(OH carboxylic) and the appearance of the band at 1665 cm^{-1} (C=O in $COOC_2H_5$).

Anal.—Calc. for $C_{15}H_{16}N_2O_5S$: C, 53.56; H, 4.79; N, 8.33; S, 9.53. Found: C, 53.06; H, 4.71; N, 8.30; S, 9.66.

Sulfanilamido-1,4-naphthoquinone Derivatives—A solution of 1,4-naphthoquinone (20 mmoles in 40 ml of 95% ethyl alcohol) was gradually added over a period of 30 min to a solution of the appropriate sulfanilamido derivative (10 mmoles in 10–30 ml of glacial acetic acid). Sodium acetate (20 mg) was added with constant stirring at 60°. Stirring was continued for 30 min, then refluxed for 1 hr, and left overnight at room temperature. A black precipitate was separated and filtered. Water was added to the filtrate when a brownish material separated. It was then filtered, washed with hot water, dried at 80°, and crystallized from 95% ethyl alcohol as light crystals. The IR spectra of the compounds showed the appearance of two bands at 1650 and 1630 cm^{-1} for the C=O of the naphthoquinone. The pair of bands of the primary NH_2 at 3300 and 3130 cm^{-1} disappeared, while the N—H stretching absorption was shown at 3245 cm^{-1} (Table IV).

4-Amino-4′-[2-(1,4-naphthoquinonyl)]aminodiphenylsulfone (XV)—This was obtained by the same procedure for the preparation of the sulfanilamido-1,4-naphthoquinone derivatives, from 1,4-naphthoquinone (20 mmoles in 40 ml of 95% ethyl alcohol) and 4,4′-diaminodiphenyl sulfone (10 mmoles in 35 ml of glacial acetic acid). The product was crystallized from 95% ethyl alcohol. Yield: 91%, mp 306–307°.

Anal.—Calc. for $C_{22}H_{16}N_2O_4S$: C, 65.34; H, 3.96; N, 6.93; S, 7.92. Found: C, 65.25; H, 4.14; N, 6.72; S, 7.71.

2-{N^4-[N^1-(*p*-Carbomethoxy)phenyl]sulfanilamido}-1,4-naphthoquinone (XVI)—A solution of IX (10 mmoles) was esterified by warming with excess methyl alcohol in the presence of concentrated sulfuric acid. The methyl ester recrystallized from glacial acetic acid was obtained in 37% yield, mp 253°. In the IR spectrum the bands at 1700 cm^{-1} (C=O in COOH) and 3245 cm^{-1} (OH carboxyl) disappeared and a band at 1750 cm^{-1} (C=O in $COOCH_3$) appeared.

Anal.—Calc. for $C_{24}H_{18}N_2O_6S$: C, 62.34; H, 3.89; N, 6.06; S, 6.90. Found: C, 61.93; H, 3.67; N, 5.64; S, 6.68.

2-[N^4-[N^1-(*p*-Carboethoxy)phenyl]sulfanilamido}-1,4-naphthoquinone (XVII)—Obtained as XVI from IX by esterification with 95% ethyl alcohol. Yield: 35%, mp 261–262°. In the IR spectrum the bands at 1700 cm^{-1} (C=O in the carboxyl group) and 3245 cm^{-1} (OH carboxyl) disappeared, and a band at 1665 cm^{-1} (C=O in $COOC_2H_5$) appeared. appeared.

Anal.—Calc. for $C_{25}H_{20}N_2O_6S$: C, 63.02; H, 4.20; N, 5.88; S, 6.72. Found: C, 62.68; H, 3.90; N, 5.48; S, 6.82.

REFERENCES

(1) H. J. Copper and M. L. Cohn, *Am. Rev. Tuber.*, **63**, 108 (1951).

(2) J. W. Cornforth, P. DA. Hart, R. J. W. Rees, and J. A. Stock, *Nature (London)*, **168**, 150 (1951).

(3) G. B. Mackaness, *Am. Rev. Tuber.*, **69**, 690 (1954).

(4) J. E. Lovelock and R. J. W. Rees, *Nature (London)*, **175**, 161 (1955).

(5) E. R. Long and F. B. Seibert, *Am. Rev. Tuber.*, **13**, 393 (1926); through *Chem. Abstract*, **20**, 2535 (1926).

(6) R. J. Anderson and M. S. Newman, *J. Biol. Chem.*, **103**, 197 (1933); through *Chem. Abstracts*, **28**, 194 (1934).

(7) A. D. Marco, B. Zachi, and V. Zavaglis, *Sperimentales*, **102**, 218 (1952); through *Chem. Abstracts*, **46**, 10435 h (1952).

