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First-Pass Effect after Rectal Administration of Thiazinamium Methylsulfate

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Received March 24, 1978, from the **Department of Pharmaceutical and Analytical Chemistry and the* ‡*Pulmonary Division of the Department of Internal Medicine, State University of Groningen, Antonius Deusinglaan 2, Groningen, The Netherlands,* and the §*Laboratory of the Dutch Pharmacists, The Hague, The Netherlands.* Accepted for publication June 5, 1978.

Abstract □ The absorption and metabolism of the quaternary ammonium compound thiazinamium methylsulfate were studied in humans using plasma concentration data and urinary excretion measurements. After giving a dose of 150 mg in suppositories, the relative bioavailability was 5.8 ± 3.2 (*SD*) % of the dose, comparable to the values obtained following oral administration. The degree of first-pass effect observed after rectal administration was comparable with that after oral administration.

Keyphrases □ Thiazinamium methylsulfate—rectal absorption and metabolism in humans □ Absorption, rectal—thiazinamium methylsulfate in humans □ Metabolism—thiazinamium methylsulfate in humans □ Phenothiazine derivatives—thiazinamium methylsulfate, rectal absorption and metabolism in humans □ Antihistaminics—thiazinamium methylsulfate, rectal absorption and metabolism in humans

It has been widely assumed that, after rectal administration, drugs mainly enter the general circulation without an initial passage through the liver, provided that the suppository does not reach the higher parts of the rectum (1, 2). The drug supposedly enters the inferior or middle rectal veins, which drain into the vena cava inferior. The blood in the superior rectal vein flows to the portal vein and subsequently enters the liver.

After oral administration, most drugs enter the portal vein, so a 100% passage through the liver is involved. Once a drug enters the general circulation, approximately 20% of the total blood flow passes through the liver during each circulation, whatever the route of administration. However, when drugs are administered parenterally, the total first passage through the liver does not occur. It has been assumed that the same condition would apply for the rectal route. For this reason, rectal administration has been recommended as a noninvasive alternative for drugs largely metabolized by the liver or excreted in the bile and for drugs subject to degradation in the GI tract.

Thiazinamium methylsulfate[1] (I) is a phenothiazine derivative with a quaternary ammonium group in the molecule. The drug is used for the treatment of some generalized obstructive lung diseases because it causes bronchodilatation (especially after intramuscular injection) as a result of substantial anticholinergic and antihistaminic properties (3–9).

[1] Multergan, Specia, Rhône Poulenc, Paris, France.

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Table I—Absorption Characteristics of Thiazinamium Methylsulfate after Rectal Administration of a 150-mg Dose

Parameter	Patient GL	Patient PH	Patient WM	Patient HH	Patient WB	Patient MF	Patient TJ	Mean ± *SD*
Age, years	57	62	52	63	26	71	68	57 ± 15
Body weight, kg	74	86	79	67	70	72	75	75 ± 6
Height, m	1.78	1.74	1.81	1.76	1.74	1.76	1.77	1.77 ± 0.02
Dose, mg/kg	2.63	2.26	2.46	2.90	2.78	2.70	2.59	2.62 ± 0.21
c_{max}[a], ng/ml	72	54	142	63	45	290	53	103 ± 89
t_{max}[b], min	45	90	45	120	105	15	20	63 ± 42
F_{rel}[c], % of dose	6.3	6.6	11.0	6.9	0.7	5.8	3.4	5.8 ± 3.2
$(F_{rel})_{tot}$[d], % of dose	6.6	6.8	11.6	8.1	0.7	5.8	3.7	6.2 ± 3.4

[a] Maximum plasma concentration. [b] Time at which c_{max} was reached. [c] Relative bioavailability (compared to intramuscular injection). [d] Estimated values obtained after extrapolation except for Patients WB and MF (measured values).

In excretion and metabolism studies (10, 11), an extensive first-pass effect apparently occurred when thiazinamium cations were administered orally. Moreover, a high proportion of both the unchanged drug and the metabolite was found in bile (12).

The purpose of this study was to investigate whether rectal administration of I would offer better systemic bioavailability.

