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Transdermal delivery of metoprolol I: Comparison between hairless mouse and human cadaver skin and effect of *n*-decylmethyl sulfoxide

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Summary

Metoprolol (MP) is a potent β_1-selective adrenergic blocking agent with proven antihypertensive activity However, it undergoes extensive hepatic first-pass metabolism and has a short biological half-life which necessitate frequent dosing to attain adequate therapeutic blood levels. To overcome this problem, the feasibility of systemic delivery of MP via the transdermal route was explored. A monolithic polyacrylate adhesive dispersion type delivery system containing MP free base was fabricated and in vitro skin permeation studies were conducted at 37°C across excised full thickness hairless mouse and dermatomed human cadaver skins. Skin permeation rate across human cadaver skin was found to be lower than that of hairless mouse. Skin permeation profiles across both types of skins showed a membrane permeation controlled Q vs t linear relationship Skin permeation rate was found to be dependent both on adhesive film thickness and loading dose of the drug in the matrix. Maximum skin permeation rate was obtained from a system having 1.5 mm thickness with a 10% (w/w) loading dose *n*-Decylmethyl sulfoxide was found to enhance skin permeation rate of MP through human cadaver skin at a loading dose of 5% (w/w)

Introduction

The transdermal delivery of drugs for the systemic treatment of disease has gained increasing interest in recent years. Some of the potential advantages include by-passing liver and/or gastrointestinal first-pass metabolism, approximation of zero-order absorption kinetics and improvement of patient compliance.

At present, β-adrenoceptor antagonists are extensively used in the therapeutic management of various cardiovascular disorders. Propranolol was the first β-adrenergic blocking agent that came into clinical use and remains the most widely used of these compounds. However, because of its blocking effect on bronchial smooth muscles and skeletal muscles, propranolol is contraindicated in individuals with bronchial asthma and must be used cautiously in a diabetic patient who

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is receiving insulin or oral hypoglycemic agents. As a consequence, there has been a search for β-adrenergic blocking agents with cardioselective properties. MP was found to be the most popular candidate in this category. After a decade of use, MP has become well established as a first choice drug in the treatment of mild to moderate hypertension and stable angina, and is beneficial in post-infarction patients (Weiner, 1985). However, MP is reported to be subjected to extensive hepatic first-pass metabolism following oral administration and has a short biological half-life (Drug Information, 1991). Controlled administration of MP via a transdermal delivery system could improve its systemic bioavailability and its therapeutic efficacy by avoiding the first-pass effect as well as decreasing the dosing frequency required for effective therapy.

Recently, a polyacrylate-adhesive based device has been developed which is capable of delivering MP in a controlled fashion over a 24 h period in our laboratory. This study investigates the in vitro skin permeation of MP from the device, using hairless mouse skin in comparison with dermatomed human cadaver skin. The effect of *n*-decylmethyl sulfoxide, a known relatively nontoxic skin permeation enhancer, on skin permeation of MP was also investigated.

Materials and Methods

Materials

Metoprolol free base was prepared from commercially available metoprolol tartrate (Sigma Chemical Co., St. Louis, MO) and used in the fabrication of the polyacrylate adhesive patch. Polyacrylate adhesive was obtained as gift from National Starch and Chemical Co. (Bridgewater, NJ) and was specially formulated in our laboratory for fabrication of patches. Scotch Pak release liner, 1022, and Scotch Pak backing membrane, 1066, were donated by 3M Co. (St. Paul, MN). *n*-Decylmethyl sulfoxide (NDMS) was obtained from Columbia Organic Chemical Co., Inc. (Camden, SC). All other reagents and solvents, either HPLC grade or reagent grade, were used as obtained (Fisher Scientific Co.).

Methods

Drug assay MP concentrations in the receptor solution were determined by HPLC with fluorescence detection (Horai et al., 1988). The HPLC system (Perkin Elmer, Series 400) consisted of a solvent pump with a fixed loop injector, and a μ-Bondapak C_{18} reverse-phase column (Alltech, Hypersil, ODS, 5 μm). The fluorescence detector (Shimadzu, Model RF 551) was set with excitation and emission wavelengths of 274 and 300 nm, respectively. The mobile phase was composed of acetonitrile : water : triethylamine (25 : 74 : 1 v/v) adjusted to pH 4.0 with phosphoric acid. A standard curve for MP was constructed using standards of 1–10 μg/ml concentrations of MP free base in phosphate buffer.

To determine the release of MP from the patch, in the absence of any interfering substances from the skin, the receptor solution was analyzed by a spectrophotometer (Milton Roy, Model 1201) at 220 nm.

Preparation of patch Weighed amount of metoprolol base was mixed thoroughly with the specially formulated polyacrylate adhesive and a uniform layer of a fixed thickness was made on heat sealable backing membrane (Scotch Pak 1006) by using a laboratory coating device (Werner Mathis USA Inc., NC). The whole system was cured at room temperature under a hood in a dust-free environment overnight. The laminate was then covered by a release liner (Scotch Pak 1022), cut out into 4 cm^2 (2 cm $\times$ 2 cm) pieces and used in the subsequent experiments.