(8) F. G. Valdecases, *Med. Clin. (Barcelona)*, **4**, 275 (1952); through *Chem. Abstracts*, **47**, 13084 d (1953).

(9) R. C. Denney, "Named Organic Reactions," Butterworth, London, 1969, p. 86.

(10) L. F. Fieser and M. Fieser, "Advanced Organic Chemistry," Rienhold, New York, N.Y., 1961, p. 853.

(11) J. H. Wilfred, "Reaction of Organic Compounds," 3rd ed. Longmans, London, 1957, p. 284.

High-Performance Liquid Chromatographic Determination of Alizapride, a New Antiemetic Compound, and Its Application to a Dose-Dependent Pharmacokinetic Study

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Received November 20, 1980, from the **Department of Pharmacology, Faculty of Medicine of Paris XII, 8 Rue du Général Sarrail—94000 Creteil*, and the ‡*Department of Medical Statistics, Hopital du Bocage—21000 Dijon, France.* Accepted for publication March 4, 1982.

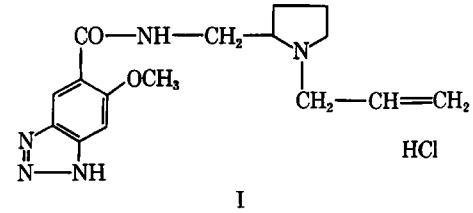
Abstract □ An assay was developed to measure alizapride (a new antiemetic compound) in biological specimens. The method involved reversed-phase high-performance liquid chromatography and fluorescence detection. The detection limit was 5 ng/ml in plasma or urine samples. The value of the assay was demonstrated with a dose-dependent pharmacokinetic study. It showed a two-phase decrease in plasma concentrations, after intravenous injection, with half-lives of 7.5-min and 2.5-hr, respectively. From plasma and urine results, pharmacokinetic parameters remained constant in the dose range of 50–200 mg.

Keyphrases □ High-performance liquid chromatography—determination and dose-dependent pharmacokinetic application of alizapride, new antiemetic compound □ Alizapride—new antiemetic compound, high-performance liquid chromatographic determination and dose-dependent pharmacokinetic application □ Antiemetic compound—high-performance liquid chromatographic determination and dose-dependent pharmacokinetic application of alizapride □ Pharmacokinetic application—dose-dependent, alizapride, new antiemetic compound, high-performance liquid chromatographic determination

Alizapride[1], *N*-[(1-allyl-2-pyrrolidinyl)methyl]-6-methoxy-1*H*-benzotriazole-5-carboxamide (I), is a new compound with antiemetic properties (1, 2). For bioavailability studies a sensitive and specific assay for plasma and urine concentrations was needed. According to the physicochemical properties of the compound, a high-performance liquid chromatographic (HPLC) assay with a fluorescence detector was selected. This study describes the procedure used for the analysis of biological samples and the preliminary results on pharmacokinetic parameters calculated in a dose-dependency study.

EXPERIMENTAL

Materials—Alizapride was obtained from commercial suppliers and showed no impurities in two different TLC systems. Methanol, chloroform, and tris(hydroxymethyl)aminomethane buffer (pH 8.1) were commercially available analytical grades and used without further purification.



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[1] Plitican, Delagrange Laboratories, Paris, France.

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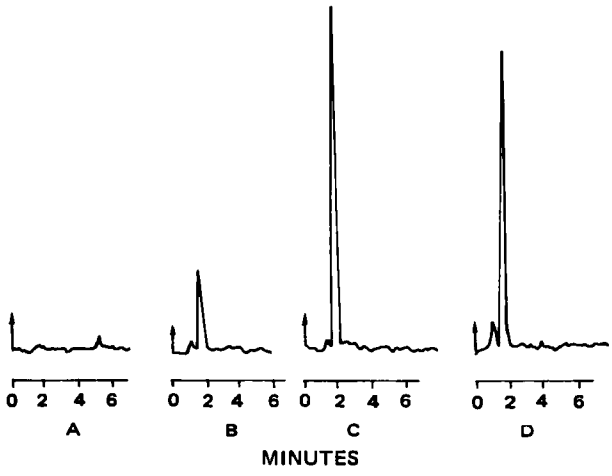

Figure 1—*High-performance liquid chromatograms of alizapride. Graph A corresponds to plasma free of alizapride, graphs B and C to plasma spiked with 50 and 250 ng of alizapride respectively, and graph D to plasma samples obtained during pharmacokinetic study.*

Apparatus—A liquid chromatograph[2] equipped with a fluorescence detector[3] and a continuous flow cell of 8-μl capacity was used. According to the fluorescence spectrum of alizapride, excitation and emission wavelengths were 323 and 380 nm, respectively. A 250-mm steel column was used, packed with a monomolecular layer of octadecyltrichlorosilane chemically bonded to Porasil beads with an average particle size of 10 μm[4]. The chromatographic solvent, methanol–pH 8.1 buffer (80:20, v/v), was delivered at a 2-ml/min flow rate.

