Drug Permeation through Membranes IV: Effect of Excipients and Various Additives on Permeation of Chlordiazepoxide through Polydimethylsiloxane Membranes

EDWARD G. LOVERING[x], DONALD B. BLACK, and MARY L. ROWE

Abstract □ Permeation experiments were used to look for interactions between chlordiazepoxide and some common excipients, nutrients, and surfactants. No effect on the permeability coefficient was observed in the presence of starch, calcium hydrogen phosphate, gelatin, lactose, silica, sucrose, cholesterol, or porcine mucin. The coefficient increased in the presence of magnesium stearate and decreased with talc, sodium saccharin, skim milk, egg lecithin, beef albumin, egg albumin, polysorbate 80, cetrimonium bromide, and sodium lauryl sulfate, indicating an interaction of these substances with the drug.

Keyphrases □ Chlordiazepoxide—permeation through polydimethylsiloxane membranes, effect of excipients and various additives □ Permeation—chlordiazepoxide through polydimethylsiloxane membranes, effect of excipients and various additives □ Membranes, polydimethylsiloxane—permeation of chlordiazepoxide, effect of excipients and various additives □ Polydimethylsiloxane membranes—permeation of chlordiazepoxide, effect of excipients and various additives □ Drug permeation—chlordiazepoxide through polydimethylsiloxane membranes, effect of excipients and various additives

A broadly based, continuing investigation of drug availability and absorption is being carried out in these laboratories. Part of the work involves dissolution and permeation studies aimed at developing relationships between physical properties measured *in vitro* and absorption parameters measured *in vivo*. Absorption of a drug is hindered by chemical or physical processes that decrease its effective concentration in the gut. Complex formation, partitioning into surfactant micelles, adsorption on surfaces, and precipitation are processes that interfere with absorption. Interactions that decrease the effective drug concentration can be detected by permeation experiments carried out *in vitro*, since the permeation rate of a drug in solution is directly proportional to its concentration. Factors affecting the permeation of chlorpromazine (1), amobarbital (2), and phenylbutazone (3) were reported previously.

This paper reports the effect of various excipients, nutrients, and surfactants on the permeation of chlordiazepoxide through polydimethylsiloxane membranes.

EXPERIMENTAL

Permeation Measurements—Cells of the type described by Garrett and Chemburkar (4) were used, except that the polydimethylsiloxane membranes were attached with silicone adhesive and the cells were stirred internally. The cells were placed in stirred beakers containing chlordiazepoxide solution, and the entire assembly was thermostated at 37 ± 1°. Drug solutions were buffered over a pH range of 3–8 using citrate or phosphate buffers (5). The desorbing solution within the cell was buffered at pH 1.2 to ensure zero concentration of unionized drug, thus preventing back-permeation. Due to an excess of drug outside the cell, concentrations were constant during the experiment and steady-state permeation rates were obtained about 1 hr after the experiment began. The desorbing solution was circulated through a UV spectrometer, and the absorption was measured at appropriate time intervals. The Beer's law molar extinction coefficient of chlordiazepoxide at 244 nm was 34,200 at pH 1.2.

Materials—Polydimethylsiloxane membranes (0.0025 cm), thought to contain silica filler, were cut from two squares as obtained from the manufacturer[1]. The squares were labeled A and B for identification purposes. The permeability coefficient of the drug measured in Membrane B was about 1.4 times greater than that measured in Membrane A under the same experimental conditions. The reason for the difference is not known. *cis*-1,4-Polybutadiene membranes (0.029 cm) containing no filler were lightly cross-linked using 2,5-bis(*tert*-butylperoxy)-2,5-dimethylhexane[2]. Chlordiazepoxide hydrochloride[3] was used as received.

Other materials used were: skim milk powder; sucrose; cholesterol[4], certified; sodium lauryl sulfate[4] USP; polysorbate 80[4]; magnesium stearate[4] USP; starch[4] USP; calcium hydrogen phosphate[4], certified; bovine albumin[5], fraction V; egg lecithin[5], 95–100%; magnesium sulfate[5], anhydrous reagent; cetrimonium bromide[5], reagent; sodium saccharin[5], reagent; sodium deoxycholate[5], reagent; gelatin[5] BP; talc[5], fine powdered, acid purified; porcine mucin[6], crude Type II; egg albumin[7], IX cryst, chicken; silica[8]; lactose[9] USP; and stearic acid[10], high purity.

