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Possible Ion-Pair-Mediated Absorption of Mixidine II: Plasma Levels and Histology

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Abstract □ Plasma intact ^{14}C-mixidine levels in rats increased when the drug was administered intraduodenally with 1:3 and 1:5 molar ratios of 2-naphthalenesulfonic acid. Upon histological examination of the duodenums, similar doses of mixidine combined with 2-naphthalenesulfonic acid produced no dose-related lesions. These and previous observations demonstrate that mixidine absorption may be enhanced by ion-pair formation.

Keyphrases □ Mixidine—absorption, effect of intraduodenal administration with various ratios of 2-naphthalenesulfonic acid as counterion □ Absorption—mixidine, effect of intraduodenal administration with various ratios of 2-naphthalenesulfonic acid as counterion □ Ion-pair formation—effect on mixidine absorption, intraduodenal administration with various ratios of 2-naphthalenesulfonic acid as counterion □ Vasodilators, coronary—mixidine, absorption, effect of intraduodenal administration with various ratios of 2-naphthalenesulfonic acid as counterion

The influence of counterions (acids) on the 1-butanol–water partitioning and the lethality in rats of mixidine, a completely ionized base, was reported (1). When the counterion concentration in an aqueous solution of mixidine was increased, mixidine partitioning into 1-butanol increased, as did the lethality of the solution in rats. This concomitant increase in partitioning with an increase in lethality led to the conclusion that mixidine absorption, as measured by lethality, was enhanced by ion-pair formation.

Also reported were the observations that the ion-pair-mediated absorption of mixidine was most easily demonstrated following intraduodenal administration, that counterions *per se* were not lethal, and that the counterions were equivalent in their ability to mediate mixidine absorption at a given pH (1).

In this study, plasma ^{14}C-mixidine levels were followed to determine if a parallel relationship to the effect of counterion concentration on partitioning and lethality occurred. In addition, rats dosed similarly to those in the plasma level study were examined histologically in an effort to show an absence of counterion-related pathology.

EXPERIMENTAL

Plasma Level Studies—*Preparations*—^{14}C-Mixidine fumarate, specific activity of 5.1 μCi/mg, was dissolved in distilled water to yield a salt concentration of 12.6 mg/ml. Rats were dosed with the equivalent of 25 mg of ^{14}C-mixidine/kg (~0.5 ml/rat).

The labeled mixidine was administered alone and in 1:1, 1:3, and 1:5 mixidine to 2-naphthalenesulfonic acid molar ratios. In experiments with 2-naphthalenesulfonic acid, the volumes of the solutions never exceeded 0.5 ml. In one experiment, 2-naphthalenesulfonic acid in a 1:3 drug to adjuvant ratio was neutralized by adding sodium hydroxide equimolar to the 2-naphthalenesulfonic acid.

Administration—Male CFN rats, 180–200 g, were fasted for approximately 17 hr. For oral administration, the rats were dosed *via* stomach tube followed by a 0.5-ml water wash. For intraduodenal administration, the rats were anesthetized with ether and dosed according to the procedure described previously (1).

Three rats were employed for each dose-route combination, except for the mixidine–2-naphthalenesulfonic acid (1:3) combination for intraduodenal administration where four rats were employed.

Analysis—The assay for intact drug was one in which the specificity had been established (2). A 100-μl blood sample was taken from the tail vein and rinsed into 0.5 ml of distilled water. Ten milliliters of chloroform, 1.0 ml of 1.0 *N* NaOH, and 1.0 ml of 0.096 mg of mixidine/ml as the fumarate were added. Then the mixture was agitated and centrifuged.

Table I—Plasma Intact Mixidine Levels (Nanograms per Milliliter) following Oral Administration of ^{14}C-Mixidine (25 mg/kg)

	1:0 Drug–Adjuvant (pH 3.7)				1:1 Drug–Adjuvant (pH 2.5)				1:3 Drug–Adjuvant (pH 1.6)			
Minutes	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
20	36	31	76	48	33	20	31	28	25	19	27	24
40	32	32	43	36	42	32	27	34	21	30	42	31
60	28	23	40	30	31	30	19	27	30	—	34	32
120	26	163	14	68	31	17	19	22	19	21	21	20
180	49	34	60	48	55	8	59	41	15	18	19	17
240	66	12	28	35	25	15	127	56	12	—	17	14
360	7	1	14	7	8	18	8	11	12	17	18	16

