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Research Article

Transdermal Controlled Delivery of Propranolol from a Multilaminate Adhesive Device

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The feasibility of transdermal controlled delivery of propranolol was investigated by conducting *in vitro* skin permeation studies using rabbit pinna (ear) skin. A new multilaminate adhesive device which is capable of releasing propranolol in a controlled fashion over a 24-hr period had been developed and was evaluated transdermally using rabbit pinna skin. Skin permeation of propranolol from the device was found to be controlled by the stratum corneum during the early phase of permeation and then by the adhesive device during steady-state permeation. The rabbit pinna skin was shown to be a good animal model for studying the transdermal permeation of propranolol from the device, when compared to human cadaver skin.

KEY WORDS: propranolol; skin permeation; controlled release; multilaminate adhesive device.

INTRODUCTION

The transdermal controlled delivery of drugs for the systemic treatment of disease has gained increasing interest in recent years. Advantages of transdermal drug delivery include the ability to control the rate and site of drug absorption over a fairly long period of time, as well as to avoid the hepatic first-pass metabolism associated with oral administration of many drugs, including propranolol.

Propranolol, a beta-adrenergic blocking agent used in the treatment of hypertension, is reportedly subjected to an extensive and highly variable hepatic first-pass metabolism following oral administration (1–3). Controlled administration of propranolol via a transdermal delivery system could improve its systemic bioavailability and its therapeutic efficacy by avoiding this first-pass effect, as well as decreasing the dosing frequency required for treatment. Transdermal delivery of propranolol has been accomplished using a gel ointment which successfully achieves therapeutic blood levels in rabbits over an extended period of time (4). However, it has been demonstrated clinically that ointment formulations do not release drug in a zero-order fashion, and therefore, they cannot maintain therapeutically effective blood levels. In addition, dosing with ointment formulations is inconvenient and often inaccurate (5).

Recently, a trilaminate adhesive device has been developed which is capable of delivering propranolol in a controlled fashion over a 24-hour period (6). This study investigates the *in vitro* skin permeation of propranolol delivered from this device, using rabbit pinna (ear) skin. For comparison, permeation of propranolol from the device has also been studied using hairless rat and human cadaver skin.

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MATERIALS AND METHODS

Materials

Propranolol free base was prepared from commercially available propranolol HCl (Sigma Chemical Co., St. Louis, Mo.) and used in the fabrication of the trilaminate adhesive device. The adhesive for the device was a silicone-based pressure-sensitive adhesive polymer which is chemically modified to retain its adhesive properties in the presence of amine drugs (BIO-PSA X7-2920/Dow Corning). All other reagents and solvents, either HPLC grade or reagent grade, were used as obtained (Fisher Scientific Co.).

Preparation of the Rabbit Pinna Skin

The pinna skin is located on the inner side of the rabbit ear. The rabbit pinna was chosen as the principle skin model for both the present *in vitro* and future *in vivo* skin permeation studies since it is easy to obtain, has a low density of hair follicles (therefore, no hair removal is necessary), and the rabbit is large enough for complete pharmacokinetic analysis. Pinna skin was obtained from male New Zealand White rabbits (approximately 6–7 weeks old) immediately after the animals were sacrificed (using T-61 Euthanasia Solution, Hoechst). Following the removal of the ear, an incision was made along the ear margin, and the pinna skin was peeled away from the underlying cartilage. The skin was free of any subcutaneous fat and, therefore, no further preparation was necessary.

Determination of Stratum Corneum Thickness

In order to obtain skin samples which would yield con-

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sistent results, the thickness of the stratum corneum along the length of the pinna was estimated using a well established stripping technique (7). The intact pinna skin which had been removed from the rabbit ear was sandwiched between two glass microscope slides of a known thickness. The skin thickness was measured using a micrometer (Brown & Sharp Corp.) at several different locations along the pinna. The skin was then stripped up to 25 times using Scotch tape (3M Co.) to remove the stratum corneum layer by layer, and the stripped skin thickness was then measured in the same manner. The difference in thickness between the whole skin and the stripped skin was determined as the stratum corneum thickness. The thickness of the stratum corneum determined by the stripping and measuring technique was also confirmed using microscopic measurements.