RESULTS AND DISCUSSION

Permeation results were plotted as the moles of drug traversing the membrane against time. Slopes, m, were measured from the steady-state portion of the curves. Permeability coefficients, P, were calculated from:

$$P = \frac{ml}{AC_0}\left[1 + e^{2.303(\mathrm{pKa}-\mathrm{pH})} + \frac{1}{T}\right] \qquad \text{(Eq. 1)}$$

where l is the membrane thickness, A is the surface area, C_0 is the total drug concentration outside the cell, pKa is the dissociation constant, and $T = D_a l/K_p h D_m$; D_a and D_m are the diffusion coefficients of the drug in water and the membrane, respectively; h is the thickness of the aqueous diffusion layer; and K_p is the partition coefficient of unionized drug between the membrane and the aqueous solution. The term T^{-1} is negligible except when the permeation rate is limited by the aqueous diffusion layer at the membrane surface.

Effect of pH—The permeation rates of chlordiazepoxide were measured over the pH range of 3.02–8.01. The permeability coefficients were calculated from Eq. 1 by taking the pKa as 4.8 and neglecting the T^{-1} term. Based on the results of 26 experiments, P was found to be independent of pH and have a value of $(6.65 \pm 0.41) \times 10^{-8}$ cm² sec^{-1} (Membrane A). If T^{-1} were not negligible, P calculated in this manner would be pH dependent. The fact that T^{-1} could be neglected indicates that the rate-limiting step in the permeation is the membrane and not the aqueous diffusion layers in contact with it (6). Phosphate buffer (pH 7.0–7.2) was used in all experiments where an excipient or other

[1] General Electric Co., Schenectady, N.Y.
[2] Cis-4 1203, Phillips Petroleum Co., Bartlesville, Okla.
[3] Hoffmann-La Roche Inc., Montreal, Canada.
[4] Fisher Scientific Co.
[5] British Drug Houses.
[6] Sigma Chemical Co.
[7] Miles Laboratories Inc.
[8] Cab-O-Sil M-5, Cabot Corp.
[9] Merck.
[10] Fine Organics Inc.

Table I—Effect of Excipients on Permeability Coefficients

Excipient	Excipient Concentration, g liter^{-1}	pH of Solution	Permeability Coefficient, (cm^2 sec^{-1}) × 10^8
Starch	1.22	7.17	6.31
	2.04	7.18	5.64
	12.9	7.15	5.53
	33.6	7.16	5.42
			Mean 5.72 ± 0.40
Calcium hydrogen phosphate	1.26	7.16	5.86
	9.9	7.16	6.01
	29.7	7.16	5.71
			Mean 5.86 ± 0.15
Gelatin	9.7	7.01	6.49
	10.1	7.02	5.92
	31.9	6.65	5.53
			Mean 5.98 ± 0.48
Lactose	1.18	7.16	6.42
	2.49	7.15	5.41
	47.1	7.14	6.24
			Mean 6.02 ± 0.54
Silica	0.05	7.17	6.39
	0.50	7.17	5.89
	4.88	7.17	5.40
			Mean 5.89 ± 0.50
Talc	2.02	7.18	5.56
	9.9	7.20	3.94
	40.0	7.22	1.95
	40.0	7.16	1.75
	40.2	7.20	1.33
Magnesium stearate	0.053	7.16	7.46
	0.061	7.20	7.27
	0.40	7.20	7.39
	0.46	7.22	8.38
	0.55	7.18	8.38
	4.87	7.18	8.64
			Mean 7.92 ± 0.61

Table II—Effect of Nutrients on Permeability Coefficients

Nutrient	Nutrient Concentration, g liter^{-1}	pH of Solution	Permeability Coefficient, (cm^2 sec^{-1}) × 10^8
Sucrose	40.0	7.10	6.12
	60.0	7.08	5.98
	100.0	7.04	5.28
			Mean 5.79 ± 0.45
Cholesterol	1.01	7.13	6.20
	1.47	7.12	5.79
	2.00	7.13	5.58
			Mean 5.86 ± 0.32
Porcine mucin	2.00	7.10	6.74
	7.00	6.98	6.93
	15.0	6.82	5.78
			Mean 6.48 ± 0.62
Sodium saccharin	5.00	7.10	5.69
	5.03	5.95	5.44
	10.0	7.08	5.27
	10.0	6.89	5.43
	16.0	7.04	4.75
	16.0	6.78	4.33
Skim milk	40.0	7.10	4.11
	60.0	7.08	3.76
	80.0	7.06	2.76
Egg lecithin	0.96	7.12	4.52
	2.07	7.11	3.39
	3.28	7.10	2.69
Beef albumin	2.02	7.13	4.72
	5.00	7.13	4.00
	8.00	7.10	3.81
Egg albumin	0.20	7.10	5.50
	0.50	7.09	5.24
	0.79	7.09	5.20

