RESEARCH ARTICLE

Development of a Transdermal Patch of **Methadone: In Vitro Evaluation Across** Hairless Mouse and Human Cadaver Skin

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ABSTRACT

A 3-day monolithic polyacrylate adhesive dispersion type delivery system containing methadone was fabricated and in vitro permeation through hairless mouse and human cadaver skins was conducted. The effect of skin permeation enhancers was also investigated. 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 cumulative amount permeated (Q) versus time (t) relationship. Skin permeation rate was found to be dependent on both adhesive film thickness and loading dose of the drug in the matrix. Effective skin permeation rate across the hairless mouse skin was obtained from a patch with 1.5 mm thickness and 15% w/w loading dose. n-Decylmethyl sulfoxide and Azone were found to produce an effective skin permeation rate of methadone through human cadaver skin at a 5% w/w concentration. These initial studies demonstrated the feasibility of methadone administration through intact skin from a transdermal patch. KEY WORDS: Adhesive patch; Azone; Hairless mouse skin; Human cadaver skin; n-Decylmethyl

sulfoxide; Methadone; Transdermal delivery.

INTRODUCTION

The high prevalence of drug abuse imposes a substantial financial burden on those affected and on society. Drug abuse is one of the major causes of widespread illness, high use of medical care services, premature death, and considerable costs to society. The substance dependency treatment program is one of the few options we are left with to halt the increasing trend of substance dependency costs. A great deal of research has been done to assess effectiveness and safety of different types of chemical agents for treatment of opioid dependency. Methadone, which has been used since 1965 for both detoxification and maintenance therapy, is still considered to be the drug of choice for treatment of opioid dependency. Methadone is a synthetic morphine agonist which, as an oral substitute for heroin or other morphine-like substances, suppresses the opiate agonist abstinence syndrome in patients who are dependent on these drugs. Peroral administration of metha-

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done is inappropriate to patients suffering from nausea, vomiting, or dysphagia, whereas parenteral administration needs medical supervision. Administration of methadone transdermally may be a possible approach to overcoming these problems. Besides patient convenience, enhanced and controlled therapeutic responses such as avoidance of variable and incomplete bioavailability and maintenance of steady-state plasma concentration with much less peak-and-trough variation have been reported as the advantages of transdermal drug delivery. Transdermal delivery of different narcotic analgesics has been reported (1-4). No study on development of a transdermal patch formulation of methadone has been reported so far except an earlier study on human cadaver skin permeability of methadone from solution (5)

This paper describes preliminary in vitro work on development of a 3-day transdermal patch of methadone. In vitro skin permeation of methadone through hairless mouse and human cadaver skins was investigated. The effect of *n*-decylmethyl sulfoxide and Azone, two known skin permeation enhancers, on human cadaver skin permeation of methadone was also evaluated.

MATERIALS AND METHODS

Materials

Methadone free base was prepared from commercially available methadone-HCl (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 the 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). Azone was obtained from Discovery Therapeutics Inc. (Richmond, VA). All other reagents and solvents, either high-performance liquid chromatography (HPLC) grade or reagent grade, were used as obtained (Fisher Scientific Co.).

Fabrication of Transdermal Patch

Transdermal monolithic patches for methadone were fabricated by a method similar to that recently described for other drugs (6-8). Pharmaceutical-grade purified acrylic pressure-sensitive adhesive (National Starch and

Chemical Corporation, NJ) was used as the adhesive to make the patches. A weighed amount of the drug was dispersed homogeneously in the polyacrylate adhesive with gentle shaking, and a single transparent layer with fixed thickness was made on heat sealable backing membrane (Scotch Pak 1066) by using a laboratory coating device (Werner Mathis USA Inc., NC). The whole system was cured at room temperature in a dustfree environment overnight. The laminate was then covered by a release liner (Scotch Pak 1022), cut into 1 cm² (1 cm × 1 cm) pieces. Optimization of the patch in terms of achieving the target delivery rate was done by trial and error method through varying the thickness of the drug loaded adhesive matrix and also by changing the amount of drugs per square centimeter of a patch. In the last phase of experiments, formulations containing either of the two enhancers were investigated in order to achieve the desired skin permeation rate and to sustain it for 3 days.

