International Journal of Pharmaceutics, 33 (1986) 37–43
Elsevier

IJP 01097

Transdermal delivery of anxiolytics: in vitro skin permeation of midazolam maleate and diazepam

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(Received 25 April 1986)
(Accepted 12 May 1986)

Key words: Midazolam maleate – Diazepam – Transdermal delivery – Azone-permeation enhancement

Summary

The skin permeation profiles of midazolam maleate and diazepam from various solvent systems were investigated. Results were computed using a program designed to manipulate skin permeation data. It was shown that the permeation rates of diazepam and midazolam maleate may be affected by using aqueous mixtures of polar organic solvents. The effect of 1% and 5% Azone on the permeation of diazepam and midazolam maleate was studied. It was found that Azone increases the permeation fluxes of both drugs, the hydrophobic diazepam and the salt midazolam maleate. In the presence of 5% Azone in a solvent system of propylene glycol (PG–ETH–H_2O) the fluxes were increased 43 times for diazepam and 86 times for midazolam maleate. The performance of a novel hydrophilic transdermal matrix containing 50 mg midazolam maleate was tested in vitro. The steady-state flux obtained was used for a coarse estimation of the feasibility of transdermal administration of midazolam maleate.

Introduction

Midazolam, synthesized by Fryer and Walser in 1976, is a relatively new benzodiazepine derivative with anxiolytic and sedative properties. The fused imidazole ringe (Fig. 1) modifies the properties of the classic benzodiazepines (e.g. diazepam) related to receptor affinity, metabolism and water solubility. Its pharmacological, pharmacodynamic and pharmacokinetic behavior have been reviewed by Dundee et al. (1984). The methyl group on the fused imidazole is very rapidly oxidized by the liver enzymes and the metabolite is further eliminated as glucuronide (Gerecke, 1983). The drug is characterized by rapid onset and short duration of pharmacodynamic effect due to the rapid elimination (half-life between 1.5 and 3.5 h) by metabolic inactivation.

This study was performed in order to investigate the skin permeation behavior of midazolam and to compare it with that of the classic derivative, diazepam.

The physical properties of midazolam, such as the water solubility of the salt, the base lipophilicity ($k_{octanol}$/buffer 7.5 = 475) (Gerecke, 1983) and its pK_a 6.15 (Walser et al., 1978) indicate a priori that the drug may permeate the skin at rates that can be controlled by drug delivery system conditions. The performance of a matrix transdermal delivery system formulated by us (Touitou, 1985) containing midazolam maleate was evaluated.

It was also of interest to learn the effect of

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Azone on the skin permeation of diazepam, a hydrophobic drug, in view of contradictory reports on the effectiveness of Azone in enhancing the permeability of hydrophobic compounds (Stoughton, 1982; Sugibayashi et al., 1985).

Materials and Methods

Materials

Midazolam maleate was a gift of Hoffman-La Roche, Basle, and Azone was a gift of Nelson, U.S.A. Diazepam was purchased from Sigma. [^{3}H]Diazepam with a specific activity of 90 Ci/mmol and a concentration of 1 mCi/ml was supplied by Amersham, and [^{3}H]midazolam with a specific activity of 200 mCi/mmol and a concentration of 4 mCi/ml was prepared by Kamag, Dimona, Israel. Propylene glycol (Sigma) and ethyl alcohol (Frutarom, Israel) were used as solvents.

Full-thickness skin

Hairless mice were obtained from Hadassah Hospital Jerusalem. The skin of 5–7-week-old male mice was excised before the experiment, washed, examined for integrity and cut for diffusion cell assembly. In most of the experiments the skin from the abdominal site was used. When dorsal skin was used for comparison purposes, it was noted in the data for that specific experiment.

In vitro skin permeation experiments in horizontal diffusion cells

The horizontal diffusion cells used have been described elsewhere (Touitou and Abed, 1985a). The cell compartments had a volume of 3 ml each, and an effective permeation area of 0.78 cm^2. Solutions of various compositions containing the drug were placed in the donor compartment. The receiver was filled with distilled water. The cells were kept at 23 ± 2°C during the experiment. 100 μl samples were withdrawn periodically from the receiver and replaced by fresh solution from 0 to a maximum of 30 h. Samples of 10 μl were withdrawn from the donor at time 0 and at the end of each experiment. The samples were mixed with Instagel scintillation cocktail (Packard) and assayed in the Kontron Betamatic scintillation counter (Lumitron Sci. Instr.). The experiments were designed to keep pseudo-sink conditions and yield steady-state diffusion. Three experiments were performed for each system.