Table III—Regression Coefficients of Nutrient: Permeability Coefficient Plots

Nutrient	Slope, cm^2 liters sec^{-1} g^{-1}	Intercept, cm^2 sec^{-1}	Correlation Coefficient
Sodium saccharin	-9.4×10^{-10} ($p < 0.05$)	6.12	−0.91
Skim milk	-3.4×10^{-10} ($p < 0.05$)	5.56	−0.96
Egg lecithin	-7.8×10^{-9} ($p < 0.05$)	5.19	−0.99
Beef albumin	-1.5×10^{-9} ($p < 0.10$)	4.94	−0.95

substance was added to the drug solution. Over this narrow pH range, the mean permeability coefficient was $(6.02 \pm 0.28) \times 10^{-8}$ cm^2 sec^{-1} (15 experiments with Membrane A). Permeability coefficients determined in the presence of nutrients, surfactants, and excipients are compared to this value.

Excipients—The effect on the permeability coefficient of excipients common in chlordiazepoxide formulations available in Canada is given in Table I. Relatively large quantities of excipients were used to increase the probability of detecting an interaction. A significant change in the permeability coefficient was deemed to have occurred if the observed coefficient differed by more than 2 *SD* ($p < 0.05$) from the coefficient for the drug itself. No significant change in the permeability was found in the presence of starch, calcium hydrogen phosphate, gelatin, lactose, or silica. The permeability decreased in the presence of large quantities of talc. A linear regression of the permeability coefficient against the concentration of talc in the slurry yielded a linear correlation ($p < 0.05$) with a regression coefficient of 0.98. The drug may be adsorbed on the surface of the talc or react with a minor impurity in it. Even though the quantities of talc used are larger then would be encountered in practice, the result suggests that talc should be used with caution in formulating chlordiazepoxide. It was recently shown that talc interferes with the *in vivo* absorption of dicumarol in dogs (7).

When magnesium stearate, a widely used excipient, was slurried in a chlordiazepoxide solution, the permeability coefficient increased from $(6.02 \pm 0.28) \times 10^{-8}$ to $(7.92 \pm 0.61) \times 10^{-8}$ cm^2 sec^{-1} (Membrane A). In subsequent experiments on the same membranes carried out without magnesium stearate in the drug solution, the permeability coefficient was $(8.09 \pm 0.59) \times 10^{-8}$ cm^2 sec^{-1}. Thus, magnesium stearate appears to have altered the properties of the membrane toward the permeation of chlordiazepoxide.

To investigate the cause of the increase in the permeability, new cells were prepared with fresh membranes. The mean coefficient for chlordiazepoxide, based on nine experiments, was $(8.72 \pm 0.50) \times 10^{-8}$ cm^2 sec^{-1} (Membrane B). Addition of magnesium sulfate to the drug solution led to some precipitation, probably of magnesium phosphate from the buffer, but to no significant change in the permeability coefficient [$(8.03 \pm 0.28) \times 10^{-8}$ cm^2 sec^{-1}; three experiments]. Therefore, the enhanced permeability does not appear to be due to the reaction of the drug with magnesium ions to form a chelate compound of greater solubility or diffusivity in the membrane than chlordiazepoxide. After the cells were allowed to stand overnight in a slurry of stearic acid and rinsed clean, the permeability coefficient of the drug increased to $(11.8 \pm 0.7) \times 10^{-8}$ cm^2 sec^{-1} (three experiments). The possibility that the enhanced permeability coefficient was the result of stearic acid adsorption on the membrane surface was eliminated by an experiment in which the drug traversed the membrane from the inside of the cell outward. The permeability was unchanged [$(11.7 \pm 0.9) \times 10^{-8}$ cm^2 sec^{-1}; three experiments]. Unequivocal confirmation that the increased permeability involved the polydimethylsiloxane membranes was obtained by replacing them with *cis*-1,4-polybutadiene membranes. The permeability coefficient of the drug through these membranes was $(8.26 \pm 0.88) \times 10^{-9}$ cm^2 sec^{-1} based on eight experiments. In the presence of stearic acid, the coefficient was unchanged [$(8.45 \pm 0.84) \times 10^{-9}$ cm^2 sec^{-1}].