Skin Permeation Studies

Either a section of the freshly excised full thickness hairless mouse or a section of the properly thawed. dermatomed human cadaver skin was mounted on a side-by-side glass diffusion cell (n = 3) with the stratum corneum side facing upward and the dermal side facing the receptor solution. After carefully removing the release liner, a patch was then placed on the stratum corneum with the drug-releasing surface of the patch in intimate contact with the stratum corneum. In order to maintain sink condition throughout the experiment, pH 4.4 acetate buffer was used as the receptor solution (5). Receptor solution at 37°C was introduced into the stirred receptor compartment, which was maintained at 37°C by a circulating water bath. 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 the HPLC procedure described below.

Assay of Methadone

The concentration of methadone in the receptor phase was measured by a stability-indicating HPLC assay method (5). The HPLC system (Perkin Elmer, Series 400) consisted of a solvent pump with a fixed loop injector, and a Chromsep Spherisorb CN column ($100 \times 3.0 \text{ mm}$, 5 µm). The ultraviolet (UV) detector (Perkin-



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Elmer, LC-95) was set at 292 nm. The mobile phase—composed of methanol:0.3% aqueous triethylamine (60:40, v/v), adjusted to pH 4.0 with phosphoric acid—was set at a flow rate of 0.6 ml/min. Under this condition methadone showed a retention time of 5.9 min. Linearity was evaluated over the methadone concentration range of 20–500 µg/ml, with a minimum detection limit of 5 µg/ml. To measure higher concentration, dilution was made with HPLC grade water prior to injection. The lowest (20 µg/ml) and the highest (500 µg/ml) samples were assayed over 3 consecutive days to determine the coefficient of variation. None of them exceeded 5%.

Data Analysis

The experimental skin permeation and release flux of methadone from the patch was calculated using a modified Fick's law equation (9):

$$J = V \left(\frac{dC}{dt} \right) / A \tag{1}$$

where J is the steady-state flux i(in micrograms/square centimeter/hour); dC/dt is the steady-state slope of the concentration versus time plot in (micrograms/cubic centimeter/hour); V is the volume of the receptor compartment (in cubic centimeters); and A is the diffusional area of the membrane (in square centimeters). Skin permeation data were analyzed by plotting the cumulative amount of methadone permeated (per unit area) versus time. The slope of the linear portion of this plot is the skin permeation rate of methadone. The skin permeation rate was computed using a LOTUS 1-2-3 spread-sheet program. The Mann-Whitney test was used to verify statistical difference on the data obtained in different experiments of the present study.

RESULTS AND DISCUSSION

Skin permeation of methadone from different formulations across the male hairless mouse skin or dermatomed human cadaver skin followed a linear Q versus t relationship, as shown in Figs. 1-3. This relationship can be explained by Fick's law of diffusion under sink conditions, as described below (9):

$$J = [(DAK/h]C_{d}t (2)$$

where J 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, h represents the thickness of the skin, and C_d is

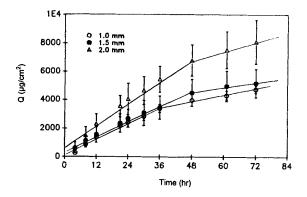


Figure 1. Effect of film thickness on in vitro permeation profile of methadone (mean $\pm SD$, n=3) following application of the patch to the abdominal skin of hairless mouse.

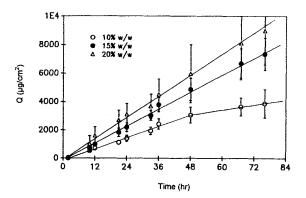


Figure 2. Effect of loading dose on in vitro permeation profile of methadone (mean \pm SD, n=3) following application of the patch to the abdominal skin of hairless mouse.

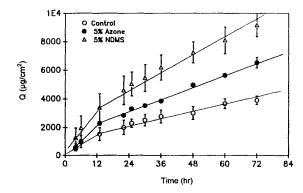


Figure 3. Effect of permeation enhancers on in vitro permeation profile of methadone (mean \pm SD, n=3) following application of the patch to the thigh region of human cadaver skin



the concentration of the drug in the system. Although the in vitro release kinetics of methadone from the systems showed a linearity of release rate as a function of square root of time, the in vitro permeation profiles through hairless mouse and human skin conform to zero-order kinetics because the systems release methadone at a rate which is greater than the skin permeation rate. Similar trends have been reported in the literature earlier (6,7,10).