Determination of the Effect of Stripping on the Stratum Corneum Thickness

Once the skin sample location had been chosen, the effect of the number of strippings on stratum corneum thickness was determined. Whole pinna skin was progressively stripped and measured at selected intervals (0, 3, 5, 7, 12, 15, 17, 20, 22, and 25 strippings) using the same technique as mentioned above.

Microscopy Studies

Fresh pinna skin samples were dehydrated serially with ethanol (50 to 95%) and then infiltrated for 24 hr with an acrylic embedding medium (JB-4, Polyscience). Following infiltration, the skin samples were embedded in JB-4 and cured for 2 days. Skin sections (5 μm) were then cut using a rotary microtome (American Optical, Model 820) with a glass knife and stained using Gill's hemotoxylin 3. Skin sections were then viewed under light microscopy (Leitz, Laborlux 11 POL) at 100× and 430×. The thickness of the stratum corneum was measured using a calibrated eyepiece in the microscope.

In Vitro Skin Permeation of Propranolol in Silicone Fluid

To investigate the skin permeation kinetics of propranolol, the *in vitro* permeation of propranolol through rabbit pinna skin from a saturated solution in silicone fluid (360 Medical Fluid, 20cs, Dow Corning) was carried out in a hydrodynamically well-calibrated horizontal-type skin permeation cell (8) at 37°C using whole and stripped skin specimens. Stripped skin specimens were obtained by stripping whole pinna skin 25 times with Scotch tape. The skin samples were mounted between the two half-cells (with an opening of 0.636 cm^2), with the dermis side facing the receptor half-cell. Then, 3.5 ml of the receptor solution (0.01 *M* acetate buffer, pH 5.6), heated to 37°C, was added to the receptor half-cell, and the donor solution (silicone fluid saturated with propranolol free base), also heated to 37°C, was added to the donor half-cell. Both half-cells were maintained at 37°C by an external circulating water bath. Aliquots of 1 ml were collected from the receptor half-cell at predetermined time intervals, and the receptor compartment was refilled with fresh buffer to its original volume throughout the 24-hr study period. Sink conditions were maintained in the receptor solution throughout the experiment (since propranolol free base has a relatively high solubility (8.99 mg/ml) in the acetate buffer used and large sample sizes (1 ml) were taken).

To ensure that the acetate buffer (pH 5.6) used did not cause skin damage and thus produce an altered skin permeation profile, the studies were repeated using isotonic phosphate buffer (0.01 *M*, pH 7.4) as the receptor solution.

Analytical Method for Determination of Propranolol Concentrations

Propranolol concentrations in the receptor solution were determined by HPLC with fluorescence detection. The HPLC system consisted of a solvent pump (Waters, Model 590), with an automated injection system (Waters, Model 710 WISP), and a μBondapak C_{18} reverse-phase column (Waters, 15 cm). The fluorescence detector (Schoeffel, Model 970) was set with an excitation wavelength of 285 nm and an emission cutoff of 417 nm. The mobile phase was composed of methanol:water:acetonitrile (90:90:30) buffered with 0.04 ml of phosphoric acid and 0.32 g of monobasic ammonium phosphate (9). A standard curve for propranolol was constructed using standards of 0.05- to 200-μg/ml concentrations of propranolol free base in acetate buffer.

Preparation of Trilaminate Adhesive Device

The adhesive device evaluated in this study is composed of three drug-adhesive laminates which were prepared individually and then laminated together, using a process discussed elsewhere (6). Each of the three adhesive layers contain different loading doses and particle sizes of propranolol free base from the inner (furthest from the skin) to the outer (closest to the skin) layers. By laminating these drug-adhesive layers together in the proper order, the release of propranolol can be controlled at a specified rate. The specific composition of the device and the resultant *in vitro* release profile of propranolol from the device are shown in Figs. 1a and b, respectively.