Skin permeation studies The freshly excised full thickness hairless mouse or properly thawed dermatomed human cadaver skin was mounted on Valia-Chien glass diffusion cells (Chien and Valia, 1984) with the stratum corneum side in intimate contact with the MP-releasing surface of the patch and the dermal side facing the receptor solution. The receptor solution (pH 7.4 Sorensen's phosphate buffer) at 37°C was introduced into the stirred receptor compartment which was maintained at 37°C by a circulating waterbath (Cole-Palmer Instrument Co., Model 1268-00). Samples from the receptor compartment were withdrawn at predetermined time intervals and

immediately replaced by an equal volume of fresh buffer solution maintained at 37°C. Initial experiments confirmed the maintenance of sink condition by this procedure. The samples were then analyzed by HPLC.

Release studies To study the release of MP from the patches, the same procedure as described above was employed with the exception that no skin sample was mounted between the donor patch and receptor solution. 2.5 ml of receptor solution was withdrawn at predetermined time intervals and was immediately replaced by fresh buffer solution maintained at 37°C. Sink condition was maintained throughout the experiment. The samples were analyzed by spectrophotometer.

Data analysis

From the concentration of MP 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

Effect of loading dose on release of MP

Diffusional release of a solute from a polymer matrix where the initial loading of solute is less than or greater than the solubility limit is seen to result in a linear plot of total amount of solute release vs the square root of time (Paul and McSpadden, 1976).

When the release of MP from the polyacrylate patch at different loading doses was studied in Sorensen's phosphate buffer (pH 7.4) at 37°C, a linear Q vs $t^{1/2}$ relationship was observed at each loading dose, which is characteristic of matrix diffusion control system. The value of drug release flux ($Q/t^{1/2}$) for each loading dose was calculated from the slope of the linear Q vs $t^{1/2}$ plot, and the data are summarized in Table 1. The results indicated that the release flux of MP from the patches proportionally increased as the drug loading increased at lower drug loadings (up to 5% w/w). However, at higher loading doses, there seemed to be a disproportional increase in the release of MP. This may be attributed to different factors. The most satisfying explanation is that the adhesive matrix gets saturated at a 5% (w/w) loading dose of MP base. At higher drug loading, it is possible that nonuniform dispersion of the drug in the polymer could have occurred, thus resulting in the rather divergent release rate differences. Change in diffusivity of MP in the adhesive matrix at higher concentrations may also be partly responsible (Martin et al., 1983).

TABLE 1

Effect of loading dose on release of metoprolol base from a polyacrylate patch [a]

Dose (% w/w)	Release rate [b] ($\mu g/cm^2$ per $h^{1/2}$)
1	8.81 (1.53)
2	16.72 (3 96)
3	24 00 (4 81)
5	50 50 (4 64)
6	127 75 (9 20)
7 5	297 98 (45.44)
9	426 64 (43.64)
10	625.55 (44.91)

[a] Thickness = 0.6 mm

[b] Mean ± S D of three determinations

Effect of loading dose on skin permeation of MP

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

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

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

skin permeation flux increased with increasing loading dose (Table 2). Like release profiles, a disproportional increase in skin permeation rate was also observed above 5% (w/w) loading dose. This was expected from the corresponding higher release rate of MP above 5% (w/w) loading dose. As more drug was released from the system, the skin permeation rate increased further and even at 10% (w/w) loading dose, the skin did not seem to have reached its saturation limit. Therefore, it is obvious that both the device and skin are controlling the steady-state permeation of MP through the full thickness of the hairless mouse skin up to the loading dose studied.

Comparison of skin permeation rate between hairless mouse and human cadaver skin

Skin permeation of MP from a polyacrylate patch having a 10% (w/w) loading dose across the human cadaver skin also followed linear Q vs t linear relationship indicating constant zero-order permeation. However, the permeation rate was found to be a little lower (35.49 ± 1.77 $\mu g/cm^2$ per h) as compared to that (45.89 ± 1.47 $\mu g/cm^2$ per h) found with hairless mouse skin from the same patch (Fig. 1). Many literature references have indicated that hairless mouse skin is more permeable than human cadaver skin and sometimes the observed difference is quite significant (Sato et al., 1991). In contrast, in vitro studies by Stoughton (1975) showed remarkable similarities in absorption for the skin of the two species for many compounds.

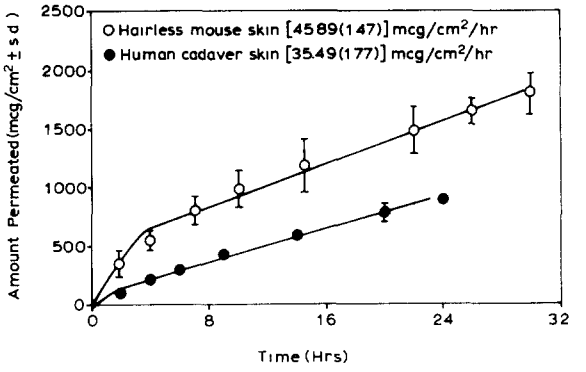

Fig 1. Comparison of skin permeation profiles of MP (10% w/w) from an adhesive patch (0.6 mm) across hairless mouse and human cadaver skins

Through both types of skins, MP was found to permeate immediately without any lag time. This may be due to leaching out of some components of the patch which caused alteration of the permeation property of the skins. Microscopic examination of the skins after removal of the patch, however, did not show any morphological change.