Effect of Film Thickness

In order to study the effect of polyacrylate film thickness, skin permeation studies were conducted with patches having 10% (w/w) loading dose and different thicknesses. Three different thicknesses (1.0, 1.5, and 2.0 mm) were studied. In all cases, biphasic skin permeation profiles were observed. In the case of the patch with 1.0 mm thickness, a steady-state skin permeation rate of 94.04 (\pm 6.02) µg·cm⁻²·hr⁻¹ was maintained up to 36 hr, followed by a drop in the skin permeation rate to 36.71 (\pm 4.21) µg·cm⁻²·hr⁻¹ maintained up to 72 hr. In case of patches with 1.5 mm and 2.0 mm thicknesses, initial steady-state skin permeation rates of 84.98 (± 22.06) and 136.81 (± 26.04) µg·cm⁻²·hr⁻¹, respectively, were maintained up to 48 hr. These high initial rates were followed by a drop in the skin permeation rates to 29.11 (\pm 2.68) and 55.02 (\pm 18.18) μg·cm⁻²·hr⁻¹, respectively, maintained up to 72 hr (Fig. 1). The results are summarized in Table 1. Higher release from patches having greater than 1.0 mm thickness was also able to sustain the initial higher skin permeation rate for a longer period (up to 48 hr in 1.5-mm and 2.0-mm patches compared to 36 hr in the case of the 1.0-mm patch). Due to differences in time intervals of phase I and phase II in patches having 1.0 mm thickness (0-36, 36-72 hr) versus patches having 1.5 and 2.0 mm thicknesses (0-48, 48-72 hr), no inference on statistical difference could be drawn. However, between patches having 1.5 mm and 2.0 mm thicknesses, skin permeation rates were significantly different at both phase I and phase II levels (p < 0.05). Film thickness above 2.0 mm was not studied as longer curing time was necessary.

Effect of Loading Dose

The next phase of the study was devoted to investigation of the effect of increasing loading dose in the formulation. Three different loading doses (10%, 15%, and

Table 1

Effect of Film Thickness on Steady-State Permeation^a of Methadone from Patches with 10% w/w Loading Dose

Thickness (mm)	Skin Permeation Rate ^b	
	Phase I	Phase II
1.0	94.04° (6.02)	36.71 ^d (4.21)
1.5	84.98e (22.05)	29.11f (2.68)
2.0	136.81e (26.04)	53.02 ^f (18.18)

^aAbdominal skin specimen from 8-week-old male hairless mouse.

20% w/w) were studied under constant patch thickness of 1.5-mm. When higher loading doses (15% and 20% w/w) were incorporated in the 1.5 mm patches, two noticeable changes occurred. Not only was the steady state skin permeation rate increased compared to the control, it was also sustained up to 72 hr. In the case of a 15% w/w loading dose, a monophasic skin permeation profile with a steady-state skin permeation rate of 103.43 (\pm 16.84) µg·cm⁻²·hr⁻¹ was observed, whereas with the 20% w/w loading dose, the same nature of profile with an even higher steady-state skin permeation rate of 122.94 (\pm 35.34) µg·cm⁻²·hr⁻¹ was found (Fig. 2; Table 2). But the difference in skin permeation rates between patches with 15% and 20% loading doses was not statistically significant (p > 0.1).

Table 2

Effect of Loading Dose on Steady-State Permeation^a of Methadone from Patches with 1.5 mm Thickness

Loading dose (% w/w)	Skin Permeation Rate ^b	
	Phase I	Phase II
10	63.58° (10.47)*	21.40 ^d (14.65)*
15	mate.	103.43 ^e (20.10)
20		122.94e (35.34)

^aAbdominal skin specimen from 14 week old male hairless mouse.



^bMean (\pm SD) of 3 determination ($\mu g \cdot cm^{-2} \cdot h^{-1}$).

^{°0-36} hr.

d36-72 hr.

e0-48 hr.

f48-72 hr.

^bMean (\pm SD) of 3 determination (μ g·cm⁻²·h⁻¹).

^{°0-48} hr.

^d48-72 hr.

^{°0-72} hr.

^{*}c and d not statistically different from values reported in Table 1 for similar patches (p > 0.05).

