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COMMUNICATIONS

In-vitro assessment of a matrix system of levobunolol

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Abstract—In order to study the feasibility of systemic delivery of levobunolol transdermally, a matrix-type delivery system was fabricated using a silicone elastomer. The relationship between loading dose and skin permeation rate was evaluated in-vitro using hairless mouse skin mounted on the stirred receptor compartment of the Keshary–Chien glass diffusion cell maintained at 37°C. The concentration of levobunolol in the receptor compartment was determined by HPLC. A similar study without using the skin was carried out to determine the effect of loading dose on the release of levobunolol from discs. It was observed that the release of drug from disc followed a matrix-diffusion controlled (Q) vs square root of time relationship at different loading doses. In contrast, the results of skin permeation of levobunolol from transdermal discs containing different loading doses showed a linear Q vs time relationship indicating a constant zero order skin permeation rate at each loading dose. Skin permeation of levobunolol appeared to reach a plateau at a 5% (w/w) loading dose in the disc indicating the attainment of equilibrium concentration of levobunolol in the skin.

Transdermal drug delivery systems have gained considerable attention recently. Some of their potential advantages include by-passing liver or gastrointestinal first-pass metabolism, approximation of zero order absorption kinetics and improvement of patient compliance.

Levobunolol, a β-adrenergic blocking agent used in the treatment of cardiovascular disorders, has a short biological half-life (4–6 h) and is effective at a very low plasma concentration (16 ng mL^{-1}) (Drug Information 1990). There is special interest in levobunolol because it is effective in cases of hypertension resistant to propranolol (Gavras et al 1977), is 50 times more potent than L-propranolol, when administered orally, inhibiting isoprenaline-induced tachycardia in conscious dogs (Commarato et al 1976), and shows greater separation than propranolol in the doses causing β-adrenergic blockade and direct myocardial depression (Robson & Kaplan 1970). In this study, a matrix-type transdermal drug delivery system for levobunolol was developed. This communication describes the evaluation of release and skin permeation characteristics of levobunolol from the system.

Materials and methods

Drugs. Levobunolol hydrochloride (PD 085130-0002, Lot #R) was a gift from Warner Lambert Company (Ann Arbor, MI, USA).

Other components. Silicone elastomer, MDX4-4210, and curing agent were gifts from Dow Corning Corporation (Midland, MI, USA).

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

Drug assay. In all skin permeation experiments, quantitation of levobunolol in the receptor compartment was performed by a modified HPLC technique (Schoenwald & Huang 1983). Using acetate buffer (0·25 M) and methanol (at a composition of 50:50 v/v) as the mobile phase at a flow rate of 1·0 mL min^{-1}, levobunolol in the sample solution was resolved by a reversed phase column (μBondapak CN) and detected at a wavelength of 254 nm at ambient temperature. The assay was linear over the concentration range 0·5–5·0 μg mL^{-1} and the minimum detection limit was 0·2 μg mL^{-1}. Receptor samples were diluted and mixed with the internal standard (metoprolol tartrate). Quantitation was by the peak height ratio method using the regression parameters of the standard curve.

To determine the release of levobunolol from the disc, in the absence of interfering substances from the skin, the receptor solution was analysed by spectrophotometry (Response UV-VIS Spectrophotometer, model 25066 × 38, Gilford) at 222·5 nm.

Preparation of a matrix-type transdermal disc. Circular transdermal discs, containing levobunolol as the hydrochloride salt, with a fixed surface area were fabricated by the method of Keshary & Chien (1984). Levobunolol hydrochloride was mixed thoroughly with the required amount of silicone elastomer. A curing agent for the elastomer was added to this mixture (0·1% w/w of the elastomer). The mixture was stirred for 5 min and then deaerated under reduced pressure (30 mm Hg) for 20 min with intermittent release of vacuum to break the entrapped air bubbles.

A plastic cup (19·5 mm i.d. 5 mm depth, red dot electrode, 3M Co., MN, USA) was used as the moulding device and holder. The inside of the cup was lined with a 2 × 2″ piece of aluminium foil. About 1 g of the deaerated blend was then cured at 65°C for 30 min.

After curing, the disc was removed from the oven, 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 skin. A male hairless mouse (6–8 weeks old) was killed by cervical dislocation of the spinal cord and a portion (about 3 × 3 cm) of the full-thickness abdominal skin was carefully excised. The dermal side of the skin was carefully cleared of any adhering subcutaneous tissues or blood vessels.

Skin permeation studies. The freshly excised full-thickness skin sample was mounted on the receptor compartment of a Keshary–Chien glass diffusion cell (Keshary & 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 drug-releasing surface in intimate contact with the skin and the donor cap was properly placed and clamped.