EXPERIMENTAL

Dosage Form—The lipophilic (fatty) base was a mixture[2] of some mono-, di-, and triglycerides of naturally occurring saturated fatty acids (C_{12}–C_{18}), with a melting range of 33.5–35.5° and a congealing range of 32.5–34.5°. The specific gravity of the base at 20° is 0.950–0.980; it has an iodine number less than three, a saponification value of 230–240, and a hydroxyl value of less than 15 (13).

Its melting behavior both *in vitro* and *in vivo* was described by Ritschel (14), who showed that a suppository of the lipophilic base without additional drug substances melted almost completely in the human rectum within 10 min.

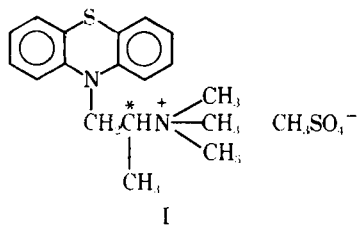
For all experiments involving rectal administration, a dose of 0.4747 m*M* (150 mg) of thiazinamium base (calculated as the hydroxide), equal to 194.6 mg of the methylsulfate, was selected. The drug was passed through a sieve to obtain particles <150 μm in size. The suppositories were prepared by the fusion method, *i.e.*, a suspension of the powder was mixed with 2 mg of colloidal silica[3] in the molten base (about 50°). The liquid then was poured into 2-ml molds at 34–35°.

The drug content in the suppositories was determined by an amphimetric titration to be 100.3% (n = 3) of the stated amount (10). The dissolution rate of the suppositories was determined as described previously (15, 16). The dissolution half-life was 6 min.

Analytical Methods—The amount of thiazinamium cations in plasma and urine was determined as described previously (17). The procedure is based on ion-pair extraction of the compound with iodide as a counterion, followed by GLC with an alkali flame-ionization detector.

Thiazinamium sulfoxide cations were isolated from the urine by means of column chromatography using an ion-exchange resin[4], followed by two-dimensional TLC (18). The sensitivity of the latter method was not sufficient to allow the measurement of metabolite concentrations in plasma.

Protocol—Seven male patients with generalized obstructive lung disease (Table I) had no abnormalities in their blood composition, kidney function, liver function, and digestive tract. None of the patients used other drugs during the investigation or the preceding week. Patients fasted overnight. After drug administration at 9.00 am, the patients stayed in bed for 1 hr.



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Blood sampling took place at 0, 3, 6, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 330, and 420 min following drug administration. The samples (~10 ml) were drawn from a permanent cannula[5] with a three-way stopcock[6] placed in the right cubital vein. The blood was collected in 15-ml glass tubes with screw caps[7]; each contained 1 drop of heparin solution (5 mg = 500 U of heparin sodium/ml of distilled water). The samples were immediately stored in a refrigerator at 4°.

Within 2 hr of collection, the samples were centrifuged for 20 min at 6000×*g*. Next, 4.0 ml of the plasma layer was transferred to a 50-ml centrifuge tube[8] and stored at −20°. It was analyzed within 2 months. Within this period, no significant decrease in the concentrations could be detected (10).

Immediately after drug administration, each patient was allowed to drink 200 ml of water. After 90 and 210 min, the patients were given a light meal (rusks with sugar and a glass of orange lemonade). During the experiment, the patients were allowed to drink water upon request.

Urine was collected every hour, if possible, during the first 7 hr of the investigation and up to 24 hr afterwards.

Determination of Relative Bioavailability—The areas under the plasma concentration–time curves were determined by cutting and weighing a standard high-quality paper. The relative bioavailability *versus* an intramuscular injection of 12.5 mg (which was found to have an absolute bioavailability of 100% during the time of the experiment) in the same patients was calculated by comparing the areas under the curves after correction for the dose. This comparison was allowed because the pharmacokinetics of this drug are not dose dependent (10).

RESULTS AND DISCUSSION

Bioavailability—Typical plasma concentration–time curves are given in Fig. 1. Plasma concentrations were generally low. In most patients, absorption started rather quickly. The t_{max} values varied from 15 to 120 min with a mean value of 63 ± 42 (*SD*) min, suggesting that thiazinamium cations are rapidly liberated from the base in the rectum. This finding is in agreement with the *in vitro* experiment.