Skin permeation determination in Franz cells

The permeation flux of midazolam maleate from a matrix transdermal delivery system formulated by us (Touitou, 1985) was measured in vertical cells (Franz diffusion cells) supplied by Crown Glass Co., N.J. The experimental system conditions were: effective area of permeation, 1.77 cm^2, receiver temperature, 37°C and receiver volume, 8 ml. Samples were collected at various intervals for 48 h post application. The samples were assayed as described above. The results are presented as a mean of 4 experiments.

Data computation

The computation was carried out on an IBM personal computer. The kinetic parameters of drug permeation were obtained using a computer program specially designed by us to manipulate data of skin permeation experiments. This program enables the calculation of the cumulative amount of drug, Q, permeating the skin directly from the sample values in counts per minute (cpm), the conversion of them into mg, and the correction of preceding samples (Touitou and Abed, 1985b). Q vs t (time) plots are drawn, and fluxes (F) and lag times are also calculated. An example of such a computer plot and data are given in Fig. 2.

Flux comparisons were made using the Student's *t*-test (two-tailed).

Results and Discussion

The time permeation courses in vitro of midazolam maleate and diazepam have been determined in horizontal diffusion cells using solutions of drug in water or in mixtures of polar organic solvents such as propylene glycol (PG) and ethanol (ETH) e.g. PG–ETH 3:7; PG–ETH–H_2O 2:2:1; PG–ETH 1:1. Plots of cumulative amount of midazolam maleate, Q. vs time, penetrated the skin from these mixtures containing 5 mg/ml drug are shown in Figs. 2 and 3.

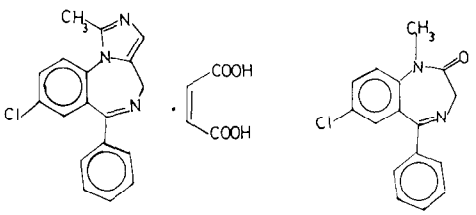

Fig. 1. A: midazolam maleate, B: diazepam.

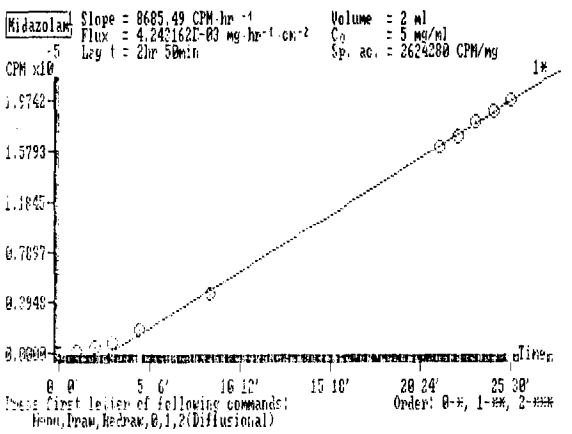

Fig. 2. Example of output of computerized data on the skin permeation profile of midazolam maleate from an aqueous solution containing 5 mg/ml drug.

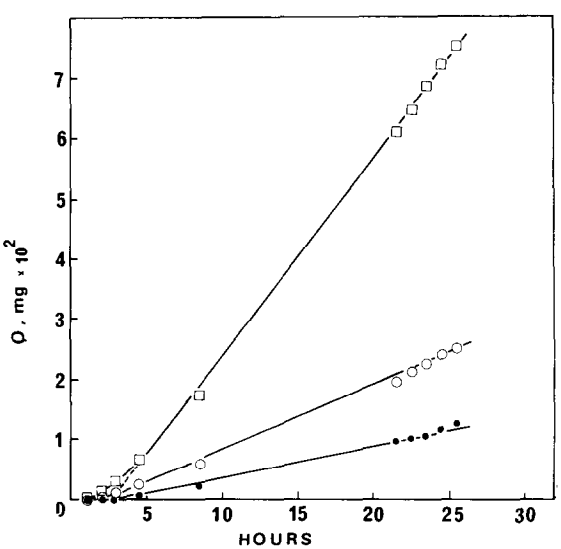

Fig. 3. Skin permeation profiles of midazolam maleate from various solvents containing 5 mg/ml drug. □, H_2O, ○, PG–Eth–H_2O 2:2:1; ●, PG–Eth 3:7.

