Accelerated Peritoneal Dialysis of Barbiturates, Diphenylhydantoin, and Salicylate

RONALD M. KUDLA*, EMAD A. EL-BASSIOUNI, and ALBERT M. MATTOCKS

Abstract □ A combination of anthranilic acid (1%) and *N*-myristyl-β-aminopropionic acid (0.25%), which had been previously found to accelerate peritoneal dialysis of salicylate, was found also to accelerate the removal of diphenylhydantoin, phenobarbital, pentobarbital, and amobarbital. *N*-Myristyl-β-aminopropionic acid alone was equally or nearly as effective as the combination for the barbiturates. Studies indicate that displacement of the drugs from binding sites on plasma proteins may be involved in the mechanism of action of these accelerators.

Keyphrases □ Peritoneal dialysis, acceleration—barbiturates, diphenylhydantoin, and salicylate □ Barbiturates, diphenylhydantoin, and salicylate—accelerated peritoneal dialysis □ Anthranilic acid and *N*-myristyl-β-aminopropionic acid—peritoneal dialysis acceleration □ *N*-Myristyl-β-aminopropionic acid—peritoneal dialysis acceleration □ Plasma protein, drug displacement—anthranilic acid peritoneal dialysis effect □ Drug displacement from plasma protein binding sites—peritoneal dialysis accelerators

In previous work in this laboratory (1), anthranilic acid and *N*-myristyl-β-aminopropionic acid[1], when added to the dialysis fluid, was found to accelerate the removal of salicylic acid by peritoneal dialysis. A combination of the two agents, called M-1 fluid, containing 1% anthranilic acid and 0.25% *N*-myristyl-β-aminopropionic acid was tested further and found to give clearances more than four times the control fluids. Anthranilic acid was initially selected for test due to its similarity in structure to salicylate. In the same laboratory, other studies of peritoneal dialysis of barbiturates and diphenylhydantoin were being conducted; although these compounds have little structural similarity to anthranilic acid, the M-1 fluid was tested on these drugs. It was found to be effective; these tests, along with similar tests of the *N*-myristyl-β-aminopropionic acid alone, are reported in this paper.

MATERIALS AND METHODS

Radioactive Injections—^{14}C-Diphenylhydantoin was synthesized and purified by a published method (2). Solutions for injection were freshly made to contain 10 mg. diphenylhydantoin as the sodium salt and 1 μc. radioactivity/ml. This was injected at a level of 10 mg./kg.

^{14}C-Phenobarbital was obtained commercially, and the injection was prepared to contain 50 mg. phenobarbital as the sodium salt and 0.1 μc. radioactivity/ml. It was injected at a level of 50 mg./kg.

^{14}C-Pentobarbital was obtained commercially, and the injection contained 18 mg. pentobarbital as the sodium salt and 4 μc. radioactivity/ml. It was injected at a level of 18 mg./kg.

^{14}C-Amobarbital and ^{14}C-butabarbital were synthesized from the appropriate malonic esters and ^{14}C-urea. Their identity and purity were checked by IR spectra and paper chromatography. The R_f values agreed with those of Smith (3). The injections were prepared and used in the same manner as pentobarbital.

^{14}C-Salicylic acid was obtained commercially, and the injection contained 50 mg. salicylic acid as the sodium salt and 1 μc. radioactivity. It was injected at a level of 100 mg./kg.

[1] A mixture of *N*-myristyl- and *N*-lauryl-β-aminopropionic acids as their sodium salts, marketed in 50% solution by General Mills, Kankakee, Ill., under the trade name Deriphat 170-C. Percentages used in this work are of the 50% solution as supplied.

Table I—Results of Intermittent Dialysis

Dialysis Fluid	Clearance, ml./min.
Diphenylhydantoin	
Control fluid	0.47
	0.39
	0.89
	0.36
Av.	0.53
M-1 fluid	1.94
	1.57
	2.05
	1.66
Av.	1.81
N-Myristyl-β-aminopropionic acid (0.25%)	1.35
	1.54
	0.96
Av.	1.28
Phenobarbital	
Control fluid	0.93
	1.12
	1.01
	0.86
	1.37
	1.06
Av.	1.06
M-1 fluid	2.48
	2.18
	2.02
	1.83
	2.35
	1.97
Av.	2.14
N-Myristyl-β-aminopropionic acid (0.25%)	2.06
	1.44
	1.90
Av.	1.80
Butabarbital	
Control fluid	1.66
	1.94
Av.	1.80
N-Myristyl-β-aminopropionic acid (0.25%)	3.26
	3.18
Av.	3.22
Pentobarbital	
Control fluid	0.94
	0.77
	1.07
	0.98
	1.16
	1.09
Av.	1.00
M-1 fluid	1.74
	2.35
	2.51
	1.98
	1.87
	1.95
Av.	2.06
N-Myristyl-β-aminopropionic acid (0.25%)	2.91
	2.62
Av.	2.77
Amobarbital	
Control fluid	0.70
	0.48
	1.06
	1.25
	1.05
Av.	0.91
M-1 fluid	2.35
	2.11
	2.58
	2.58
	2.49
Av.	2.36
N-Myristyl-β-aminopropionic acid (0.25%)	2.21
	2.16
Av.	2.19
Salicylate	
Control fluid[a]	0.44
	0.52
	0.46
Av.	0.47
M-1 fluid[a]	2.54
	1.88
	2.38
Av.	2.27
N-Myristyl-β-aminopropionic acid (0.25%)	0.99
	1.03
	0.97
Av.	1.00