The values for c_{max} varied from 54 to 290 ng/ml, with a mean value of 103 ± 89 (*SD*) ng/ml. In most patients, a more or less pronounced plateau

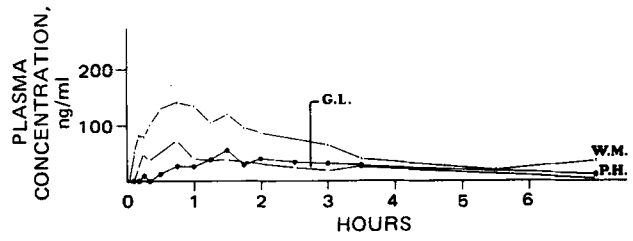

Figure 1—*Typical examples of individual plasma concentration–time curves obtained after rectal administration of a suppository containing 150 mg of thiazinamium.*

[2] Witepsol H-15, Dynamit Nobel A.G., Chemische Werke Witten, Witten, German Federal Republic.
[3] Aerosil.
[4] Amberlite XAD-2.
[5] Indwelling catheter, Braunule T with LOK, B. Braun, Melsungen, German Federal Republic.
[6] K-75a, Pharmseal, Herstal, Belgium.
[7] Sovirel, Levallois-Perret, France.
[8] Quickfit.

Table II—Cumulative Urinary Excretion of Thiazinamium Cations (I) and Thiazinamium Sulfoxide Cations (II) after Rectal Administration of a 150-mg Dose[a]

Parameter	Patient GL	Patient PH	Patient WM	Patient WB	Patient MF	Patient TJ[b]	Mean ± *SD*[c]
I, % of dose	2.2	1.9	5.3	0.6	2.5	1.4	2.5 ± 1.7
I, % of F_{rel}	35.1	28.7	47.9	96.9	43.4	33.3	55.9 ± 16.5
II, % of dose	2.0	1.8	4.6	1.0	1.2	1.6	2.1 ± 1.5
II, % of F_{rel}	32.1	26.7	42.2	161.5	20.4	38.0	56.6 ± 59.2
I + II, % of dose	4.2	3.7	9.9	1.6	3.7	3.0	4.0 ± 3.1
I + II, % of F_{rel}	67.2	55.4	90.1	258.4	63.8	71.3	106.9 ± 85.6
I to II ratio	1:0.92	1:0.93	1:0.88	1:1.67	1:0.47	1:1.14	1:1.01
Intramuscular I to II ratio[d]	1:0.16	1:0.25	1:0.29	1:0.17[e]	1:0.26	1:0.34	1:0.25

[a] No urinary excretion data are available for Patient HH. [b] Until 390 min after administration. [c] Mean of data of five patients (TJ omitted). [d] For comparison, the ratio between unchanged drug and metabolite in urine as observed after intramuscular injection of a 12.5-mg dose is also given. [e] Until 420 min after injection.

level was found where the plasma concentration remained relatively constant. This finding may indicate that the absorption of the drug continues for an extended period.

From the plasma concentration–time curves during the experiments, a mean relative bioavailability, F_{rel}, of 11.5 ± 6.0 (*SD*) mg of thiazinamium methylsulfate was calculated, which equals 5.8 ± 3.2 (*SD*) % of the dose. In two patients, the curve had already declined to zero at 7 hr after administration; in five patients, extrapolation to zero was possible, which led to an estimated total relative bioavailability, $(F_{rel})_{tot}$, of 6.2 ± 3.4 (*SD*) % of the dose. These data are of the same order of magnitude as those following oral administration (11).

Biotransformation and Urinary Excretion—Earlier investigations (10) showed that only one metabolite, thiazinamium sulfoxide, was formed in humans. Figure 2 represents the cumulative renal excretion of thiazinamium cations and thiazinamium sulfoxide cations in Patient GL. Table II lists the quantities of these compounds excreted during the experiment.

The excretion of thiazinamium cations appeared to be rapid. In general, the patients had already excreted in 7 hr ~90% of the total amount excreted in 24 hr. The amount of unchanged drug excreted over 24 hr varied from 28.7 to 96.9% of the calculated value of the bioavailability.