It is unlikely that the observed increase in the permeation rate is due to plasticization of the membrane by stearic acid because no increase was observed in previous experiments with amobarbital (2) or phenylbutazone (3). The increased permeability may arise from the competitive interaction of the drug and stearic acid with the surface of the silica filler contained in the membrane, but the nature of the interaction is not known.

Nutrients—The permeability coefficient (Membrane A) of chlordiazepoxide was measured in the presence of various food constituents, either dissolved or slurried in the drug solution (Table II). Sucrose, cholesterol, and porcine mucin had no effect on the permeability coefficient. Sodium saccharin at concentrations much higher than would be encountered *in vivo* led to a small but definite decrease in the permeability, perhaps the result of ion-pair formation between chlordiazepoxide cations and saccharin anions. The protein-containing materials, skim milk, egg lecithin, and beef albumin, produced substantial decreases in the coefficient while a slight decrease was noted in the presence of small amounts of egg albumin. Plots of the permeability coefficient *versus* the concentration of protein yield linear correlations, the regression coefficients of which are given in Table III. The correlations suggest that adsorption or protein binding is taking place. Chemical reaction seems unlikely because the large excess of nutrient would probably drive any reaction far toward completion and, consequently, the permeability coefficient virtually to zero.

Surfactants—The decrease in the observed permeability coefficient with increasing concentrations of surfactant was used to calculate the partition coefficient, K_p, of drug partitioning between the surfactant micellar phase and the aqueous solution. Data were analyzed according to the equation:

$$\frac{Q}{C_u W_w} = \frac{K_p W_m}{\rho_m W_w} + \frac{1}{\rho_w} \qquad \text{(Eq. 2)}$$

where Q is the total quantity of drug in the system; C_w is the quantity of drug in the aqueous phase; W_m and W_w are the weights of the micellar and water phases, respectively; and ρ_m

Table IV—Effect of Surfactants on Permeation of Chlordiazepoxide

Total Drug, Q, (moles $liter^{-1}$) $\times 10^4$	pH	Weight of Surfactant, (g $liter^{-1}$)	Apparent Permeability Coefficient, P_a, (cm^2 sec^{-1}) $\times 10^8$
Sodium Lauryl Sulfate			
1.725	7.14	0.104	6.19
1.868	7.14	0.276	6.05
1.856	7.10	0.461	5.81
1.808	7.11	1.208	2.58
1.808	7.11	3.019	0.96
Cetrimonium Bromide			
1.880	7.15	0.116	4.60
1.904	7.14	0.512	3.40
2.153	7.03	2.00	1.51
1.915	7.12	5.00	0.85
Polysorbate 80			
1.999	7.15	0.552	4.42
1.832	7.14	1.236	5.00
1.796	7.14	4.26	3.21
2.011	7.14	10.88	1.52

Table V—Surfactant Regression Coefficients and Partition Coefficients

Surfactant	$K_p \rho_m^{-1}$, liter g^{-1}	ρ_w^{-1}, (liter g^{-1}) $\times 10^3$	r	K_p
Sodium lauryl sulfate	1.87	0.44	0.99	1880
Cetrimonium bromide	1.19	1.28	0.99	1190
Polysorbate 80	0.27	0.97	0.99	270

and ρ_w are the respective densities (2, 3); C_w was estimated from:

$$C_w = \frac{QP_a}{P} \qquad \text{(Eq. 3)}$$

where P_a is the apparent permeability coefficient measured in the presence of surfactant. The decrease in the permeability coefficient from 6.02×10^{-8} cm^2 sec^{-1} (Membrane A) upon addition of surfactant is assumed to be directly attributable to the disappearance of free drug. Plots of Q/C_wW_w against W_m/W_w were constructed from the experimental data (Table IV). The density of the surfactant was taken as unity. When assuming no volume change upon mixing, W_w and W_m were obtained directly. The regression coefficients of these plots are given in Table V, along with the partition coefficient estimated from Eq. 2.