[a] From previous work (1).

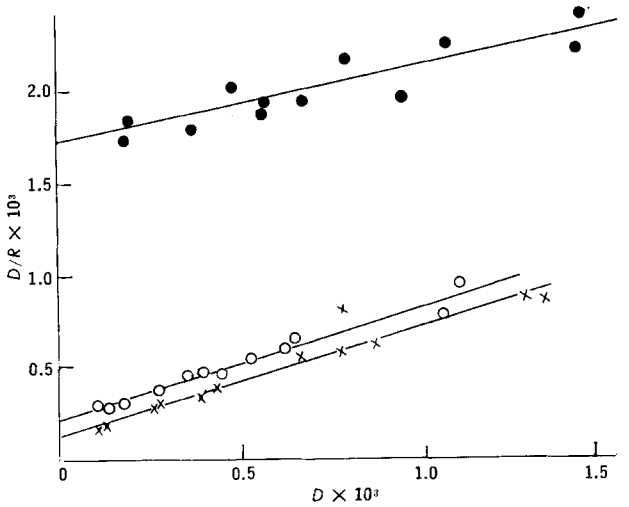

Figure 1—*Typical plots of protein binding data for salicylate. Key: ×, control points; ○, points with 0.25% N-myristyl-β-aminopropionic acid added; and ●, points with 1% anthranilic acid added. Lines are computed by least squares.*

Dialysis Fluids—The control dialysis fluid was a typical formula with 377 milliosmoles/l. It contained dextrose (1.5%), sodium lactate (0.5%), sodium chloride (0.56%), calcium chloride (0.026%), and magnesium chloride hexahydrate (0.015%). The M-1 fluid was prepared by dissolving 1% anthranilic acid and an equivalent amount of sodium bicarbonate in sufficient water to make it isotonic with the control fluid, then dissolving 0.25% of the commercial *N*-myristyl-β-aminopropionic acid solution, and making up to volume with control fluid. *N*-Myristyl-β-aminopropionic acid dialysis fluid was prepared simply by dissolving 0.25% of the commercial solution in the control fluid.

Intermittent Dialysis—Healthy, mature, male, albino rabbits were used as the test animals. They were administered the ^{14}C-tagged drug intravenously, and 1 hr. was allowed for distribution in the body before beginning dialysis. Then 60 ml./kg. of dialysis fluid was introduced into the peritoneum *via* a pediatric size catheter over a period not exceeding 1 min. The fluid was allowed to remain in the peritoneum for 19 min. and to drain by gravity for 10 min., thus giving a cycle time of 30 min. The fluid was drained into a graduated cylinder, its volume was read, it was then mixed, and a sample was taken for counting. Blood samples were taken from the marginal ear vein at the time of introduction of the dialysis fluid and at the completion of drainage of each dialysate, thus affording calculation of an average plasma concentration for each 30-min. cycle. Five such cycles were performed on each animal.

Measurements of Drug Concentrations—For salicylate and phenobarbital, the amount of drug metabolized in the 3-hr. experiment was negligible; 0.5-ml. samples of plasma and dialysate were added directly to the counting fluid [0.01% 1,4-bis-2-(5-phenyloxazolyl)-benzene and 0.4% 2,5-diphenyloxazole in a solvent of 33% nonionic surfactant[2] and 67% toluene]. Samples thus prepared were counted in a liquid scintillation counter[3] using the channels ratio method for quench correction.

