In Vitro Adsorption of Diphenoxylate Hydrochloride on Activated Charcoal and Its Relationship to Pharmacological Effects of Drug *In Vivo* I

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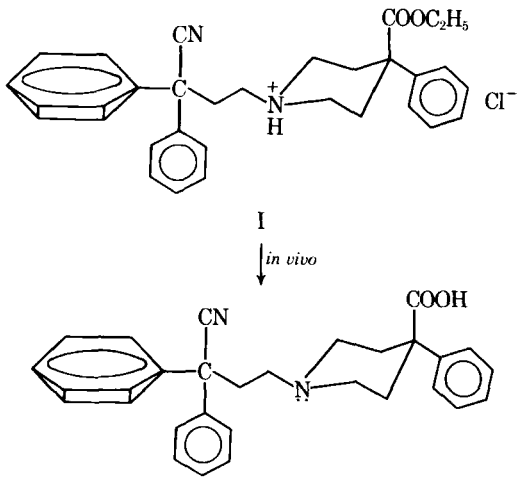
Abstract □ The adsorption of diphenoxylate hydrochloride, a potent antidiarrheal agent, on activated charcoal powder was studied *in vitro*. Langmuir adsorption isotherms were established at pH 4 and 7, and the maximum adsorption capacity of charcoal for this drug was estimated using these values. Activated charcoal modified the bioavailability of diphenoxylate hydrochloride *in vivo*. The antipropulsive action of diphenoxylate in the mouse was strongly inhibited in the presence of activated charcoal. A comparative evaluation of charcoal and chromium oxide used as inert, nonabsorbable markers revealed that chromium oxide may be the marker of choice in GI transit studies in laboratory animals since it does not influence the bioavailability of diphenoxylate hydrochloride.

Keyphrases □ Diphenoxylate hydrochloride—adsorption on activated charcoal and chromium oxide, effect on bioavailability □ Bioavailability—diphenoxylate hydrochloride, effect of activated charcoal and chromium oxide □ Adsorption—diphenoxylate hydrochloride on charcoal and chromium oxide, pH 4 and 7 Langmuir isotherms

It has been established that the presence of a solid adsorbent, such as activated charcoal, interferes with the drug absorption process, resulting in a decrease in bioavailability of some drugs (1–5). Such an interference in the systemic availability of a drug is brought about by its adsorption on the activated surface of the solid adsorbent, thus preventing the adsorbed fraction of the drug from permeating through the GI mucosa into the bloodstream. In general, under *in vitro* experimental conditions the drug adsorption phenomenon exhibits Langmuir adsorption isotherm characteristics (4–8). Indeed, this principle of surface adsorption has found clinical usefulness in the management of acute toxicity in drug overdosing of pediatric as well as adult patients (9).



Scheme I

Diphenoxylate hydrochloride (I), a potent antidiarrheal agent, has been shown to be clinically effective for the management of severe diarrhea (10). In pediatric use, however, there is a persistent concern about the overdosage of this drug given in combination with atropine sulfate (11, 12). One aspect of this problem is the rapid absorption of the drug as manifested by possible enterohepatic circulation of diphenoxylic acid (II), the major metabolite (Scheme I), from the lower intestinal tract (13).

It was of interest, therefore, to explore the possibility of altering the *in vitro* availability of diphenoxylate hydrochloride (I) in the presence of activated charcoal powder and to assess its relationship to the pharmacological activity of this drug *in vivo*. This report presents the results of these investigations and their clinical implications in the management of overdosage problems related to this drug.

EXPERIMENTAL

Materials—All reagents used were analytical grade. Diphenoxylate hydrochloride[1] was tested for its structure identity and purity prior to use. Activated charcoal powder[2], chromium oxide[3], methylcellulose[4], and absolute alcohol[5] were used as supplied.

Methods—All *in vitro* adsorption studies were conducted at 25°. Studies with activated charcoal were conducted at pH 2, 4, and 7 with a 0.2% solution of diphenoxylate hydrochloride in hydroalcoholic mixtures (50:50 v/v). Accurately weighed amounts of activated charcoal powder, 0.02–1 g, were transferred to 25-ml test tubes with screw caps. After 25 ml of a 0.2% solution of diphenoxylate hydrochloride was added to each test tube, the charcoal suspensions were continuously mixed[6] for 20 hr. The suspensions were then rapidly centrifuged, and the clear supernatant liquid was assayed spectrophotometrically at 258 nm[7].

Appropriate control solutions were also run and assayed to ensure control over experimental conditions. The amount of diphenoxylate hydrochloride adsorbed on the charcoal was determined on the basis of a previously established standard curve. Studies with chromium oxide were conducted in a similar manner with the adsorbent at 0.5 and 1.0 g in suspension with a 0.2% solution of diphenoxylate hydrochloride and the blank solvent. The following *in vivo* studies were performed.