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Table II—Plasma Intact Mixidine Levels (Nanograms per Milliliter) following Intraduodenal Administration of ^{14}C-Mixidine (25 mg/kg)

Minutes	1:0 Drug–Adjuvant (pH 3.7)				1:1 Drug–Adjuvant (pH 2.5)				1:3 Drug–Adjuvant (pH 1.7)				1:5 Drug–Adjuvant (pH 1.3)				Neutralized 1:3 Drug–Adjuvant (pH 3.5)			
	A	B	C	Mean	A	B	C	Mean	A	B	C	D	Mean	A	B	C	Mean	A	B	C	Mean
20	492	410	440	444	227	280	357	305	865	901	510	645	731	1981	2594	772	1782	54	65	44	54
40	329	203	213	248	246	204	266	239	—	—	254	365	310	1395	989	340	908	34	54	39	43
60	247	90	158	165	184	169	190	181	428	401	167	185	296	1034	595	197	609	28	39	33	33
120	105	50	70	75	86	70	78	78	198	168	85	89	135	—	204	—[a]	—	18	15	14	16
180	66	37	37	47	35	23	42	33	130	61	49	46	72	983	111	—	547	8	9	7	8
240	43	38	34	38	23	14	23	20	50	37	40	42	42	—[a]	63	—	—	8	2	6	5
360	27	56	54	46	9	6	9	8	26	28	21	10	21	—	10	—	—	8	2	4	5

[a] Died.

Figure 1—*Plasma intact mixidine levels following intraduodenal administration of ^{14}C-mixidine (25 mg/kg) with various molar ratios of 2-naphthalenesulfonic acid. Key: A, 1:5; B, 1:3; C, 1:1; D, 1:0; and E, neutralized 1:3.*

The aqueous layer was aspirated, and 7 ml was transferred to a counting vial. The chloroform was evaporated, and the residue was redissolved in 0.1 ml of chloroform. A 20% ethanol scintillator was added, and the solution was counted for 10 min. Average efficiencies were determined on all samples.

The samples were collected at 20, 40, 60, 120, 180, 240, and 360 min following administration.

Histological Studies—*Preparations*—The solutions administered to the rats were identical to the solutions employed in the plasma level studies, except that unlabeled mixidine fumarate was used instead of ^{14}C-mixidine fumarate. Each rat received the equivalent of 36 mg of mixidine/kg instead of 25 mg/kg. Molar ratios of 1:0, 1:1, 1:3, and 1:5 mixidine fumarate to 2-naphthalenesulfonic acid were prepared to test the effect of increasing the sulfonic acid dose on the duodenal integrity.

Administration—Male CFN rats, 170–220 g, were fasted for approximately 17 hr. They then were anesthetized with ether and dosed intraduodenally as described for the plasma level studies.

Five rats were examined to evaluate each mixidine to 2-naphthalenesulfonic acid ratio.

Examination—Rats were killed 24 hr after surgery by exsanguination under chloroform anesthesia. GI tracts were opened and examined for gross effects. Tissues with gross lesions were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned at 6 μm, stained with hematoxylin and eosin, and examined histologically.

RESULTS AND DISCUSSION

Plasma intact mixidine concentrations are given in Tables I and II. After oral administration, the plasma intact mixidine levels were low and were essentially equivalent regardless of the amount of 2-naphthalenesulfonic acid. To some degree, this result was anticipated from the lethality studies (1) in which oral doses four times greater than intraduodenal doses were not lethal.

For intraduodenal administration, a vehicle-to-vehicle variation in counterion concentration was detected by differences in the plasma levels

Table III—Histological Examination Results [a]

Mixidine–2-Naphthalene-Sulfonic Acid	Animal	Serositis	Myositis	Enteritis	Granuloma (Foreign Body)
1:0	1	—	—	—	—
	2	—	2	2	—
	3	—	2	2	X
	4	—	—	—	—
	5	—	1	1	—
1:1	6	—	—	—	—
	7	—	—	—	—
	8	1	—	—	—
	9	—	—	—	—
	10	—	—	—	—
1:3	11	—	—	—	—
	12	—	—	—	—
	13	2	2	2	—
	14	—	—	—	—
	15	—	—	—	—
1:5	16	1	1	—	—
	17	—	—	—	—
	18	t	t	—	—
	19	—	—	—	—
	20	3	3	3	X

[a] X = present, t = trace, 1 = slight or small, 2 = moderate, 3 = marked, and 4 = severe.

with 1:5 ≫ 1:3 > 1:1 ≈ 1:0 > neutralized 1:3 for the mixidine to 2-naphthalenesulfonic acid ratios. This result is consistent with the trend observed in the lethality studies.