From the profiles drawn in these figures it is clear that the highest permeation was obtained when the drug was dissolved in water. The fluxes calculated from the linear segments at steady-state in Figs. 2 and 3 are 4.2 and 1 $\mu g \cdot cm^{-2}\ h^{-1}$, for water and propylene glycol : ethanol 3 : 7 mixture, respectively; these values indicate a decrease of more than 4 times in the permeation rate of midazolam maleate from systems containing the organic polar solvents.

The effect of solvents on the skin permeability of diazepam was measured using solvent mixtures with the same compositions as those used for midazolam maleate. Unlike the midazolam maleate solutions, the diazepam solutions contained different concentrations of drug due to the very low solubility of diazepam in water. The aqueous solution of diazepam had a concentration of 10^{-2} mg/ml, while the concentration of diazepam in organic solutions ranged between 4.2 and 4.8 mg/ml.

The fluxes and permeation constants of diazepam from these systems are given in Table 1. From water, diazepam permeated the skin at lower rates than from the PG–ETH mixtures. Furthermore, in the organic solvent systems, an increase in the donor drug concentration led to an increase in the amount that permeated the skin. The permeation could be increased by up to 7 times;

TABLE 1

PERMEATION FLUXES AND PERMEABILITY CONSTANTS OF DIAZEPAM THROUGH HAIRLESS MOUSE SKIN FROM VARIOUS SOLVENTS TO WATER

PG, propylene glycol.

Concentration (mg/ml)	Solvents	Flux ($mg \cdot cm^{-2} \cdot h^{-1} \times 10^4$)	K_p ($cm \cdot h^{-1} \times 10^2$)
$1.0\ 10^{-2}$	Water	1.2	1.20
$1.0\ 10^{-2}$ *	Water	0.6	0.60
4.2	PG–Ethanol 3:7	8.4	0.02
4.4	PG–Ethanol–H_2O 2:2:1	7.6	0.02
4.8	PG–Ethanol 1:1	5.1	0.01

* Dorsal skin.

however, in order to obtain this permeation the concentration of diazepam was increased 420 times. To obtain a more complete picture of the skin permeability of diazepam from these systems the effect of drug concentration on the steady-state fluxes was determined. Fluxes were obtained from the slopes of the linear regression lines of the plots Q vs t at various donor concentrations and plotted against drug initial concentration. An example of these plots is given in Figs. 4 and 5.

Fig. 4 shows that by increasing the concentration of diazepam in PG–ETH–H_2O 2:2:1 from 8×10^{-3} to 1.9 mg/ml the cumulative amount, Q, of drug released in the receiver was directly increased. A classic time course for a molecule permeating the skin in vitro, linearity with a lag time, can be seen for each donor concentration tested. No concentration related change in lag time can be observed.

The fluxes (F) vs concentration plot in Fig. 5 is linear and intersects the origin. This indicates that Fick's law can be applied and the permeability constant K_p, may be obtained from the slope of the regression line. For the PG–ETH–H_2O (2:2:1) mixture the permeability constant of diazepam through hairless mouse skin reaches a value of 2.3×10^{-4} cm/h. The values of K_p for diazepam in the different solvents systems are given in Table 1. The data presented in this table show a skin permeability of up to 120 times greater for diazepam from water than from a mixture of PG–ETH (1:1) 1.2×10^{-2} vs 1×10^{-4} cm/h respectively. These results indicate a low activity of diazepam in the polar organic solvent mixtures tested. From a formulative perspective, the permeation rates of diazepam can be monitored using aqueous mixtures of organic solvents in which the drug concentration and the activity of the drug are brought to an optimum for each system.

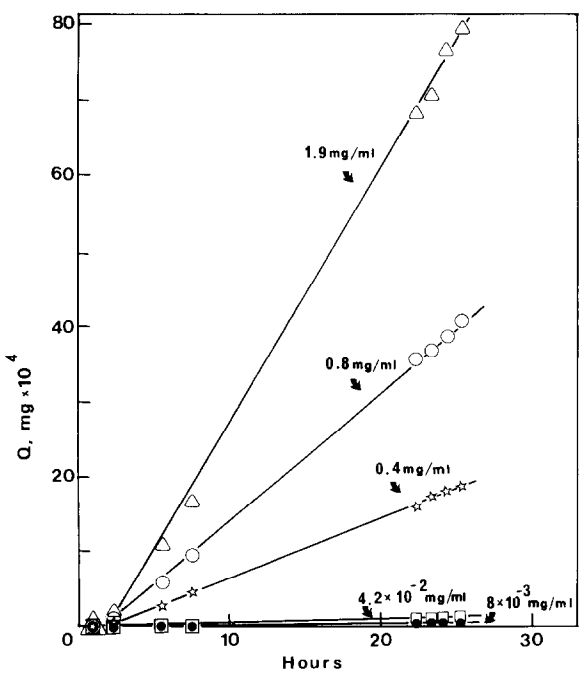

Fig. 4. Permeation profiles of various concentrations of diazepam in PG–Eth–H_2O (2:2:1) through hairless mouse skin.