The large partition coefficients found for cetrimonium bromide and sodium lauryl sulfate solutions are due to their ionic nature. At the pH of these experiments, chlordiazepoxide exists in the unionized and cationic forms. In addition to dissolution of unionized drug in the hydrocarbon interior of the micelles, reaction with the charged exterior occurs. Chlordiazepoxide cations react with lauryl sulfate anions to form ion-pairs while unionized drug forms dipole complexes with cetrimonium ions. The strong interaction of chlordiazepoxide with ionic surfactants suggests that the latter should be avoided in preparing formulations of this drug.

CONCLUSIONS

The interaction of chlordiazepoxide with talc and ionic surfactants suggests that care should be exercised in preparing formulations with these substances to avoid conditions under which absorption may be restricted by drug–excipient interaction.

The interaction of magnesium stearate with the polydimethylsiloxane membrane highlights the need for careful membrane selection. The ideal material would be a nonporous, homogeneous, polymeric membrane that contains no filler, crystallites, or other internal microphases which influence the diffusion rate or solubility of the drug in the membrane. Most commercially available, thin elastomeric membranes contain filler to impart physical strength.

REFERENCES

(1) M. Nakano, *J. Pharm. Sci.*, **60**, 571(1971).

(2) E. G. Lovering and D. B. Black, *ibid.*, **62**, 602(1973).

(3) *Ibid.*, **63**, 671(1974).

(4) E. R. Garrett and P. B. Chemburkar, *J. Pharm. Sci.*, **57**, 944(1968).

(5) "Documenta Geigy, Scientific Tables," 6th ed., Geigy Pharmaceutical Co., Manchester, England, p. 314.

(6) A. Suzuki, W. I. Higuchi, and N. F. H. Ho, *J. Pharm. Sci.*, **59**, 644(1970).

(7) M. J. Akers, J. L. Lach, and L. J. Fischer, *ibid.*, **62**, 391(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 3, 1973, from the *Drug Research Laboratories, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario K1A OL2, Canada.*

Accepted for publication March 27, 1974.

The cross-linked Cis-4, *cis*-1,4-polybutadiene, membranes were a gift from the Phillips Petroleum Co., Bartlesville, Okla.

[x] To whom inquiries should be directed.

Synthesis of Substituted Piperidino Carbamides: Correlation between CNS Effects and Selective Inhibition of NAD-Dependent Oxidations

A. K. GUPTA*, C. DWIVEDI*, T. K. GUPTA*, SURENDRA S. PARMAR*‡[x], and WILLIAM E. CORNATZER‡

Abstract □ Several 1-(*N*-acetyl-substituted piperidino)-3-aryl carbamides were synthesized. All carbamides possessed anticonvulsant activity, as exhibited by protection against pentylenetetrazol-induced seizures, and potentiated pentobarbital-induced hypnosis. Inhibitory effects of these carbamides on the respiratory activity revealed selective inhibition of NAD-dependent oxidation of the various substrates by the rat brain homogenate. Such a selective inhibition of respiratory activity was in no way related with the pharmacological properties of the compounds that were tested.

Keyphrases □ Carbamides of substituted piperidines—synthesis, relationship between anticonvulsant activity and inhibition of respiratory activity □ Piperidine carbamides—synthesis, relationship between anticonvulsant activity and inhibition of respiratory activity □ Anticonvulsant activity—synthesis and evaluation of 1-(*N*-acetyl-substituted piperidino)-3-aryl carbamides □ Respiratory activity—synthesis and evaluation of carbamides as inhibitors □ CNS activity—synthesis and evaluation of 1-(*N*-acetyl-substituted piperidino)-3-aryl carbamides □ Structure–activity relationships—substituted piperidino carbamides, CNS effects

Continuing interest in the synthesis of substituted carbamides (1–3) and the study of their central nervous system (CNS) effects led to the synthesis of some 1-(*N*-acetyl-substituted piperidino)-3-aryl carbamides. In the present study, the anticonvulsant activity of these compounds against pentylenetetrazol-induced seizures and their ability to potentiate pentobarbital hypnosis were determined. The *in vitro* inhibitory effects of these carbamides on the respiratory activity of the rat brain homogenate were also investigated to elucidate their biochemical mechanism of action. These carbamides were synthesized by following the methods outlined in Scheme I.