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The receptor solution (pH 7·4 Sørensen's phosphate buffer) was then introduced into the stirred receptor compartment which was maintained at 37°C in a circulating waterbath (Cole-Palmer Instrument Company, Model 1268–00). The donor compartment was maintained at 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 analysed by HPLC. Each data point represents the average of six experiments.

Release studies. To study the release of levobunolol 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. Receptor solution (2 mL) was withdrawn at predetermined time intervals and was immediately replaced by fresh buffer solution. Sink conditions were maintained throughout the experiment. The samples were analysed by spectrophotometry.

Data analysis. From the concentration of levobunolol 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 $\mu g\ cm^{-2}\ h^{-1}$ or in $\mu g\ cm^{-2}\ hg^{-1/2}$, respectively, depending on the cases discussed in the following section.

Results and discussion

Effect of loading dose on levobunolol release from silicone discs. 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:

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

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

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

$$Q = (2AD_PC_Pt)^{1/2} \quad (2)$$

When the drug release at different loading doses from the silicone disc was studied in Sørensen's phosphate buffer solution (pH 7·4), a linear Q vs $t^{1/2}$ was observed at steady-state at each loading dose (Fig. 1). The magnitude of the slope ($Q/t^{1/2}$) increased with an increase in loading dose, which is characteristic of matrix diffusion control systems. The steady state flux values plotted against $A^{1/2}$ gave a linear relationship (Fig. 2), as expected from the modified equation given below:

$$Q/t^{1/2} = (2D_PC_P)^{1/2}\ A^{1/2} \quad (3)$$

This plot can be used in the prediction of the optimum loading dose for specific conditions.

Effect of loading dose on skin permeation of levobunolol from a silicone disc. Skin permeation of levobunolol from a silicone disc across male hairless mouse skin showed a linear steady state Q vs t relationship at each loading dose of levobunolol hydrochloride. This indicates that levobunolol permeates through the intact hairless mouse abdominal skin at a constant rate. The magnitude of the skin permeation rates, as determined by the slopes (Q/t), increased with an increase in loading dose. This relationship can be explained by Fick's law of diffusion under sink conditions (Michaels et al 1975), as described below:

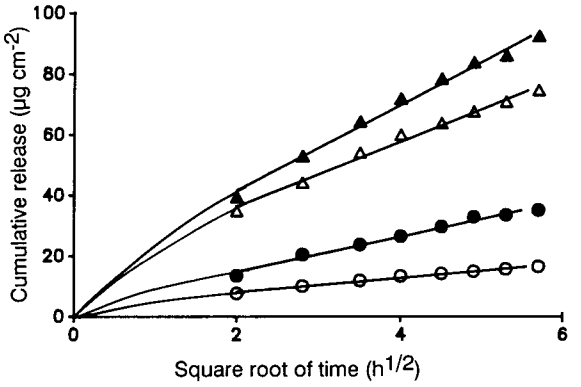

FIG. 1. The release profile of levobunolol from silicone discs at different loading doses. The numbers in parentheses represent the steady-state flux value ($\mu g\ cm^{-2}\ h^{-1/2}$) ± s.d. of 6 determinations. ○, 1% (2·41 ± 0·49); ●, 3% (5·95 ± 1·66); △, 5% (9·43 ± 1·09); ▲, 10% (15·10 ± 1·94).

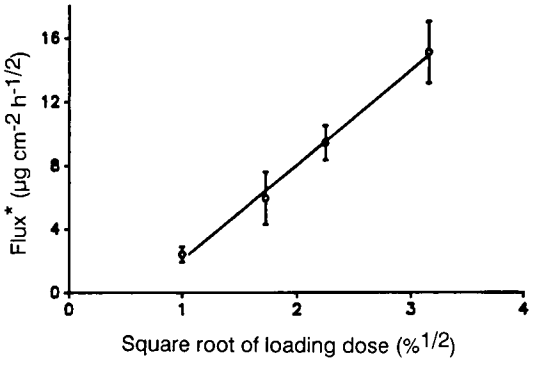

FIG. 2. Relationship between flux and (loading dose)$^{1/2}$ of levobunolol in silicone discs. *n = 6.

$$Q = [(DAK)/h]C_dt \quad (4)$$

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

The observed constant rate of skin permeation may be attributed to the fact that the skin permeation thickness of the drug depletion zone formed during the skin permeation did not add substantially to the total thickness of the diffusional path (Keshary & Chien 1984).