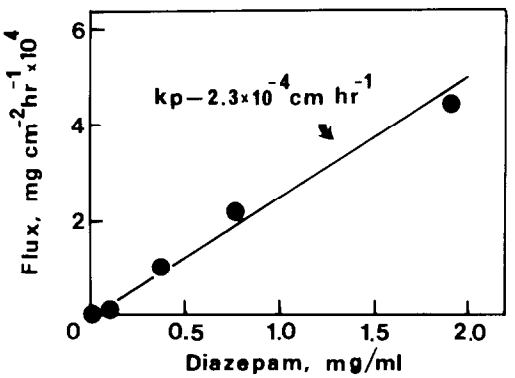

Fig. 5. Plot of permeation flux vs. concentration of diazepam in PG–Eth–H_2O (2:2:1) solutions.

Effect of Azone

From the data presented it is noteworthy that in all the systems tested, the skin permeation of diazepam is low and probably will need to be enhanced in order to enable transdermal administration. One approach to promote drug transport through the skin is to use compounds that may alter skin permeability. Azone (1-dodecylazacycloheptan-2-one) is a relatively new molecule that has been reported to dramatically enhance the permeation of many compounds such as: clindamycin phosphate, erythromycin base, fusidate sodium, fluorouracil, desomide, amcinomide, triamcinolone acetonide (Stoughton, 1982), iodo-

deoxyuridine (Touitou et al. 1985), metronidazole (Wotton et al., 1985) and trifluorothymidine (Sheth et al., 1986). The drugs listed are either hydrophilic or hydrophobic molecules. The exact mechanism of permeation enhancement by Azone is not clarified. In a study on the enhancing effect of Azone on the permeation of fluorouracil, Sugibayashi et al. (1985) reported that results obtained in vitro with 3.3% w/v Azone on hairless rat skin suggest that Azone mainly affects the stratum corneum. Further, the authors conclude that Azone might not be effective for enhancing the permeability of lipophilic compounds.

In the present work the effect of 1 and 5% Azone on the permeation of diazepam and midazolam maleate was investigated. These concentrations have been chosen based on reported results indicating that the appropriate concentrations of Azone are in the range of 2–10% (Stoughton and McClure, 1983). The results are presented in Fig. 6 and Table 2. Fig. 6 shows the permeation profiles of diazepam from systems containing 0, 1 and 5% Azone and 5 mg/ml drug in PG–ETH–H_2O mixture. The amount of diazepam that permeated the hairless mouse skin was drastically increased in the presence of 5% Azone. It is interesting to observe that although the Q vs t plot shows a classic profile, the lag time was increased from about 2.5 to 6 h. The calculated fluxes given in Table 2 indicate that 5% Azone highly and significantly ($P < 0.001$) increases the skin permeation rates of both drugs, diazepam and midazolam maleate from the PG–ETH–H_2O mixture containing 5 mg/ml drug, e.g. 43-fold increase for diazepam and 86-fold for midazolam maleate fluxes. At a concentration of 1% Azone the enhancement was relatively low, e.g. 2-fold for diazepam and 3-fold for midazolam fluxes.

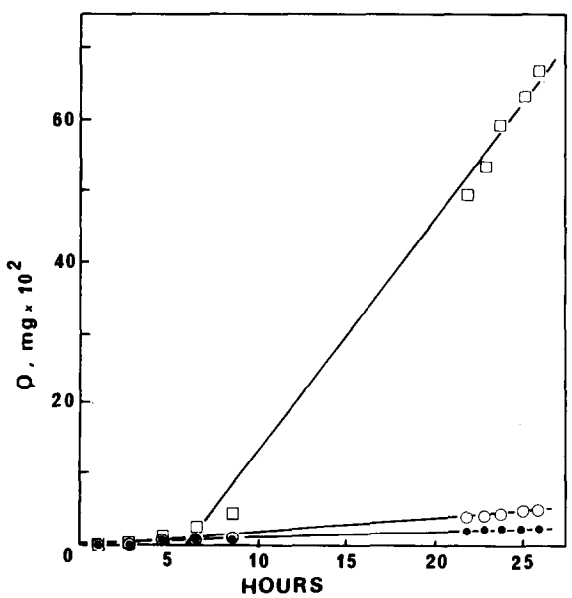

Fig. 6. Effect of Azone on the skin permeation profile of diazepam from a 5 mg/ml solution in PG–Eth–H_2O (2:2:1). ●, no Azone; ○, 1% Azone; □, 5% Azone.

