of *in*-vivo antihypertensive activity

In the experimental rats, hypertension was induced by subcutaneous injection of dexamethasone (10 µg/kg/d) in the evening. Each rat was educated and familiarized to the restrainer and transducer, for about 15 mins before the experiment. The rat was nonaggressive in a low-stress environment and allowed to enter the holder freely at least 10–15 min prior to obtaining BP measurements[19]. The animal's nose was made to protrude through the front of the nose cone permitting for relaxed breathing and the tail of the animal was fully prolonged to exit through the rear hatch opening of the holder. The rat was warmed but not heated using restrainer, the room temperature was maintained about 32–35.4°C, reduce stress and the blood flow to the tail was enhanced to acquire a BP signal[20](Vijayan *et. al.,* 2011). The rat never had its head bent sideways or its body compressed against the back hatch. The animal's temperature was monitored throughout the experiment as the procedure suggested by Vijayan *et. al.,* 2011[20].

Estimation of systolic and Diastolic blood pressure

Systolic blood pressure (SBP) measurements were recorded weekly by the same investigator, between 10-12 AM, using the integrated BIOPAC instrument (Non-Invasive Blood Pressure 200A system). The basic software setup is done, and IR sensors are calibrated prior to starting the measurement. An IR sensor is then connected to the tail of the animal inside the restrainer. After the required setup and calibration of IR sensors, SBP was recorded.

Statistical analysis

Statistical analysis was carried out by two-way analysis of variance followed by Bonferroni post-tests. All values were expressed as mean ± standard error of the mean. All groups were compared with hypertensive control animals. A p value of <0.05 was considered to be statistically significant.

Results and discussion

Skin Irritation Study

The skin irritation test of the transdermal patch showed a skin irritation score (erythema and edema) of less than 2 (Table 1). According to Trush*et.al.*[21], compounds producing scores of 2 or less are considered negative (Table 2) (no skin irritation).

Table 2: Skin irritation Draize score of transdermal patches

Draize score	Erythema (before)	Erythema (after)
1	0	1
2	0	1
3	0	0
4	0	0
5	0	1
6	0	1
Average	0	0.66±16

(n=6±SD)

Evaluation of *in*-vivo antihypertensive activity

Among diverse models available, for the existent study, the dietary induction of hypertension in Wistar rats was employed using dexamethasone according to methods described by Jena *et al*[22]. The animal's temperature was monitored throughout the experimentMalkoff, 2005[23]. After 14 days treatment SLN patch showed maximum significant attenuation in Blood pressure as compared to hypertensive control animals.

Table:2 Measured Systolic blood pressure of different groups of rats

Sr. No.	Group	0^{th} day	7^{th} day	14^{th} day
1	Control	110±2.11	109±3.25	105±3.77
2	Hypertensive control	107.2±2.25	123.5±3.11	132.5±2.24
3	Standard	108.5±1.75	113.5±2.42*	110.6±3.06*
4	Test I Patch	111.6±2.46	118.4±2.25	117.5±3.60*
5	Test II SLN Patch	109.5±2.35	114.2±3.15	112.6±2.60*

Table: 3 Measured Diastolic blood pressure of different groups of rats

Sr. No.	Group	0^{th} day	7^{th} day	14^{th} day
1	Control	76.2±2.50	77.5±2.85	78.6±2.30
2	Disease control	78.2±3.15	88.8±3.47	92.2±3.25
3	Standard	77.6±2.85	81.3±2.11	79.2±3.10*
4	Test I Patch	75.8±2.01	85.2±2.50	83.4±2.78*
5	Test II SLN Patch	77.2±3.22	84.3±3.10	81.5±2.15*