In Vitro Skin Permeation of Propranolol from the Trilaminate Adhesive Device

The *in vitro* skin permeation of propranolol from the adhesive device was carried out in the skin permeation cells outlined above, at 37°C, using pinna skin samples with and without stripping. Stripped skin samples were prepared by stripping the whole skin 3, 7, 13, 20, and 25 times using Scotch tape. For comparison the release of propranolol from the device was also studied in the same permeation cells without skin.

The adhesive devices were carefully applied onto the stratum corneum surface of each skin specimen and pressed, under a 1.5-kg weight for 5–10 sec, to facilitate the adhesion of the pressure-sensitive adhesive device to the skin. The device–skin combination was then positioned between two half-cells with the dermis side of the skin facing the receptor half-cell, into which 3.5 ml of receptor solution (0.01 *M* acetate buffer at pH 5.6) was added. During the course of the permeation study, aliquots of 1 ml were taken at regular time intervals over a period of 24 hr.

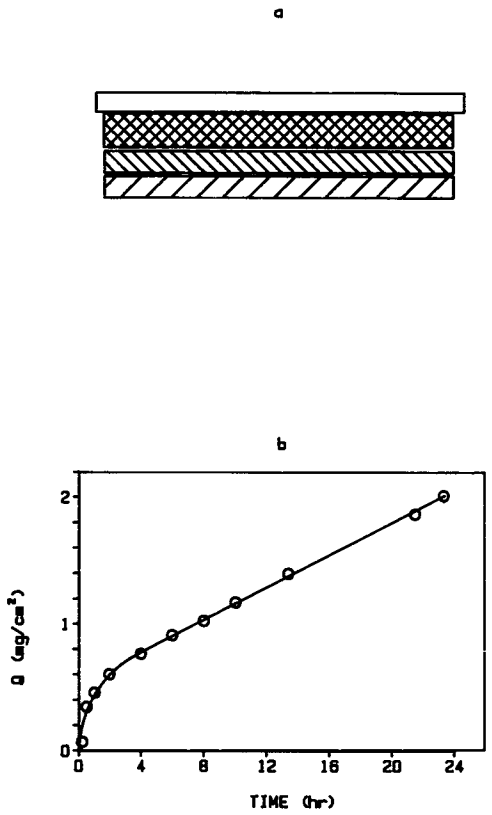

Fig. 1. (a) Schematic representation of the trilaminate adhesive device used in the present study. Shown: release liner (□); inner laminate containing 50% (w/w) propranolol (130-μm particle size) in adhesive (4.0 mils thick) (▨); middle laminate containing 30% (w/w) propranolol (130-μm particle size) in adhesive (3.0 mils thick) (▧); outer laminate containing 20% (w/w) propranolol (1.5-μm particle size) in adhesive (2.5 mils thick) (▨). (b) Release profile of propranolol from the trilaminate adhesive device shown in a. Reproduced from Ref. 6.

In an attempt to determine if the steady-state rate of drug permeation from the device is controlled by the skin or the device, several other trilaminate adhesive devices with different release rates were also evaluated. The same type of trilaminate adhesive devices were prepared, with the outer laminate (closest to the skin) containing 2–28% (w/w) loading doses of propranolol. All of the devices prepared were capable of releasing propranolol in a similar controlled fashion but with different release rates.

Comparison of the Skin Permeation of Propranolol from the Device Across the Rabbit, Hairless Rat, and Human Cadaver Skin

Permeation studies across the skin of rabbit pinna, hairless rat, and human cadaver were conducted to determine any species difference in the skin permeation profile of propranolol delivered by the transdermal adhesive device. Again, devices were placed in intimate contact with the fresh full-thickness skin specimens of rabbit (pinna), fresh hairless rat (abdominal), and frozen split-thickness skin of a human cadaver (anterior torso) and then mounted between the half-cells. During the course of the permeation study, aliquots of 1 ml were then removed from the receptor solution at predetermined time intervals over the 24-hr study period and assayed for propranolol.