The permeation rate of levobunolol through the intact skin, as found by the magnitude of the slope (J), was observed to increase steadily as the loading of levobunolol in the silicone disc was increased from 1 to 5%. Loading the disc with 10% drug did not show any significant increase in the flux (Fig. 3). The results therefore show that up to a loading dose of 5%, both the release and corresponding permeation rates increased simultaneously. This indicates that both the device and skin are controlling the steady-state permeation of levobunolol through the full-thickness hairless mouse skin. If the skin was the only controlling factor in the steady-state permeation rate, there would be no change in the permeation rate with an increase in the release rate. Therefore, the results suggest that the skin permeation of levobunolol can be increased by increasing the drug release rate

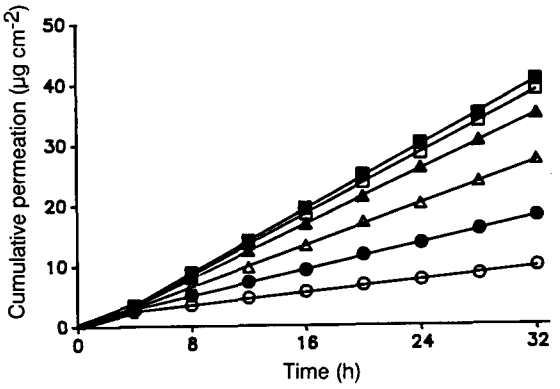

FIG. 3. The permeation profile of levobunolol across hairless mouse skin from silicone discs at different loading doses. The numbers in parentheses represent the steady-state flux value ($\mu g\ cm^{-2}\ h^{-1}$) ± s.d. of 6 determinations. ○, 1% (0·25 ± 0·12); ●, 2% (0·56 ± 0·14); △, 3% (0·85 ± 0·10); ▲, 4% (1·10 ± 0·20); □, 5% (1·29 ± 0·24); ■, 10% (1·44 ± 0·38).

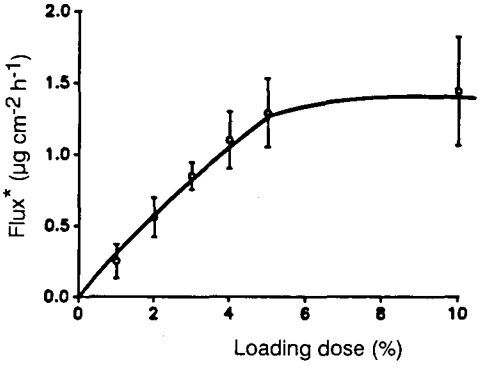

FIG. 4. Relationship of flux and levobunolol loading dose in silicone discs across hairless mouse skin. * n = 6.

from the device, and that the rate of skin permeation from the device obtained thus far has not yet reached the maximum achieveable skin permeability of levobunolol in hairless mouse skin. Increasing the loading dose beyond 5% showed a still greater increase in release rate but no significant change in the permeation rate. This indicates that maximum skin permeability is attained at a loading dose of 5%.

When the skin permeation rate ($\mu g\ cm^{-2}\ h^{-1}$) was plotted against the loading dose (Fig. 4), a hyperbolic relationship was obtained as predicted from the equation developed earlier (Keshary & Chien 1984). For diffusion through intact skin where the stratum corneum is the rate limiting barrier and a pseudo steady-state is established, the total flux (J_S) can be described by the following relationship:

$$J_S = \frac{aA}{1+bA} \quad (5)$$

where A is the loading dose, and a, b are constants.

The above equation can be rearranged to

$$1/J_S = (1/a \times 1/A) + b/a \quad (6)$$

Accordingly, a plot of $1/J_S$ against 1/A will yield a straight line with a slope of 1/a and an intercept of b/a. The data obtained in this study followed this relationship. From the slope and intercept, the values of a and b were found to be 0·26 and 0·018, respectively.

The hyperbolic relationship between the permeation rate of levobunolol (J_S) across the intact skin of male hairless mouse, and the loading dose (A) of levobunolol in the matrix diffusion-type delivery system can, therefore, be expressed by:

$$J_S = \frac{0{\cdot}26A}{1+0{\cdot}018A} \quad (7)$$

It has been found that following oral administration of a single 3 mg dose of levobunolol hydrochloride in healthy adults, a peak blood concentration of about 16 ng mL^{-1} occurs within 1–3 h. Total body clearance of levobunolol from plasma has been reported to be 11 mL $min^{-1}\ kg^{-1}$ (Drug Information 1990). Based on these data, the skin permeation rate obtained from this system does not seem to be high enough to reach the required input rate. One of the reasons may be due to poor solubility of levobunolol hydrochloride in the silicone elastomer. Different polymers may perform better. However, this preliminary study has demonstrated the potential of transdermal delivery of levobunolol.

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