GI Transit Time in Mice—The method used was adapted from previous studies (14, 15). In these experiments, the extent of GI propulsion was measured with the aid of nonabsorbable and visually identifiable markers such as charcoal. Six male Charles River mice, 20–25 g, were fasted in screen-bottom cages with water supplied *ad libitum* for 24 hr prior to the test. The animals were orally pretreated with logarithmically graded doses (0.3–18 mg/kg) of diphenoxylate hydrochloride in a 0.5% solution of methylcellulose in water. The total volume of the suspension administered to

[1] Lot D538049, Searle Laboratories, Chicago, IL 60680
[2] Mallinckrodt Chemical Co., St. Louis, Mo.
[3] Matheson, Coleman and Bell, Norwood, Ohio.
[4] Dow Chemical Co., Midland, Mich.
[5] Union Carbide Co., South Charleston, W. Va.
[6] Model 150V rotating mixer, Scientific Industries, Springfield, Mass.
[7] Model 124D dual-beam spectrophotometer, Perkin-Elmer Corp., Maywood, Ill.

Table I—Effect of Charcoal on the Antipropulsive Activity of Diphenoxylate Hydrochloride in the Mouse

Treatment Condition	$ED_{50} \pm SE$, mg/kg po	Relative Potency
Diphenoxylate hydrochloride administered 30 min prior to charcoal	3.12 ± 1.29	1.0
Diphenoxylate hydrochloride and charcoal administered simultaneously	14.77 ± 6.59	0.22 ± 0.14[a]

[a] Statistically significant at $p = 0.02$ level.

these animals was kept constant at 10 ml/kg. The control group received the same volume of 0.5% methylcellulose solution.

Thirty minutes after drug administration, the animals were given a single 0.2-ml dose of a 10% suspension of activated charcoal powder in 1% methylcellulose solution. The animals were sacrified 3.5 hr later, and the cecum was examined for the presence or absence of charcoal on an all-or-none basis. In 95% of all control animals, charcoal was present in the cecum. Antidiarrheal drugs, such as diphenoxylate hydrochloride, interfere with the GI propulsive activity, so the presence or absence of charcoal in the cecum provides a measure for the pharmacological response to these drugs. Consequently, the median effective dose (ED_{50}) can then be calculated by using the logistic method of Berkson (16).

In a second experiment, logarithmically graded doses (4.6–46.0 mg/kg) of diphenoxylate hydrochloride mixed with charcoal powder in methylcellulose solutions were administered to the animals; the extent of charcoal translocation also was evaluated in a similar manner. The relative potencies for the two experiments were calculated using the logistic method of Berkson (16).

Effect of Chromium Oxide as a Marker on Antipropulsive Activity of Diphenoxylate Hydrochloride in Mice—In this study, the antipropulsive activity of diphenoxylate hydrochloride was evaluated using a second nonabsorbable and visually identifiable marker, chromium oxide. The experimental design was essentially the same as described previously. Aqueous solutions of methylcellulose (0.5%) were used to prepare 10% suspensions of charcoal or chromium oxide. Thirty minutes after the administration of diphenoxylate hydrochloride as a suspension in 0.5% methylcellulose, the animals were given a single 0.2-ml dose of the marker preparation. The animals were sacrificed 3.5 hr later, and the presence or absence of the marker in the cecum was evaluated on an all-or-none basis.

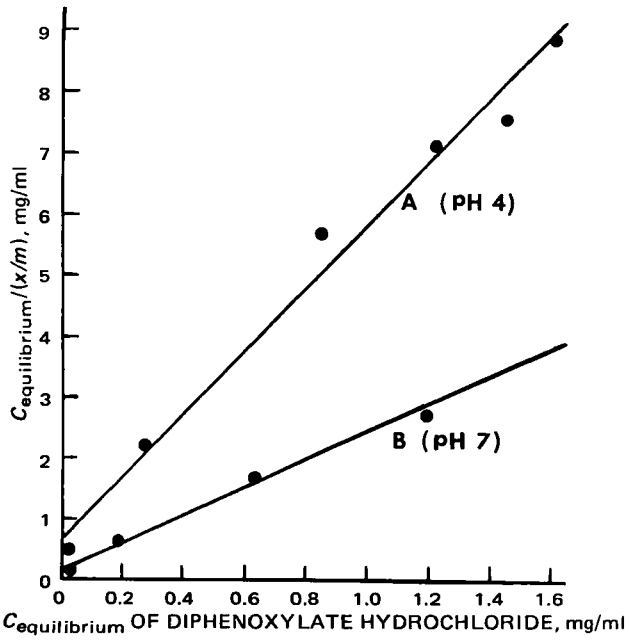


Figure 1—*Langmuir isotherm for the adsorption of diphenoxylate hydrochloride on activated charcoal at pH 4 (A) and pH 7 (B).*