Pentobarbital and its metabolites were not extracted completely by conventional methods, and a new procedure was developed for these experiments. A 0.3-ml. sample of dialysate or 50-μl. sample of plasma was counted directly for total radioactivity. A second sample of 2 ml. dialysate or 1 ml. plasma was transferred to a vial, acidified with 0.4 ml. 0.5 *N* HCl, and extracted with 4 × 3 ml. ether. The aqueous residue, containing only metabolites, was counted directly. The ether residue, containing all the unchanged drug and small amounts of metabolite, was spotted on a 20 × 20-cm. sheet of Whatman No. 2 chromatography paper. The chromatogram was developed in an Eastman chromatogram chamber with ammonium hydroxide–amyl alcohol (10:90). This gave average R_f values of 0.90 for pentobarbital and 0.10 for metabolites. After drying, the chromatograms were cut into strips, taped together, and scanned on a radiochromatogram scanner. The ratio of the area of pentobarbital peak to the sum of the two areas gave the fraction of unchanged drug present in the ether extract. This ratio, when multiplied by total count of the sample after correction for that remaining in the aqueous residue, gave the concentration of unchanged drug in the original sample. Application of this method to samples of known concentrations gave recoveries of 97% or better.

Amobarbital and butabarbital were extracted completely from acidified samples with 4 × 5 ml. ether. Contamination by metabolites was less than 3%. Extracts were evaporated directly in counting vials.

For diphenylhydantoin, the samples were acidified with 0.5 *N* HCl and extracted with 2 × 5 ml. chloroform, which was evaporated in counting vials and counted.

In each case, a sample of the radioactive injection was counted with the samples as a standard.

Binding Studies—The degree of binding of the drugs to plasma proteins was measured by equilibrium dialysis. One milliliter of pooled human plasma was placed in a bag made of 8-mm. dialysis tubing, which had been soaked overnight in pH 7.4 isotonic buffer. The plasma bags were tied and placed in 12 × 100-mm. test tubes. Outside the bags was added 6 ml. of isotonic phosphate buffer, pH 7.4, to which had been added a given concentration of ^{14}C-tagged drug. The tubes were allowed to stand at refrigerator temperature (to avoid bacterial putrefaction) for 48 hr. Each drug had been previously tested for the time required to establish equilibrium, and between 24 and 48 hr. was required in each instance. At the end of this period, the bags were removed, quickly rinsed with distilled water, wiped, and then cut open. Samples of 0.5 ml. plasma and outer fluid were pipeted into vials and counted.

For displacement tests, the agent, anthranilic acid or *N*-myristyl-β-aminopropionic acid, was dissolved in water to make an isotonic solution. Then the drug was added, and the solution was made to volume with isotonic buffer.

RESULTS

The M-1 fluid was tested for removal of diphenylhydantoin, phenobarbital, pentobarbital, and amobarbital. *N*-Myristyl-β-aminopropionic acid was tested with these drugs and also with butabarbital and salicylate. Previously reported results for control and M-1 fluids on salicylate were added for comparison. Intermittent dialysis results are shown in Table ', where each entry represents the average clearance for five exchanges on one animal.

From Table I, it is seen that the M-1 fluid accelerated removal of diphenylhydantoin to about three times the control; it accelerated phenobarbital, pentobarbital, and amobarbital to about twice the control. These results were quite unexpected, since the M-1 formula had been developed specifically for salicylate, and anthranilic acid bears little structural resemblance to diphenylhydantoin and the barbiturates. When anthranilic acid alone had been tested previously, the results were quite variable; thus the wetting agent, *N*-myristyl-β-aminopropionic acid, was tested alone to see if it might account for the full activity of the M-1 fluid. Although the wetting agent acted as an accelerator in each case, it appeared to represent the full effect of M-1 fluid only with pentobarbital and amobarbital and nearly the full effect with diphenylhydantoin and phenobarbital (Table I).

One possible effect of anthranilic acid might be the displacement of bound drug from binding sites on plasma proteins; thus the protein binding studies were performed. Because of the availability of human plasma, it was used for the binding studies, although rabbits were used for the dialyses. There may be some species differences in the degree of binding to plasma, but experiments in this laboratory demonstrated close agreement in binding of salicylate to bovine serum albumin and human plasma and in binding of pentobarbital to rabbit and human plasma. Thus, the species differences probably are small with these drugs and the displacing agents would have similar effects, since the proteins involved are the same in the different species.

The ranges of drug concentration used in plasma binding tests were selected to represent the plasma levels encountered in therapeutic doses and toxic reactions of the drugs. With diphenylhydantoin, the range that could be studied was limited by the solubility of the drug in pH 7.4 buffer. In all cases except salicylate, the fraction of drug bound was essentially constant throughout the range studied; a simple average represents adequately the degree of

[2] Triton X-100, Rohm & Haas, Philadelphia, Pa.
[3] Packard 3320 scintillation spectrometer, Packard Instrument Co., LaGrange, Ill.

