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In vitro release and permeation of levobunolol from various transdermal formulations

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Summary

Levobunolol (LB) is a potent β-blocker with proven antihypertensive activity. However, it has a short biological half-life and requires frequent dosing. To overcome these problems, the feasibility of systemic delivery of LB via the transdermal route was explored. A series of in vitro skin permeation studies of the drug were conducted at 37°C across hairless mouse skin. It was found that the skin permeation potential of LB free base was much higher than that of its hydrochloride salt. Release of LB from different systems showed a characteristic matrix diffusion-controlled Q vs $t^{1/2}$ linear relationship, whereas skin permeation profiles from all those systems showed a membrane permeation-controlled Q vs t linear relationship. The in vitro skin permeation flux values ranged from 1.09 to 60.92 $\mu g/cm^2$ per h depending upon the system at a 5% (w/w) loading dose of LB base. Azone showed a skin permeation enhancing effect on LB at a 10% (w/w) concentration. The flux value obtained from an indirect in vivo method using a polyacrylate patch showed an excellent in vitro/in vivo correlation.

Introduction

Transdermal drug delivery (TDD) offers several advantages over other routes of drug administration. Besides patient convenience, enhanced and controlled therapeutic responses have been reported (Barry, 1983).

Controlled delivery of a β-blocker via transdermal system can improve its systemic bioavailability and therapeutic efficacy by avoiding first-pass effects. It will also decrease the dosing frequency required for the treatment. Therefore, several β-blockers are under investigation for administration in TDD systems (Cargil et al., 1986; Corbo et al., 1989).

Levobunolol [(−)-5-(3-(*tert*-butylamino-2-hydroxypropoxy-3,4-dihydro-1(2H)-naphthalenone; LB] is the levorotatory isomer of bunolol. It is related to propranolol in structure and in nonselectivity towards β-adrenoceptor antagonist activity. Several therapeutic advantages of LB over propranolol have been reported (Robson and Kaplan, 1970; Commarato et al., 1976; Gavras et

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al., 1977). But like propranolol, LB also has a short biological half-life of 5–6 h (Drug Information, 1990). Frequent dosing is therefore required for effective hypertension therapy. The high transdermal permeability of LB was recently recognised, and a therapeutic system containing LB hydrochloride in silicone elastomer was developed.

The present study is an attempt to evaluate the performance of different transdermal formulations of LB. Satisfactory skin permeation of LB from all these preparations was found across the intact skin of male hairless mouse.

Experimental

Materials

Drug. Levobunolol hydrochloride (PD 085130-0002, lot no. R) was obtained as a gift from Warner Lambert Co., Ann Arbor, MI. Oily levobunolol free base was prepared and characterized in our laboratory from the hydrochloride salt.

Other components. Silicone elastomers (MDX4-4210 and 3100), curing agents and Bio-PSA X7-2920 were obtained as gifts from Dow Corning Corp. (Midland, MI). Polyacrylate adhesive (9871), Scotch Pak release liner (1022), and Scotch Pak backing membrane (1066), were donated by 3M Co. (St. Paul, MN). Azone was procured as a gift from Nelson Research (Irvine, CA). All other reagents and solvents, either HPLC grade or reagent grade, were used as obtained (Fisher Scientific Co., NJ).

Animal. Breeding pairs of hairless mouse (Skh : Sr-1) were purchased from the Skin Cancer Research Institute, Temple University, Philadelphia. All experimental animals (6–8 week) used were obtained from the established in-house breeding colony.

Methods

Drug assay. In all skin permeation experiments, quantitation of LB in the receptor compartment was performed by a modified HPLC technique (Schoenwald et al., 1983). The receptor solution was mixed with a suitable amount of metoprolol tartrate as internal standard and the mixture was injected directly into the variable loop injector. The mobile phase consisting of 0.25 M acetate buffer (50%) and methanol (50%) was used at a flow rate of 1.0 ml/min. The column effluent was passed through the detector set at 254 nm at ambient temperature. The detector sensitivity was set at 0.01 absorbance units full scale (aufs). The retention times of metoprolol and LB were found to be 4.2 and 5.5 min, respectively. Linearity existed over the concentration range of 0.5 to 5.0 μg/ml and the minimum detection limit of LB was found to be 0.2 μg/ml. Receptor samples were diluted prior to mixing with the internal standard, if needed.