Table II—Effect of Chromium Oxide and Charcoal on the *In Vivo* Antipropulsive Effect of Diphenoxylate Hydrochloride in the Mouse

Marker	$ED_{50} \pm SE$, mg/kg po	Relative Potency ± *SE*
Charcoal	3.12 ± 1.29	1.0
Chromium oxide	0.68 ± 0.18	6.07 ± 2.97[a]

[a] Statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Figure 1 illustrates the Langmuir adsorption isotherm for the uptake of diphenoxylate hydrochloride by activated charcoal powder suspended in pH 4 and 7 solutions. The reciprocal of the slope of the two lines (A and B) gives the maximum binding capacity of charcoal at the given pH. A twofold increase in the binding capacity was observed for diphenoxylate hydrochloride at pH 7 as compared to pH 4 solutions. No significant difference in the extent of drug adsorption was observed between pH 2 and 4 solutions.

These data indicate that the nonionized species of diphenoxylate hydrochloride (pKa = 4.4) is more preferably adsorbed than its ionized species in solution. The lipophilic moieties (phenyl and piperidyl) of this drug probably promote drug adsorption through hydrophobic interaction with the activated surface of the charcoal particles. Such a possibility is consistent with previous findings on the nature of the activated carbon surface (7, 17). Furthermore, the lipophilic nature of diphenoxylate hydrochloride, as evidenced by its partition coefficient[8] ($PC_{CHCl_3-H_2O} \geq 68.5$) supports the occurrence of such an interaction between diphenoxylate hydrochloride and activated charcoal. Under given experimental conditions, the maximum adsorption capacity of charcoal was estimated at 200 and 416 mg/g of charcoal in suspension at pH 4 and 7, respectively.

To assess whether charcoal modifies the pharmacological activity of diphenoxylate hydrochloride *in vivo*, GI transit studies of the drug with and without activated charcoal were carried out in the mice. Table I illustrates the effect of charcoal on the antipropulsive activity of diphenoxylate hydrochloride in the mouse; coadministration of the two compounds significantly affected the potency of diphenoxylate hydrochloride as a constipating agent. In the presence of activated charcoal, there was a fourfold decrease in the relative potency of diphenoxylate hydrochloride. These results strongly suggest that the adsorption of diphenoxylate to activated charcoal can occur *in vivo*, resulting in a modification of the antipropulsive activity of this drug.

In view of these findings, it was of interest to examine whether a different marker, such as chromium oxide, which shares with charcoal the property of being pharmacologically inert, can affect the observed antipropulsive activity of diphenoxylate hydrochloride. Furthermore, investigations on sterol balance in humans (18) have provided ample evidence that chromium oxide exhibits superior performance as an inert, nonabsorbable marker for GI transit studies. *In vitro* studies showed that a 2–4% suspension of chromium oxide adsorbs diphenoxylate hydrochloride only to the extent of 10–18%, whereas activated charcoal at the same level in suspension adsorbs virtually all drug in solution.

As evident from Table II, the quantitative response of diphenoxylate hydrochloride was strongly influenced by the choice of the marker in the mouse studies. With chromium oxide, diphenoxylate hydrochloride showed a sixfold increase in its relative potency as compared to charcoal used conventionally to screen for antipropulsive activity of antidiarrheal agents. The important implication of these findings is that charcoal may not provide the desired "inert" nature as a marker since it can modify the apparent pharmacological activity of antidiarrheal agents such as diphenoxylate hydrochloride.

The clinical implications of these adsorption studies are obvious when one considers the maximum adsorption capacity of activated charcoal for diphenoxylate hydrochloride. Although the use of activated charcoal for the management of accidental overdosage of

[8] The partition coefficient was estimated on the basis of saturation solubility of this drug in the two solvents.

diphenoxylate hydrochloride has been suggested previously, the present studies provide *in vitro* as well as *in vivo* evidence to support the possibility that activated charcoal may significantly modify the bioavailability of this drug.

REFERENCES

(1) D. L. Sorby, *J. Pharm. Sci.*, **54**, 677(1965).

(2) D. L. Sorby and G. Liu, *ibid.*, **55**, 504(1966).

(3) G. Levy and T. Tsuchiya, *Clin. Pharmacol. Ther.*, **13**, 317(1972).

(4) T. Tsuchiya and G. Levy, *J. Pharm. Sci.*, **61**, 586(1972).

(5) *Ibid.*, **61**, 624(1972).

(6) H. Nogami, T. Nagai, E. Fukuoka, and H. Uchida, *Chem. Pharm. Bull.*, **16**, 2248(1968).