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Effect of Permeation Enhancers in Human Cadaver Skin

Skin permeation studies were conducted across dermatomed human cadaver skin using methadone patches of 1.5 mm thickness and 15% w/w loading dose. A loading dose of 20% w/w or a patch with a thickness of 2.0 mm seemed to compromise the adhesiveness of the formulation to some extent. Therefore the patch with 15% loading dose and 1.5 mm thickness was chosen for human cadaver skin permeation studies. To study the effect of permeation enhancers, two commonly used skin permeation enhancers, Azone and n-decylmethyl sulfoxide (NDMS), were incorporated separately in the above-mentioned formulation at a 5% w/w concentration.

In all three formulations, biphasic skin permeation profiles were observed. But a higher skin permeation rate was found to continue up to 12 hr followed by a drop in the skin permeation rate which was then maintained up to 72 hr (Fig. 3). In the formulation without any enhancer, an initial permeation rate of 98.29 (± 1.30) μg·cm⁻²·hr⁻¹was observed up to 12 hor followed by a constant permeation rate of 42.74 (± 8.59) μg·cm⁻²·hr⁻¹ maintained up to 72 hr. The change in the skin permeation profiles with these formulations may be due to inherent difference between hairless mouse and human cadaver skins. The same formulation with 5% Azone showed the initial rate of $196.82 (\pm 6.50)$ μg·cm⁻²·hr⁻¹ up to 12 hr followed by a maintenance rate of 72.33 (\pm 6.80) µg·cm⁻²·hr⁻¹ sustained up to 72 hr. In the formulation containing 5% NDMS, the initial and final rates were found to be 227.47 (± 26.89) and 91.59 (\pm 20.03) µg·cm⁻²·hr⁻¹, respectively. Therefore, both the enhancers were found to significantly enhance the skin permeation rate in both the initial and final phases (p < 0.10). The enhancement factors were calculated and listed in Table 3. Comparing the two enhancers studied, NDMS was found to be significantly more effective than Azone in enhancing skin permeation of methadone across the human cadaver skin in both the initial and the final phases (p < 0.10).

The first phase of the study was devoted to observation of the effect of drug-loaded adhesive film thickness on maintainenance a constant rate of skin permeation over 3 days. By increasing thickness, maintenance of initial skin permeation rate could be increased from 36 hr to 48 hr, but the target of 72-hr maintenance could not be reached. The next phase of the project dealt with changing the loading dose in the formulation. The formulation with 10% w/w loading dose and 1.5 mm thick-

Table 3

Effect of Permeation Enhances (5% w/w) on Steady-State
Permeation* of Methadone from Patches with 15% w/w
Loading Dose and 1.5 mm Thickness

Enhancer	Skin Permeation Rate ^b	
	Phase I ^c	Phase IId
Control	98.28 (1.30)	42.74 (8.59)
Azone	196.82 (6.50)	72.33 (6.80)
	[2.0] ^e	[1.7]¢
NDMS	227.47 (26.89)	91.59 (20.03)
	[2.3] ^e	[2.1]e

aSkin specimen from the thigh region of a 52-year-old Caucasian male human cadaver

ness was chosen as control based upon permeation rate and adhesiveness. The target maintenance period of 72 hr was achieved when loading dose was increased from 10 % to 15 % w/w. The same 72-hr maintenance was also observed in the formulation with 20% w/w loading dose with even higher steady-state skin permeation rate. But these observations were based on hairless mouse skin. In order to correlate the hairless mouse skin permeation data obtained with that of human, the third phase of the experiment was conducted using human cadaver skin. In that phase, the effect of skin permeation enhancers on skin permeation rate of methadone was also investigated. Enhancement of skin permeation rate could mean lower loading dose and/or smaller patch size for this controlled drug. Compared to the hairless mouse skin, the permeation profile of methadone across human cadaver skin was different. In human cadaver skin, the initial higher skin permeation rate could not be maintained more than 12 hr with the formulation studied. But a constant skin permeation rate from 12 to 72 hr was observed. The enhancement effect of Azone and NDMS on skin permeation of methadone was also significant.

To investigate the possible reason for biphasic skin permeation profiles observed in some formulations across the hairless mouse skin, the contents of the patches were analyzed before use, at the time point where slope changed, and at the end of 72 hr. It was observed that as long as the patches were retaining about 40% of the original loading dose, the initial steady-state skin permeation could be maintained. When the loading dose



bMean (± SD) of 3 determination (µg·cm⁻²·h⁻¹).

^{°0-12} hr.

d12-72 hr

eEnhancement factor compared to control under the same condition.

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