Table II—Effects of Anthranilic Acid and *N*-Myristyl-β-aminopropionic Acid on Binding of Drugs to Plasma Proteins

Drug	Plasma Concentration Range, mcg./ml.	Displacement Agent	Fraction Bound	$K^a \times 10^4$	n
Diphenylhydantoin	2–11	None	0.86		
		Anthranilic acid (1%)	0.71		
		N-Myristyl-β-aminopropionic acid (0.25%)	0.70		
Phenobarbital	40–300	None	0.32		
		Anthranilic acid (1%)	0.16		
		N-Myristyl-β-aminopropionic acid (0.25%)	0.26		
Pentobarbital	6–40	None	0.47		
		Anthranilic acid (1%)	0.37		
		N-Myristyl-β-aminopropionic acid (0.25%)	0.47		
Amobarbital	6–40	None	0.41		
		Anthranilic acid (1%)	0.35		
		N-Myristyl-β-aminopropionic acid (0.25%)	0.40		
Butabarbital	6–40	None	0.30		
		Anthranilic acid (1%)	0.20		
		N-Myristyl-β-aminopropionic acid (0.25%)	0.30		
Salicylate	130–370	None	0.45–0.80	1.87	1.40
		Anthranilic acid (1%)	0.22–0.30	40.8	2.23
		N-Myristyl-β-aminopropionic acid (0.25%)	0.42–0.70	3.91	1.61

[a] Binding constants were calculated only for salicylate, since the other drugs showed no significant difference in fraction bound within the range studied.

binding. With salicylate, the fraction bound varied throughout the range studied, and the data were plotted by an equation attributed to Klotz and cited by Goldstein (4):

$$D/r = K/n + 1/n(D) \qquad \text{(Eq. 1)}$$

where D is the molar concentration of drug in plasma, r is the ratio of moles of drug bound per mole of protein, n is the number of binding sites on the protein molecule, and K is the binding constant. This equation was found, in this laboratory, to give better definition of binding curves with a number of drugs, probably because of the range of drug concentrations utilized.

Binding results are presented in Table II, and the salicylate binding curves are illustrated in Fig. 1.

Table II shows that anthranilic acid reduced the protein binding of each of the drugs studied. *N*-Myristyl-β-aminopropionic acid reduced the plasma binding of diphenylhydantoin and phenobarbital but had no significant effect on the binding of the other drugs.

DISCUSSION

The broad effect of the M-1 fluid as an accelerator for peritoneal dialysis of drugs makes the understanding of its mode of action important in order to devise more effective and less toxic accelerators. The general effect of anthranilic acid to reduce plasma binding is of interest but is not yet conclusively shown to represent its action as an accelerator.

If displacement of the drug from protein occurred equally throughout the circulatory system, it would be expected that the free drug would rapidly be equilibrated throughout its compartments of distribution, possibly most of the body water, giving a rapid drop in plasma level and a rather small increase in free drug concentration in plasma. If this occurred, the increase in dialysis rate would be smaller than that observed in the laboratory. Thus, it is believed more likely that the displacement from binding sites occurs chiefly in the vessels of the mesentery where the agent is chiefly absorbed and where dialysis is taking place. Therefore, the full effect of drug displacement is reflected in dialysis rate increase, and results might certainly be as high as those observed. This concept is supported in part by an experiment conducted in this laboratory. Anthranilic acid was infused intravenously while dialyzing salicylate with control fluid. No acceleration of dialysis could be detected. Other evidence is needed, of course, to determine whether this is truly the mode of action of these accelerators.

It is conceivable that the displacement of drugs from plasma proteins might reflect a more general effect, including displacement from attachment to the peritoneal membrane. This points to another possible mechanism by which accelerators may act.

Preliminary evidence found in this laboratory indicates that the amounts of anthranilic acid and *N*-myristyl-β-aminopropionic acid needed for the full accelerating effect may be greatly reduced by special techniques. This work is continuing and offers promise of making these agents quite useful with greatly reduced danger of toxicity.

REFERENCES

(1) A. M. Mattocks, *J. Pharm. Sci.*, **58**, 595(1969).

(2) A. Murray and D. L. Williams, "Organic Syntheses With Isotopes," part 1, Interscience, New York, N. Y., 1958, p. 693.

(3) I. D. Smith, "Chromatographic and Electrophoretic Techniques," vol. 1, Interscience, New York, N. Y., 1960, p. 500.

(4) A. Goldstein, *Pharmacol. Rev.*, **1**, 102(1949).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 8, 1971, from the *School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514*

Accepted for publication March 15, 1971.

The authors acknowledge the valuable technical assistance of Sherman Riggsbee.

This work was supported in part by Grant DS00013 from the U.S. Public Health Service, Bethesda, Md.

* Present address: Lederle Laboratories, Pearl River, N. Y.