*Significant P‹0.05 as compared to disease control

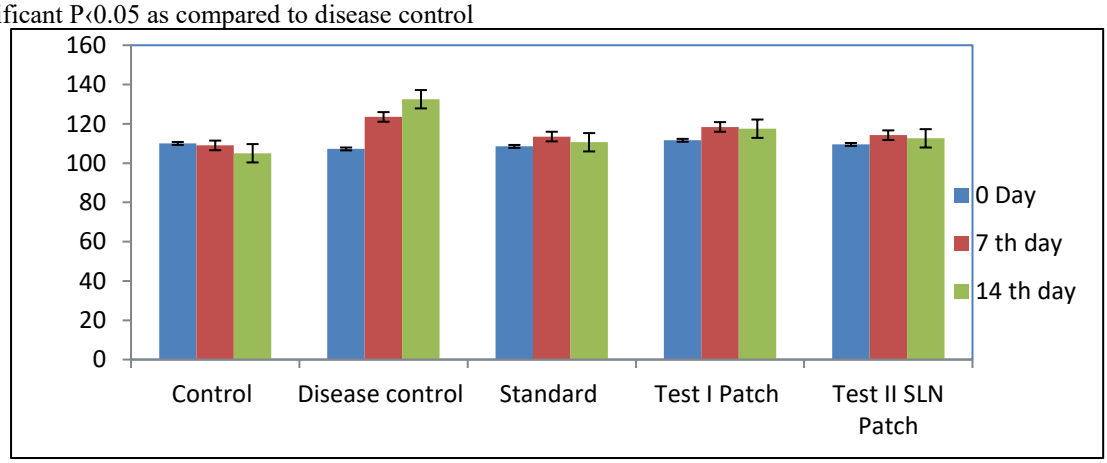

Figure 2: Graphs showing Systolic blood pressure of rats

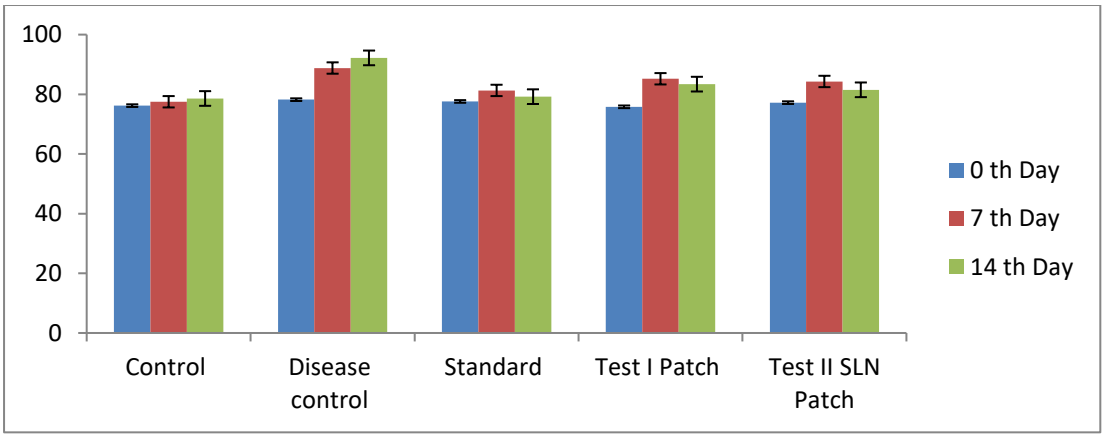

Figure 3: Graphs showing Diastolic blood pressure of rats

Results revealed that as hypertension induced rats along with prepared nano-particle formulation of TM is a non-skin irritant. When rats were treated with SLNs along it produce irritation with minimal erythema after 10 days and definite erythema, readily visible edema was produced after 12 days. Compared with this both the placebo and optimized batch was not show any type of irritation up to 10 days after that there was little erythema found with light redness at the site of application. These results of ***in-vivo*** skin irritation study suggested that optimized batch TM19 does not show any type of major irritation on rat skin up to 14 days and it was safely used up to 24 hrs., photographs of optimized batch TM19 after in-vivo skin irritation study shown in Figure. 4.

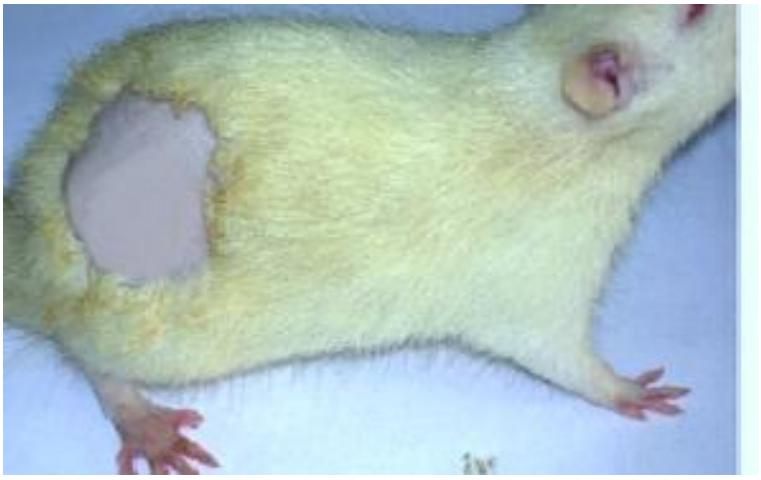

Figure 4: Showing no any redness or spot before and after patch application

Conclusion

In this present study, Transdermal patch evaluated for anti-hypertensive study and a skin irritation study. No any marked skin irritation observed before and after applying the patch.Henceforward, this study can be considered as a new report that focuses on the problem of the side-effects associated with the modern medicaments toward hypertension.

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