The 1:5 combination produced lethality in two out of three rats. This finding was not anticipated from the lethality studies in which much higher doses in very acidic solutions failed to produce lethality. Possibly, the extensive absorption of the drug combined with the surgical trauma associated with intraduodenal administration and the prolonged administration of anesthetic was fatal to the animals. This hypothesis was not investigated.

The means of the plasma levels following intraduodenal administration are illustrated in Fig. 1. The figure describes more dramatically the differences in the plasma levels, especially the extremely high levels observed during the 1st hr following administration of the 1:5 mixidine–2-naphthalenesulfonic acid combination.

Of particular interest are the low levels of intact mixidine observed following administration of the sodium hydroxide-neutralized 1:3 mixidine–2-naphthalenesulfonic acid combination. The levels were even lower than with mixidine without adjuvant. It may be postulated that the sodium hydroxide has a greater effect on mixidine absorption across the duodenal wall than just neutralization of the sulfonic acid.

Histologic lesions were present in all groups without any apparent relationship to ratios employed (Table III). Some lesions, such as the foreign body granulomas (trichogranuloma), obviously were related to surgical technique, but the cause of the other lesions could not be ascertained. Certainly, the pathology discovered upon histological examination failed to demonstrate a trend that implicates increases in the doses of 2-naphthalenesulfonic acid with increases in the number and severity of lesions.

It is apparent from the lack of counterion lethality (1) and the absence of counterion-related lesions that dose-related lethality and dose-related plasma intact mixidine levels are the results of increased mixidine absorption across the duodenal wall and are not related to toxic or corrosive effects of the counterions. These observations, coupled with the observation of ion-pair-mediated partitioning of mixidine (1), substantiate the conclusion that mixidine absorption may be ion-pair mediated.

Under the proper circumstances, improved drug absorption through ion-pair mediation across the GI tract may occur. However, the application of ion-pairs to improve the absorption of mixidine or other drugs in humans remains a challenge. Ion-pairs exist only in nonaqueous media. Casually releasing a quantity of drug with counterions into the aqueous GI tract environment is not likely to improve drug absorption, as demonstrated by the oral administration studies.

Increasing the quantity of counterion relative to the quantity of drug to overload the GI tract with counterion presents problems regarding dosage form size, particularly if complete absorption is necessary for the clinical activity of a low potency compound. Also, if ion-pair-mediated absorption is site specific, as it appears to be, then a novel means of delivering the drug and counterion simultaneously to that site must be developed since conventional enteric release dosage forms are generally regarded as problematic. Future studies with ion-pair-enhanced absorption will have to address these problems.

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Simple GLC Analysis of Anticonvulsant Drugs in Commercial Dosage Forms

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Abstract □ A simple, specific GLC procedure is described for the analysis of one sedative and six anticonvulsant drugs in pharmaceutical dosage forms. Sample aliquots of ethotoin, glutethimide, mephenytoin, methsuximide, and phensuximide were shaken with or extracted into ethyl acetate, diluted with the internal standard (diphenyl phthalate) solution, injected into a gas chromatograph, and eluted from a methylsilicone column. Primidone and phenytoin samples (extracted as the free acid) required derivatization with *N,O*-bis(trimethylsilyl)acetamide prior to chromatography. The same temperature programming conditions and flow rate settings were used for all seven drugs. The GLC results agreed well with those obtained using the pharmacopeial methods.

Keyphrases □ GLC—analyses, various anticonvulsants and one sedative in commercial dosage forms □ Anticonvulsants, various—GLC analyses in commercial dosage forms □ Sedatives—glutethimide, GLC analysis in commercial dosage forms

The pharmacopeial procedures (1–3) for the assay of several important anticonvulsant drugs in commercial dosage forms generally involve multiple steps. The sample preparation and measurement techniques vary, depending on the chemical entity and its associated physicochemical characteristics. UV spectrophotometry is employed for the

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