Quantitation was performed by the peak height ratio (PHR) method. PHRs of drug vs internal standard were plotted against different concentrations of drug to construct the standard curve. Unknown concentration was determined using the regression parameters of the standard curve.

To determine the release of LB from the disc, in the absence of any interfering substances from the skin, the receptor solution was analyzed by a spectrophotometer (Response ^{TM} UV-Vis Spectrophotometer, model 25066 × 38, Gilford) at 222.5 nm.

Preparation of matrix-type transdermal disc. Circular transdermal discs containing LB base with a fixed surface area were fabricated by a method similar to that described by Keshary et al. (1985). LB free base was mixed thoroughly with the required amount of silicone elastomer (3110). A curing agent (RTV catalyst 1) for the elastomer was added to this mixture (5.0% w/w of the elastomer) and the mixture was stirred for 5 min.

A plastic cup (19.5 mm inner diameter and 5 mm depth, red dot electrode, 3M Co, MN) was used as the molding device and holder. The inside of the cup was lined with a 2 inch × 2 inch piece of aluminum foil. About 1 g of the blend was poured into the mold and the system was cured at room temperature for 6 h.

After curing, the disc was removed from the mold, and the edge of the disc was carefully trimmed to produce a transdermal disc with a fixed surface area. The disc was then used in the skin permeation and release studies.

Preparation of silicone cream. LB base (5% w/w) was mixed with the MDX4–4210 silicone elastomer (95% w/w), and the mixture was stirred thoroughly for 5 min.

Preparation of ointments. Hydrophilic petrolatum ointment (USP) and PEG ointment (USP) were prepared at a 5% (w/w) loading dose of LB base according to the method described in USP XXI.

Preparation of adhesive patches. Silicone adhesive, Bio-PSA (X7-2920) was used to make a pressure sensitive adhesive dispersion type silicone patch system using a direct coating process (Musolf, 1987). A 20% w/v solution of pharmaceutical grade purified acrylic pressure sensitive adhesive in *n*-hexane was used as the adhesive solution to make polyacrylate patches. In both types of patches, two uniform laminates (125 μm each) were made separately on heat-sealable backing membrane (Scotch Pak 1006) at 30-min intervals and the whole system was cured at room temperature in a dust-free environment overnight. The laminate was then covered by a release liner (Scotch Pak 1022), cut out into 2.25 cm^2 (1.5 × 1.5 cm) pieces and used in the subsequent experiments.

Preparation of skin. Male hairless mouse (6–8 week) was killed by cervical dislocation of the spinal cord and a portion (about 3.0 × 3.0 cm) of the full-thickness abdominal skin was carefully excised. The dermal side of the skin was carefully cleared of any adhering subcutaneous tissues and/or blood vessels.

Skin permeation studies. The freshly excised full-thickness skin sample was mounted on the receptor compartment of the Keshary-Chien glass diffusion cell (Keshary and Chien, 1984) with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. One unit of the transdermal disc was placed with the LB-releasing surface in intimate contact with the skin and the donor cap was properly placed and clamped.

The receptor solution (pH 7.4 Sorensen's phosphate buffer) was then introduced into the stirred receptor compartment which was maintained at 37°C by a circulating water-bath (Cole-Palmer Instrument Co., Model 1268–00). The donor compartment was maintained at the ambient temperature of 25 ± 1°C.

Samples (200 μl) from the receptor compartment were withdrawn at predetermined time intervals and immediately replaced by an equal volume of fresh buffer solution. Initial experiments confirmed the maintenance of sink condition by this procedure. The sample withdrawn from receptor compartment was then analyzed by HPLC. Each data point represents the average of six determinations.