TABLE 2

EFFECT OF AZONE ON THE PERMEATION RATES OF MIDAZOLAM MALEATE AND DIAZEPAM THROUGH HAIRLESS MOUSE SKIN FROM SOLUTIONS CONTAINING 5 mg/ml DRUG IN PG–ETH–H_2O (2:2:1)

Drug	Azone (%)	Flux ($mg \cdot cm^{-2} \cdot h^{-1} \times 10^3$)
Midazolam maleate	0	1.0
	1	3.0
	* 5	86.1
Diazepam	0	1.1
	1	2.1
	* 5	47.4

* $P < 0.001$ when tested vs 0% Azone.

These results indicate that in the solvent system tested Azone enhances the skin permeation in vitro of diazepam, a lipophilic drug. However, it is also interesting to note that at both concentrations tested the effect on midazolam maleate was stronger than the effect on diazepam.

Transdermal delivery of midazolam maleate from a matrix delivery system

The steady-state fluxes of midazolam maleate may be modulated by altering the donor system characteristics such as pH and solvents.

Midazolam maleate was incorporated in a novel transdermal patch. The patch is a flexible hydrophilic polymeric gel matrix which enables the incorporation of oily and hydrophilic solvents (Touitou, 1985). Patches containing midazolam maleate at a concentration of 100 mg drug/1 g

patch were tested in vertical Franz diffusion cells. In these studies patches of 550 mg containing 50 mg drug were applied to stratum corneum of the hairless mouse skin with an effective area of 1.77 cm^2 and the amount that permeated the skin was assayed in the receiver solution. Four experiments were performed and the time courses of the mean cumulative amount of midazolam delivered transdermally for these systems are presented in Fig. 7. Following a lag time of about 14 h a steady-state regime was reached where the drug permeates the skin with a flux of 3.9×10^{-1} mg · $cm^{-2} \cdot h^{-1}$.

A first approximation of the feasibility of midazolam maleate for transdermal administration in humans can be made by estimating the steady-state plasma concentrations C_{ss} using Eqn. 1. This equation was already used by Guy and Hadgraft (1985) for clonidine administered transdermally. This approximation can be made if the in vitro skin permeation kinetic parameters of the drug from the device and two pharmacokinetic parameters, the volume of distribution, V_d, and the systemic elimination kinetics, k_4 are available.

$$C_{ss} = Ak^{\circ}/V_d k_4 \quad (1)$$

where V_d and k_4 are defined above and A is the surface area of the delivery system. If V_d and k_4 are drug pharmacokinetic characteristics nonrelated to the mode of administration, k° must reflect the flux of drug permeating the skin and not only the pure release rate from the delivery system. Thus, k_o will be best expressed by the steady-state flux of the drug through human skin.

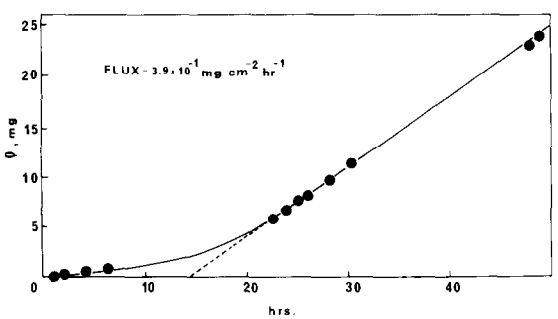

Fig. 7. Skin permeation profile of midazolam maleate from the transdermal patch tested in Franz cell assembly at 37°C.

Midazolam maleate values of 50.2 liter and 0.313 h^{-1} were reported for V_d and k_4 respectively (Heizmann et al., 1983). The application area, A, is arbitrarily taken as 20 cm^2. Using the value of $3.9.10^{-1}$ mg · $cm^{-2} \cdot h^{-1}$ for k°, a steady-state concentration of 496 ng/ml is estimated by means of Eqn. 1. This estimated plasma value of midazolam is high enough (Heizmann et al., 1983) to allow for a possible correction for permeation through human skin.

This coarse estimation given here indicates that midazolam maleate may be an interesting candidate for transdermal administration.

Acknowledgement

The author would like to thank Mrs. Michaela Engel for her excellent technical assistance.

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