Effect of film thickness

In order to study the effect of polyacrylate film thickness, both release and skin permeation studies were conducted with patches of varying thicknesses. Constant Q vs $t^{1/2}$ release and a Q vs t skin permeation profiles were observed at each thickness. Both the release and skin permeation rates were found to increase proportionally with increasing thickness (Table 3). It is suggested that as more drug was released from the patches of greater thickness, correspondingly higher skin permeation rates were observed. Film thickness was not increased beyond 1.5 mm as longer period of time was required to cure the patches.

Effect of n-decylmethyl sulfoxide

Recently, NDMS, a non-ionic surfactant, has been studied as a percutaneous absorption enhancer. It is reported to increase the permeability of compounds by denaturing keratin or by perturbing the lipid structures in skin. It has been clinically used in a tetracycline formulation for its

TABLE 2

Effect of loading dose on permeation of metoprolol base from a polyacrylate patch [a] *across hairless mouse skin*

Dose (% w/w)	Permeation rate [b] ($\mu g/cm^2$ per h)
1	0 35 (0.06)
2	1.25 (0 34)
3	1.91 (0 71)
5	6.62 (0.68)
6	14.01 (0 53)
7.5	34.11 (3 83)
9	39 51 (3 35)
10	45 89 (1 47)

[a] Thickness = 0.6 mm.
[b] Mean ± S.D of three determinations.

TABLE 3

Effect of polyacrylate film thickness on skin permeation of metoprolol base from a polyacrylate patch [a] *across human cadaver skin* [b]

Film thickness (mm)	Release rate [c] ($\mu g/cm^2$ per $h^{1/2}$)	Permeation rate [c] ($\mu g/cm^2$ per h)
0 6	625 55 (44 91)	35 49 (1.77)
0 9	706.61 (8 79)	68.55 (3.09)
1 2	777.28 (23 89)	90.68 (3.69)
1 5	834.28 (9.82)	118.43 (5.87)

[a] 10% (w/w) loading dose

[b] Skin specimen from the thigh region of a 34 year old female cadaver

[c] Mean ± S D. of three determinations

enhancing action, a factor which suggests it is safe to use on live skin (Wechsler et al., 1978). In order to evaluate the effect of NDMS on skin permeation rate of MP across human cadaver skin, polyacrylate patches (10% w/w, 1.5 mm) containing varying concentrations of NDMS were fabricated and tested for skin permeation potential. The results are summarized in Table 4. It was found that incorporation of NDMS up to 3% w/w concentration did not produce any significant change in skin permeation rate. A concentration of 5% w/w of NDMS showed 40% enhancement in MP permeation rate. This may be attributed to the fact that at 5% NDMS, the epidermal concentration reached a critical value

TABLE 4

Effect of NDMS on skin permeation of metoprolol base from a polyacrylate patch [a] *across human cadaver skin* [b]

NDMS (% w/w)	Permeation rate [c] ($\mu g/cm^2$ per h)
Control	118.43 (5 87)
1	109.58 (6.35)
3	121.51 (21.04)
5	166 92 (7.45)
7.5	159.58 (10 70)
10	160.12 (35 28)

[a] 10% (w/w) loading dose and 1 5 mm thickness

[b] Skin specimen from the thigh region of a 34 year old female cadaver.

[c] Mean ± S.D. of three determinations.

Enhancement ratio = 1 4.

to exert pharmacological activity. Increasing the NDMS concentration above 5% did not produce any further increase in the MP concentration rates but rather decreased slightly. This may be due to reduced thermodynamic activity of MP in the patch as the result of solubilization of MP with higher concentration of NDMS. In a similar study, it was reported that the skin permeation rate of methotrexate was not increased up to a concentration of 1%, whereas a dramatic increase was observed at 2.5% (McCullough et al., 1976).

Conclusion

The results obtained from the in vitro studies show that MP permeated through both hairless mouse and human cadaver skin quite efficiently when applied from the polyacrylate patch and no significant lag time was observed. It has been reported (Drug Information, 1991) that the effective therapeutic concentration of MP after oral administration is 25 ng/ml and the calculated total body clearance of MP from plasma is approx. 18 ml/min per kg. Based on these data, it is estimated that an input rate of approx. 1.9 mg/h is needed from the patch to achieve that plasma concentration in normal human subjects. This means that a 16 cm^2 patch without any enhancer or a 11 cm^2 patch with 5% (w/w) NDMS should be able to maintain the target input rate for 24 h.

In summary, our initial studies demonstrate the feasibility of MP administration through intact skin from the developed transdermal patch. The primary concerns in the development of a viable transdermal patch are in vivo bioavailability and efficacy. Studies are in progress to evaluate the systemic bioavailability of MP following topical application of the patch. In addition, elucidation of the mechanism of action of NDMS and its potential skin irritation problems are currently under investigation in our laboratory.

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