Release studies. To study the release of LB from discs, the same procedure as described above was employed with the exception that no skin sample was mounted between the donor and receptor compartments. 2 ml of receptor solution was withdrawn at predetermined time intervals and immediately replaced by fresh buffer solution. Sink conditions were maintained throughout the experiment. The samples were analyzed by spectrophotometer.

In vivo study. An indirect method ('mass balance' or 'difference method') was used for this study (Weiss et al., 1987). All patches employed for the experiments were obtained from the same batch. A polyacrylate patch was applied on the abdominal skin of the live hairless mouse. Food and water were supplied ad libitum. The patch was withdrawn from the body at predetermined time intervals. The animal was killed and the skin was carefully cut out. The levobunolol content of the patch after removal and the levobunolol uptake by the skin were then determined separately. From the data obtained, the amount of levobunolol permeated through the skin into the body was calculated.

Determination of the skin uptake during in vivo study was carried out by a modified method described in the literature (Keshary et al., 1985). The square area of the skin directly in contact with the patch was carefully removed and its dermal surface was dried with a Kimwipe® paper. The skin sample was then cut into small pieces and immersed in 2 ml of methanol. The methanol-skin mixture was shaken for 24 h in a wrist-action shaker. The mixture was centrifuged at 5000 rpm for 15 min. The clear supernatant

was then separated and analyzed by HPLC after subsequent dilution.

Data analysis

From the concentration of LB in the receptor solution, the amount permeated per unit area ($\mu g/cm^2$) was calculated and plotted as a function of either time or square root of time to obtain the flux in $\mu g/cm^2$ per h or in $\mu g/cm^2$ per $h^{1/2}$, respectively, depending on the conditions discussed below.

Results and Discussion

Release and skin permeation of LB from different transdermal systems

According to Higuchi (1963), the release of a drug at steady state, from a matrix-type drug delivery system into a sink condition follows the relationship:

$$Q = \left[D_P(2A - C_P)C_P t \right]^{1/2}$$

where Q is the cumulative amount of drug released after time t, D_P denotes the diffusivity of the drug in the matrix system, A is the initial loading of the drug in the matrix and C_P represents the solubility of the drug in the matrix.

In the case where the loading dose A is much larger than C_P, the above equation can be simplified to:

$$Q = (2AD_P C_P t)^{1/2}$$

This equation describes a linear relationship between Q and $t^{1/2}$. Release of LB from discs and patches followed a linear Q vs $t^{1/2}$ relationship which confirmed a matrix diffusion-controlled release process (Tables 1 and 3).

Skin permeation of LB from different systems across the male hairless mouse skin showed a linear Q versus t relationship. This indicates that LB permeates through the intact hairless mouse abdominal skin at a constant rate. This relationship can be explained by Fick's law of diffusion under sink conditions (Michaels et al., 1975), as described below:

$$Q = \left[(DAK)/h \right] C_d t$$

where Q is the cumulative amount of drug permeated through the skin at time t, D and K correspond to the diffusivity and partition coefficients of the drug in the skin, respectively, A is the effective surface area of the skin, h denotes the thickness of the skin, and C_d is the concentration of the drug in the donor side.

TABLE 1

Steady-state release and skin permeation [a] of levobunolol base and salt

System	Release rate [b] ($\mu g/cm^2$ per $h^{1/2}$)	Permeation rate [b] ($\mu g/cm^2$ per h)
5% (w/w) salt in silicone disc	14.98 (2.20)	1.35 (0.27)
5% (w/w) base in silicone disc	321.56 (3.54)	17.47 (0.97)

[a] Skin specimen from 6–8 week old male hairless mouse.
[b] Mean (± SD) of 6 determinations.