However, the 96.9% value (Patient WB) was much higher than the values obtained from the other patients, so WB may represent a special case. Without this value, the excretion varied between 28.7 and 47.9% of the calculated bioavailability [mean value of 38.8 ± 8.6 (*SD*) %].

Figure 2 shows that the renal excretion rate of thiazinamium sulfoxide cations was almost identical to that of the unchanged drug. The mean value of the amount of thiazinamium sulfoxide cations found in the urine of Patients GL, PH, WM, WB, and MF accounted for 30.4 ± 9.2 (*SD*) % of the bioavailability.

The mean ratio between the unchanged drug and metabolite (Table II) was 1:1.01. Without the value for Patient WB, a mean ratio of 1:0.78 was found. After an intramuscular injection of a dose of 12.5 mg in the same patients, the mean value for the ratio between the unchanged drug and metabolite was much lower, namely 1:0.25.

The difference between these ratios strongly suggests that a substantial first-pass effect occurs after rectal administration. Comparison with data obtained after oral and intramuscular administrations in another group of patients (10, 19) indicates that the first-pass effect following rectal administration is of the same degree as that after oral administration (ratio 1:0.94).

This finding implies that thiazinamium cations only partially bypass the first liver passage when administered rectally. This conclusion is rather surprising because, although there is no concrete evidence, it is currently accepted (1, 2) that rectally administered drugs enter the systemic circulation largely without initial passage through the liver (provided that the suppository does not reach the higher parts of the rectum).

To explain a first-pass effect after rectal administration of thiazinamium cations, it must be assumed that this drug enters the systemic circulation almost exclusively by way of the smaller rectal vessels, which drain into the portal vein and subsequently the liver, just as in the case of oral administration. As pointed out previously, only the veins of the higher part of the rectum drain directly into the portal veins. Therefore, the drug must have been transported largely by way of the superior rectal veins.

If part of the drug is absorbed in the inferior or middle rectal veins, it probably then reaches the superior rectal veins by way of the numerous connections (anastomoses) between them. Another possibility is that the molten suppository quickly reaches the higher parts of the rectum and that the drug is directly absorbed into the superior rectal veins.

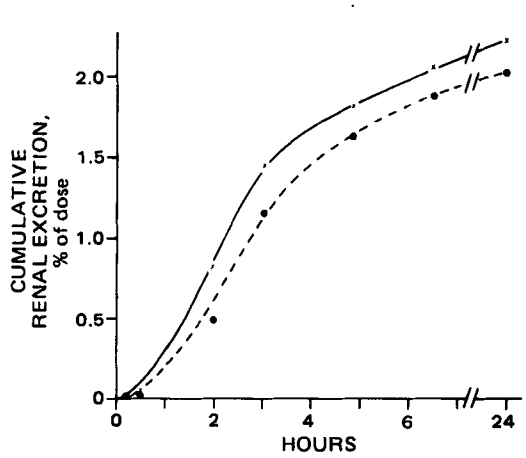

Figure 2—*Typical example of cumulative renal excretion curves of thiazinamium cations* (×) *and thiazinamium sulfoxide cations* (●) *after rectal administration of 150 mg to Patient GL.*

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Determination of Submicrogram Quantities of Clonidine in Biological Fluids

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Abstract □ A sensitive and specific GLC method using electron-capture detection was developed for clonidine in plasma and urine. Di-perfluoroacyl derivatives of both clonidine and the 4-methyl analog of clonidine (used as an internal standard) were formed, and an extraction process was developed for the removal of excess derivatization reagent and endogenous biological compounds; the assay permitted quantification of 25 pg of clonidine/ml in a 4-ml plasma sample. The assay was used to elucidate the time course of plasma concentrations in a normotensive subject following oral administration of 50, 100, and 200 μg of clonidine hydrochloride and also to determine unchanged drug excreted in the urine.

Keyphrases □ Clonidine—GLC analysis in plasma and urine □ GLC—analysis, clonidine in plasma and urine □ Antihypertensives—clonidine, GLC analysis in plasma and urine

Clonidine, 2-[(2,6-dichlorophenyl)imino]imidazolidine, is a potent drug used in the treatment of arterial hypertension. The sympatico-inhibitory activity of clonidine is predominantly due to its influence on α-adrenergic receptors in the brain stem (1). The hypotensive effect of clonidine is absent in tetraplegic subjects with transection of the cervical spinal cord above the sympathetic outflow (2). The absence of the hypotensive effect in these subjects indicates that the action of the drug is centrally mediated in humans.