RESULTS AND DISCUSSION

Determination of Stratum Corneum Thickness of the Pinna Skin

It was found that the thickness of stratum corneum along the pinna skin varies from 18.1 μm near the top of the ear to 8.1 μm at the base (Fig. 2). However, an area of approximately 9 cm^2 in the middle portion of the ear was found to have a consistent thickness of approximately 13 μm (boxed area in Fig. 2). Skin samples for all of the skin permeation studies were therefore taken from this area.

Microscopy Studies

Results of the microscopic examination of the rabbit pinna skin shown in Fig. 3 indicate that the pinna skin is composed of three basic layers: the stratum corneum (13–14 μm thick), a viable epidermis (45–65 μm thick), and a dermis layer (220–260 μm thick). This structure is quite similar to that of human skin (10).

Skin Permeation Kinetics of Propranolol from a Suspension

The passive diffusion of compound through the skin can be described by Fick's first law of diffusion under sink conditions (11–13):

$$Q = [(DK)/h]C_d t \quad (1)$$

where Q is the cumulative amount of drug which has permeated through the skin, D and K are the diffusivity and partition coefficient of the drug in the skin, respectively, h is the skin thickness, and C_d is the concentration of drug in the donor compartment.

The time lag before steady-state permeation has been reached can be approximated by (13,14)

$$t_l = h^2/6D \quad (2)$$

The skin permeation profiles of propranolol through the rabbit pinna skin from a suspension of propranolol free base in

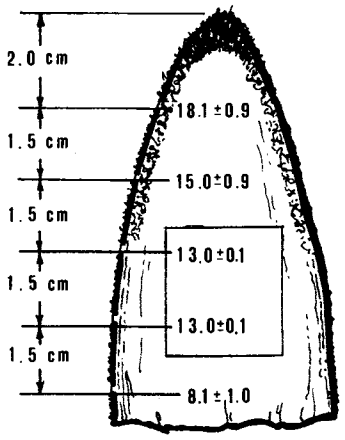

Fig. 2. Variation in stratum corneum thickness along the rabbit pinna skin (N = 6).

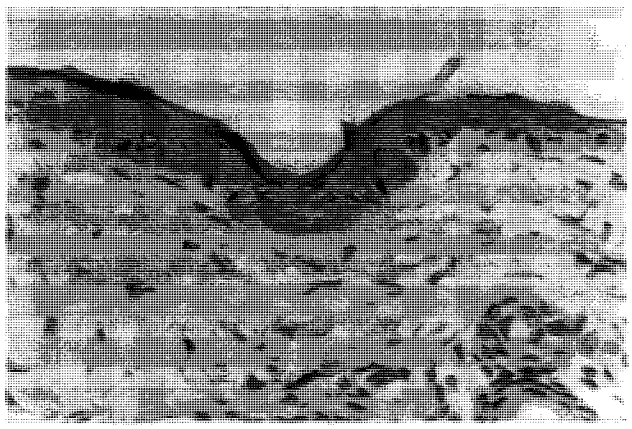

Fig. 3. Light micrograph of the rabbit pinna skin. Cross-sectional view. 430×; reduced 45% for reproduction.

silicone fluid are shown in Fig. 4. The results indicate that propranolol permeates through both whole and stripped skin in a zero-order fashion as expected from Eq. (1), with steady-state permeation rates of 69.2 and 228.1 μg/cm² hr, respectively.

When these studies were repeated using isotonic phosphate buffer (pH 7.4) as the receptor solution, no significant difference in the skin permeation profiles of propranolol was observed between the pH 5.6 and the pH 7.4 solutions. These results indicate that acetate buffer (pH 5.6) produces no damaging effect to the skin and the skin permeation characteristics. Therefore, the acetate buffer could be used as the receptor solution for the skin permeation studies.

In Vitro Skin Permeation of Propranolol from the Adhesive Device

The permeation of propranolol delivered by the trilaminate adhesive device across the intact rabbit pinna skin was observed to follow a zero-order process with a steady-state permeation rate of 62.1 μg/cm² hr (Fig. 5). This permeation rate is somewhat lower than that of the permeation of propranolol from the silicone fluid donor (69.2 μg/cm² hr).