Comparison of the release and skin permeation profiles of LB base and LB HCl from silicone discs (Table 1) showed that both the release and permeation rates increased dramatically when the free base was used instead of hydrochloride salt. An increase of approx. 13-fold in skin permeation rate was observed with the base compared to the salt, although little change in the lag times was observed (Fig. 1). This indi-

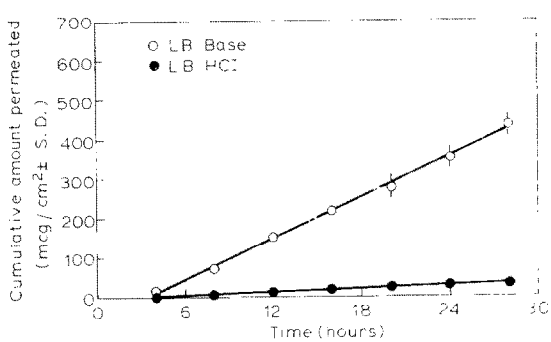

Fig. 1. Comparison of skin permeation profiles of LB base and LB HCl from silicone discs.

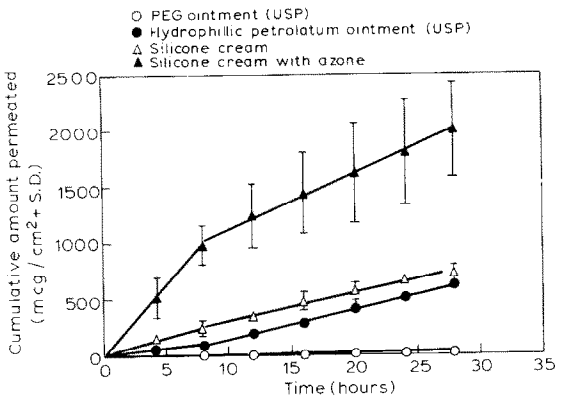

Fig. 2. Skin permeation profiles of LB base from various creams and ointments.

cates that LB was released and permeated much faster in its free base form than its hydrochloride salt from the silicone discs.

Evaluation of creams

The skin permeation profiles of LB base from a silicone cream containing 5% (w/w) LB base across hairless mouse skin showed zero-order permeation at a constant penetration rate of 24.31 (±6.05) $\mu g/cm^2$ per h (Fig. 2).

In order to evaluate the effect of azone on the skin permeation of LB, 10% (w/w) azone was added to the silicone elastomer cream. More than 2-fold increase in flux value [52.73 (±14.47) $\mu g/cm^2$ per h] was observed (Fig. 2).

Analysis of the skin permeation profiles of LB from silicone creams with or without azone indicates that characteristically both showed the same kind of zero-order release after 8 h. The profile with azone indicates that incorporation of azone in the formulation might have changed the inherent barrier property of the skin to some extent as evident from the sudden release of drug up to 8 h. The effect diminished after 8 h which is evident from the later steady state portion of the profile (Table 2). It was reported that an azone-containing ophthalmic formulation of LB did not produce better ocular bioavailability in rabbit (Tang-Liu and Burke, 1988). However, this study has demonstrated the effectiveness of azone in enhancing the permeability of LB across hairless mouse skin.

TABLE 2

Steady state skin permeation [a] *of levobunolol base from different topical formulations*

Formulation	Skin permeation rate [b] ($\mu g/cm^2$ per h)
Silicone cream	24.31 (6.05)
Silicone cream with 10% (w/w) azone	52.73 (14.47)
Hydrophilic petrolatum ointment (USP)	27.02 (1.49)
PEG ointment (USP)	1.09 (0.32)

[a] Skin specimen from 6–8 week old male hairless mouse.
[b] Mean (±SD) of 6 determinations.

Evaluation of hydrophilic petrolatum ointment and PEG ointment

When the constant permeation rates of LB base across the hairless mouse skin from two different ointment preparations having 5% (w/w) loading dose were compared, a significant difference in flux values was observed (Fig. 2). A mean steady state flux value of 27.02 (±1.49) $\mu g/cm^2$ per h was observed from hydrophilic petrolatum ointment, whereas a flux value of 1.09 (±0.32) $\mu g/cm^2$ per h was found from PEG ointment (Table 2). The difference may be due to the lower solubility of LB base in the PEG ointment. Analysis of steady-state skin permeation rates from different creams and ointments shows that LB base permeated through intact hairless mouse skin at different rates from different formulations having the same loading dose (5% w/w). The observed differences may be due to differences in the solubilities of LB base among the various formulations, which in turn give rise to differences in thermodynamic activities of LB base in those formulations.