The therapeutic dose of clonidine is a few tenths of a milligram per day. Clonidine is lipophilic in nature with a large volume of distribution (3). Thus, the resulting plasma concentrations following oral administration of therapeutic doses are in the submicrogram range.

BACKGROUND

The metabolism and disposition of clonidine in various animal species were studied (4) using ^{14}C-labeled clonidine. The time course of total radioactivity in the plasma following oral administration of either 390 or 1440 μg of clonidine was determined. The plasma concentrations of unchanged drug were not ascertained, although approximately 45% of the orally administered dose was excreted in the urine as unchanged drug.

The plasma levels, renal excretion, and urinary metabolic pattern were determined (5) in normotensive subjects. Three hours following oral administration of 300 μg of ^{14}C-labeled clonidine, clonidine contributed less than 50% of the total ^{14}C-radioactivity in the plasma.

A recently developed GLC–mass spectrometric method permits the measurement of clonidine in plasma and urine following administration of unlabeled therapeutic doses to humans (6). The method is based on the selective monitoring of mass fragments produced from clonidine and from a deuterated internal standard under electron-impact ionization. The sensitivity of the method and the chromatographic properties of clonidine and the internal standard were improved by formation of their dimethyl derivative through on-column methylation with trimethylanilinium hydroxide (7). The precision of the method at 0.25 ng/ml was ±11% *SD* with 4 ml of plasma. Davies *et al.* (8) used the improved GLC–mass spectral method to study the pharmacokinetics and pharmacodynamics of clonidine following oral and intravenous administration of 300 μg of clonidine hydrochloride[1].

Cho and Curry (9) developed a GLC method using a ^{90}Sr-ionization detector to determine clonidine levels in the blood and tissues of rats administered 500 μg of drug/kg iv. The minimum detectable quantity of clonidine was 2–3 ng. The drug, which was not derivatized, tended to adsorb onto the chromatographic support, especially with the longer columns, thus decreasing the sensitivity of the assay.

A GLC method with electron-capture detection of the pentafluorobenzyl derivative of clonidine was reported (10). This method was used to determine the temporal pattern of plasma concentrations in rats following administration of 500 μg of clonidine hydrochloride/kg, about 100 times the dose received by humans.

This paper reports an electron-capture GLC method for the determination of clonidine in plasma and urine using a di-heptafluorobutyryl derivative of clonidine and an internal standard. The electron-capture-sensitive background is reduced through the use of silica gel columns. This method was used to elucidate the time course of plasma concentrations in a normotensive subject following oral administration of 50, 100, and 200 μg of clonidine hydrochloride.

EXPERIMENTAL

Materials—Methylene chloride, benzene, and hexane, employed in the extraction procedures, were used as obtained[2]. Ethyl acetate[3], purchased in quart bottles and sealed under nitrogen, was used as the solvent for the derivatization of clonidine and the internal standard.

The carbonate buffers, 1 *M* (pH 9.75) and 0.1 *M* (pH 9.2), were made from potassium bicarbonate and adjusted to the appropriate pH with freshly prepared potassium hydroxide solution. Sulfuric acid (0.1 *N*) was diluted from the concentrated acid.

Heptafluorobutyric anhydride[4] was obtained in 1-ml glass ampuls. After the ampul was opened, the contents were stored in a 3-ml minivial for protection from moisture. A new ampul was used daily.

The internal standard, 2-[(2,6-dichloro-4-methylphenyl)imino]imidazolidine, was used as received[5].

Dimethyldichlorosilane, 5% in toluene, was used to silanize all glassware for at least 2 hr; the glassware was then rinsed with toluene, methanol,

[1] Catapres, Boehringer Ingelheim.
[2] Nanograde solvents, Mallinckrodt, St. Louis, Mo.
[3] Burdick & Jackson Laboratories, Muskegon, Mich.
[4] Pierce Chemical Co., Rockford, Ill.
[5] C. H. Boehringer Sohn, Ingelheim, Germany.

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