To investigate the possible rate-limiting role of the stratum corneum in the skin permeation of propranolol from the device, permeation across stripped skin was also studied. As the stratum corneum was progressively removed by consecutive stripping, the skin permeation profile of propranolol in the first 4–6 hr changed proportionally, however, there was no significant change in the steady-state permeation rate (Fig. 6). At greater than three strippings, a burst phase in the initial skin permeation profile was observed. This suggests that the stratum corneum plays a significant role in controlling the initial non-steady-state permeation of the propranolol from the device. After approximately 18 strippings, the skin permeation profiles became equivalent to that of the device release profile. This indicates that after a majority of the stratum corneum is removed, the skin permeation rate is controlled by the release kinetics of propranolol from the device. The effect of stepwise stripping on the thickness of the skin is shown in Fig. 7. These results confirmed that the stratum corneum was totally removed after 18–25 strippings.

The development of the burst-phase permeation as a function of the number of strippings indicates that the stratum corneum is acting as a barrier to the non-steady-state skin permeation of propranolol from the device. This barrier function can be demonstrated by plotting the burst and lag

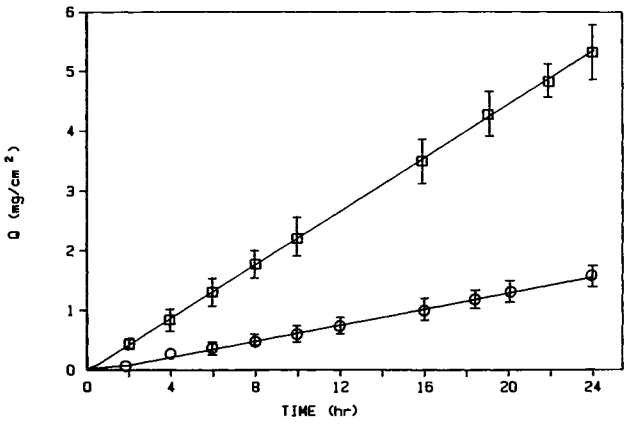

Fig. 4. Permeation profiles of propranolol through whole (○) and stripped (□) rabbit pinna skin, from a saturated solution of propranolol (free base) in silicone fluid (N = 4).

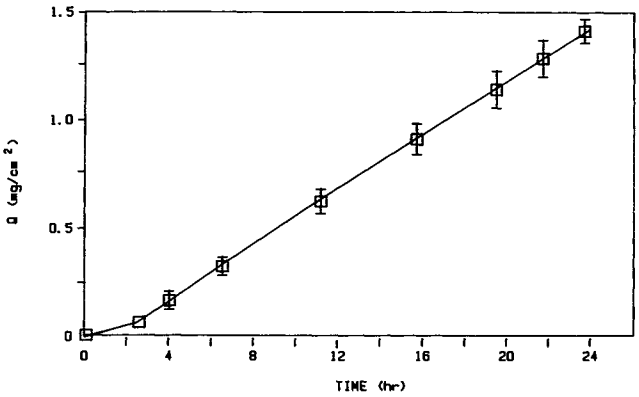

Fig. 5. Permeation profile of propranolol through the full-thickness pinna skin of rabbits from the trilaminate adhesive device (N = 6).

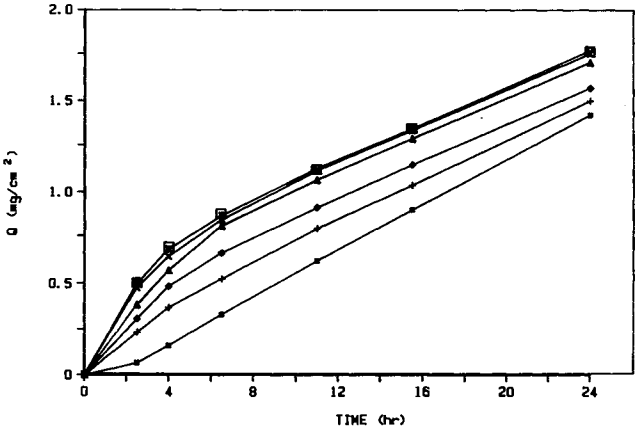

Fig. 6. Permeation profiles of propranolol from the trilaminate adhesive device through pinna skin without stripping (■), and with stripping for 3× (+), 7× (♦), 13× (▲), 20× (×), and 25× (▽), and the release of propranolol from the device (□) (N = 3). Standard deviations <10%.