(7) H. Nogami, T. Nagai, and H. Uchida, *ibid.*, **17**, 168(1969).

(8) H. Nogami, T. Nagai, and S. Wada, *ibid.*, **18**, 342(1970).

(9) G. R. Wilkinson, *Clin. Toxicol.*, **3**, 249(1970).

(10) P. A. Janssen, A. J. Jageneau, and J. Huygens, *J. Med. Chem.*, **1**, 299(1959).

(11) B. H. Rumack, *Pediatrics*, **53**, 495(1974).

(12) G. Wasserman, Medical News Section Interview, *J. Amer. Med. Ass.*, **230**, 14(1974).

(13) A. Karim, R. E. Ranney, K. L. Evensen, and M. L. Clark, *Clin. Pharmacol. Ther.*, **13**, 407(1972).

(14) D. I. Macht and J. Barba-Gose, *J. Amer. Pharm. Ass.*, **20**, 558(1931).

(15) P. A. Janssen and A. J. Jageneau, *J. Pharm. Pharmacol.*, **9**, 381(1957).

(16) J. Berkson, *J. Amer. Statist. Ass.*, **48**, 565(1953).

(17) H. Nogami, T. Magai, and N. Nambu, *Chem. Pharm. Bull.*, **18**, 1643(1970).

(18) B. A. Kottke and M. T. R. Subbiah, *J. Lab. Clin. Med.*, **80**, 530(1972).

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Electron-Capture GLC Determination of Timolol in Human Plasma and Urine

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Abstract □ A GLC procedure was developed for measuring nanogram quantities of timolol in plasma and urine. The unchanged drug was extracted into heptane–4% isoamyl alcohol from alkalinized plasma or urine, together with a homolog of timolol which served as the internal standard. The compounds were subsequently back-extracted into 0.1 *N* HCl and then into chloroform following adjustment of the acid phase to an alkaline pH. The compounds in the chloroform extract were derivatized with heptafluorobutyrylimidazole to form the diheptafluorobutyryl derivatives; these were quantitated by electron-capture GLC. Recovery of timolol added to normal plasma and urine was quantitative and reproducible, and no interfering substances were observed in normal biological samples. The method is capable of measuring concentrations as low as 2 ng/ml in plasma or 20 ng/ml in urine. After a 10-mg oral dose of ^{14}C-timolol, peak plasma levels of approximately 30 ng/ml were observed in 1–2 hr.

Keyphrases □ Timolol—extraction, derivatization, GLC analysis, biological fluids □ Adrenergics—timolol, extraction, derivatization, GLC analysis, biological fluids □ GLC—analysis, timolol, biological fluids

Timolol maleate, (−)-1-(*tert*-butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol maleate, is a new β-adrenergic receptor blocking agent (1). More recently, it was shown to reduce the frequency of anginal episodes and thus have a place in the treatment of angina pectoris (2). When administered orally to rats and dogs, the compound is several times more potent than propranolol in blocking isoproterenol-induced cardiac acceleration (3). Its activity in humans also has been demonstrated (4, 5).

This report is concerned with the determination of timolol in biological fluids. In view of the small therapeutic doses used (generally 5–15 mg po), a sensitive as well as a specific analytical method is required. The procedure involves extraction of the compound from biological fluids followed by derivatization to form the diheptafluorobutyryl analog and determination by electron-capture GLC. The procedure is capable of quantitating timolol in concentrations as little as 2 ng/ml in plasma and 20 ng/ml in urine. Accordingly, the method should prove useful in clinical applications where quantitation and diagnostic confirmation are necessary.

EXPERIMENTAL

Reagents—Pesticide quality *n*-heptane[1] was used without further purification. Reagent grade isoamyl alcohol[2] was glass distilled prior to use. Methylene chloride[3], reagent grade, was washed successively with 1 *N* HCl, 1 *N* NaOH, and three times with water and then glass distilled. Distilled, deionized water was used in the preparation of 0.1 *N* HCl and 2.0 *N* NaOH. Heptafluorobutyrylimidazole[4], in 1-ml ampuls stored under nitrogen, was diluted 1:10 with pesticide quality ethyl acetate[5] immediately before use. Timolol (I) was used as the maleate salt, and desmethyltimolol, 1-isopropylamino-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol hydrochloride (II) served as the internal standard. All concentrations were expressed in terms of the free base.

Apparatus—Samples were analyzed on a gas chromatograph[6]

[1] Matheson, Coleman and Bell.
[2] J. T. Baker.
[3] Fisher Certified, ACS Spectranalyzed.
[4] Pierce Chemical Co.
[5] Fisher Certified, ACS.
[6] Hewlett-Packard model 5750.