Evaluation of adhesive patches

The release fluxes obtained from silicone and polyacrylate patches were 245.50 (±51.67) and 309.10 (±26.90) $\mu g/cm^2$ per $h^{1/2}$, respectively. The corresponding skin permeation fluxes were found to be 43.61 (±2.06) and 60.92 (±7.63) $\mu g/cm^2$ per h, respectively (Table 3). Again the differences in flux values may be due to differences in the solubilities of LB bases in two dis-

TABLE 3

Steady state release and skin permeation [a] *of levobunolol base from various transdermal systems*

System	Release rate [b] ($\mu g/cm^2$ per $h^{1/2}$)	Permeation rate [b] ($\mu g/cm^2$ per h)
Silicone patch	245.50 (51.67)	43.61 (2.61)
Polyacrylate patch	309.10 (26.90)	60.92 (7.63)
Polyacrylate patch (in vivo)	–	60.43 (4.62)

[a] Skin specimen from 6–8 week old male hairless mouse.
[b] Mean (±SD) of 6 determinations.

tinct adhesives. The higher release and permeation rates obtained from the polyacrylate patch indicate its suitability as the polymer of choice of the two polymers studied for the development of transdermal levobunolol delivery system.

In vivo study

The results of our indirect in vivo study show an excellent agreement between in vivo and in vitro data. When the cumulative amount of LB permeated into the body was plotted vs time, a good linearity was obtained. The mean flux value obtained from this indirect in vivo method was 60.43 (±4.62) $\mu g/cm^2$ per h which is almost identical to that of 60.92 (±7.53) $\mu g/cm^2$ per h determined in vitro (Fig. 3).

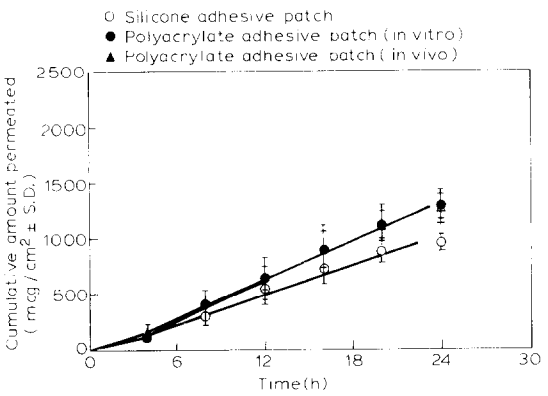

Fig. 3. Skin permeation profiles of LB base from different adhesive patches.

A mass-balance method was employed to determine the in vivo flux value. The blood level of LB after transdermal application was not measured. Therefore, any possible degradation in the serum and/or protein binding were not considered. The indirect in vivo method determines the amount of LB permeated into the body by subtracting the amount remaining in the patch and that retained by the skin from the initial amount of drug contained in the patch. Therefore, it excludes the possibility of any degradation of LB by the skin enzymes.

Conclusion

The results obtained from the in vitro studies show that LB permeated through hairless mouse skin very rapidly when applied from different formulations studied in its free base form. The skin permeation profiles of LB showed that the steady state was reached in less than 5 h for all the formulations. The results of in vitro/in vivo correlation showed excellent agreement. It has been found that following oral administration of a single 3 mg dose of LB hydrochloride in healthy adults, a peak blood concentration of about 16 ng/ml occurs within 1–3 h. Total body clearance of LB from plasma has been reported to be 11 ml/min per kg (Drug Information, 1990). Based on these data, it has been calculated that an input rate of approx. 740 $\mu g/cm^2$ per h is needed from the patch to achieve that plasma concentration. This means that a 12 cm^2 polyacrylate patch should be able to reach the target input rate.

In summary, these initial studies demonstrate the feasibility of LB administration through intact skin from various transdermal delivery systems.

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