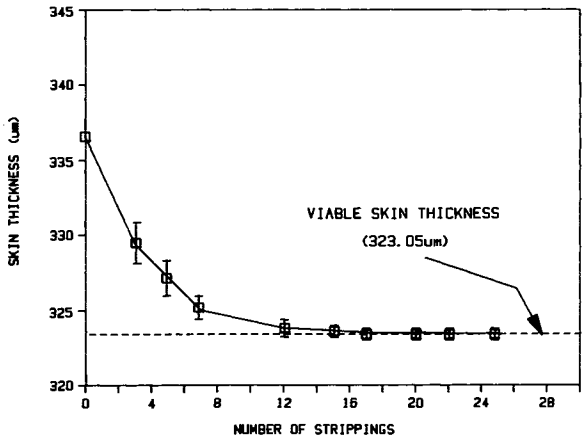

Fig. 7. Thickness of the rabbit pinna skin after various strippings (N = 4).

time for the permeation profile versus the square of its corresponding stratum corneum thickness. The data in Fig. 8 show that as the thickness of the stratum corneum decreases, the duration of the lag time decreases and the burst time increases. The duration of the burst effect is described by (11)

$$t_b = -h^2/(3D) \quad (3)$$

The linear relationship observed between the burst and lag time and the square of the stratum corneum thickness demonstrates that the barrier function of the skin is directly proportional to the square of the stratum corneum thickness (h^2_1) (14,15), as expected from Eqs. (2) and (3).

To investigate the role that the trilaminate adhesive device plays in controlling the steady-state skin permeation of propranolol, three other devices were fabricated to release drug in a zero-order fashion, but at rates different from the original device investigated above. The release rate was varied by loading the outer laminate of the device (closest to the skin) with different drug loading doses. The relationship between the steady-state skin permeation rates and the steady-state release rates obtained is shown in Fig. 9. A linear relationship exists between the release and the corresponding permeation rates, indicating that the device is controlling the

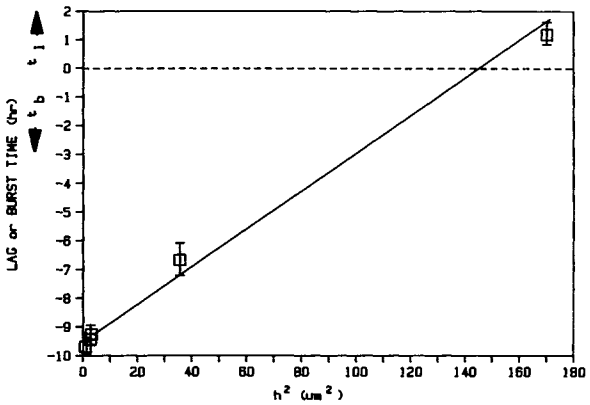

Fig. 8. Plot of the duration of burst and lag times for the permeation profiles of propranolol from the trilaminate adhesive device versus the square of the stratum corneum thickness (N = 3).

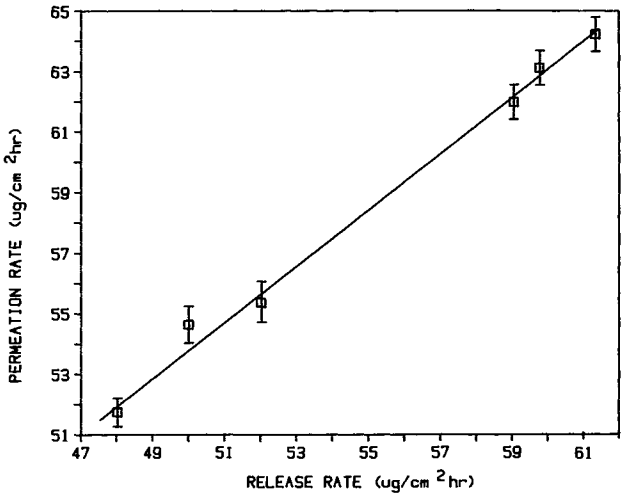

Fig. 9. Relationship between the steady-state permeation across whole skin and the steady-state release rate of propranolol from trilaminate devices containing different drug loads in the outer laminate (closest to the skin) (N = 3).

steady-state permeation through the full-thickness rabbit pinna skin. If the skin is 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 propranolol can be increased by increasing the drug release rate from the device, and the rate of skin permeation from the device obtained thus far has not yet reached the maximum achievable skin permeability of propranolol in the rabbit pinna.

The results obtained thus far indicate that in the early, non-steady-state phase of permeation, the skin permeation of propranolol is controlled by the stratum corneum, as reflected by the thickness-dependent variation in the lag or burst time, while in the later (steady-state) stages of permeation, it is controlled by the release of propranolol from the device. The data in Fig. 6 also support this mechanism of transdermal drug delivery, where the steady-state permeation rate of propranolol does not change as the stratum corneum is progressively removed.

Comparison of Transdermal Delivery of Propranolol in Various Species

The permeation profiles of propranolol through rabbit, human, and hairless rat skin following delivery from the trilaminate adhesive device are compared in Fig. 10. The results demonstrate that the rabbit pinna skin and the human cadaver skin have nearly identical propranolol permeation profiles, indicating that the rabbit pinna skin could be a good animal model for the *in vitro* permeation of propranolol from the device. On the other hand, permeation of propranolol across the hairless rat abdominal skin is significantly lower and also has a longer lag time than that found in the rabbit and human cadaver skin. The differences in the lag times for the different species could be related to the variation in stratum corneum thickness and drug diffusivity in the skin.

In summary, the results of the *in vitro* skin permeation of propranolol from the trilaminate adhesive device indicate

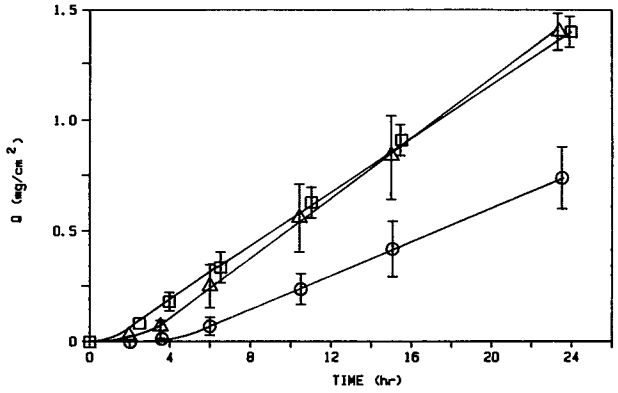

Fig. 10. Comparison of the permeation profiles of propranolol, delivered by the adhesive device, through skin from rabbit pinna (□), hairless rat abdominal (○), and human cadaver skin, anterior torso (△) (N = 3).

that the device is capable of delivering propranolol in a zero-order fashion over a 24-hr period. The skin permeation of propranolol delivered by the device was found to be controlled by the stratum corneum in the initial stage of skin permeation and then by the device once steady-state permeation had been reached. In addition, this investigation has found that the rabbit pinna skin is a useful skin model for studying the *in vitro* skin permeation of propranolol. While subtle differences could have been masked by the use of the controlled-release adhesive device, the human cadaver and rabbit pinna skin showed no significant difference in steady-state permeation rate or lag time, whereas hairless rat skin showed a significantly lower permeation rate with a longer lag time.

The primary concerns in the development of a viable transdermal delivery system are *in vivo* bioavailability and efficacy. Studies are currently under way in our laboratory to investigate the systemic bioavailability of propranolol in rabbits following topical application of the device. In addition, the potential need for skin permeation enhancement and the possibility of skin irritation are being investigated.

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