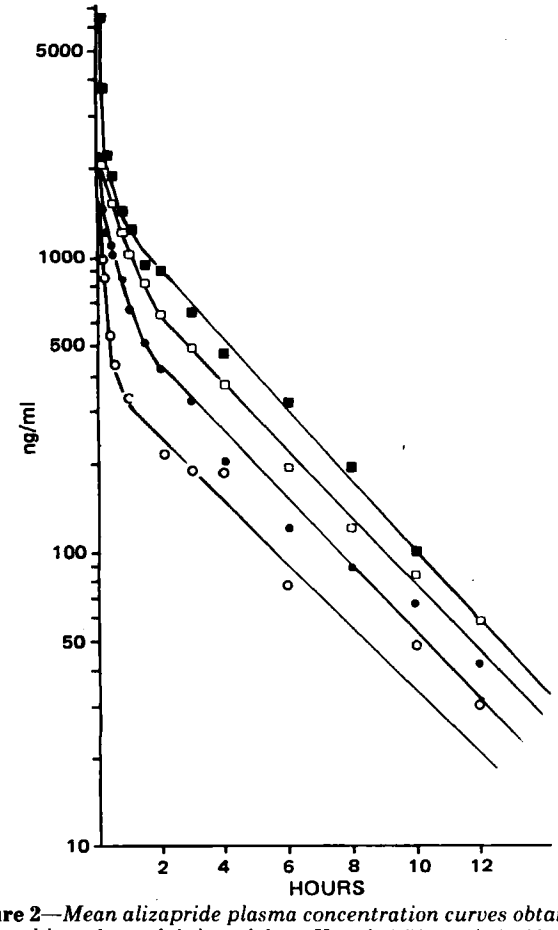

Figure 2—*Mean alizapride plasma concentration curves obtained in three subjects for each injected dose. Key: (○) 50 mg; (●) 100 mg; (□) 150 mg; (■) 200 mg.*

[2] Waters Associates, Paris, France.
[3] JY3, Jobin et Yvon, Paris, France.
[4] Bondapak C-18, Waters Associates, Paris, France.

Table I—Alizapride Recovery from Spiked Plasma Samples[a]

Recovered, ng	Added, ng						
	25	50	100	250	500	1000	2000
$\bar{m}$	25.5	49.5	99.8	248.6	501.2	1001.3	2003.0
SD	1.6	3.0	6.2	7.0	8.5	14.7	64.0
CV%	6.1	6.0	6.2	2.8	1.7	1.5	3.2

[a] Total of five assays.

Sample Preparation Procedure—For statistical analysis, human plasma was spiked with known amounts of alizapride to reach 5–2000 ng/ml and equilibrated by shaking for 30 min. In human experiments, blood samples were immediately centrifuged, and the plasma was stored at −20° until analysis.

Extraction Procedure—To a 1-ml plasma sample, 1 ml of pH 8.1 buffer and 5 ml of chloroform were added. The mixture was shaken at room temperature for 1 min on a vortex-type mixer and centrifuged at 3000 rpm for 10 min. A 4.8-ml volume of the organic phase was evaporated to dryness under a smooth nitrogen stream. The residue was reconstituted with 35 μl of the chromatographic solvent; 25 μl of the mixture was injected onto the HPLC column.

Quantitation—Calibration curves were calculated using peak height and amounts of drug added to plasma, using the least-squares method.

Human Experiments—Six subjects gave informed consent to participate in the study. They were free from cardiac, renal, hepatic, and respiratory diseases and allergies according to clinical and biological examinations. None of the subjects received any drugs for at least 15 days prior to the study. Each subject received four doses (50, 100, 150, and 200 mg) at one-week intervals, three of them intravenously and the others orally, in tablet form.

A 7-ml heparinized blood sample was withdrawn at 0, 3, 6, 10, 15, 20, 30, 45, 60, 90, and 120 min and 3, 4, 6, 8, 10, 12, and 24 hr after administration. Urine samples were collected every 2 hr during the first 12 hr, once during the next 12 hr, and every 24 hr during the next 4 days.

Calculation—Statistical and pharmacokinetic calculations were performed with a table microcomputer[5] using programs developed previously (3).

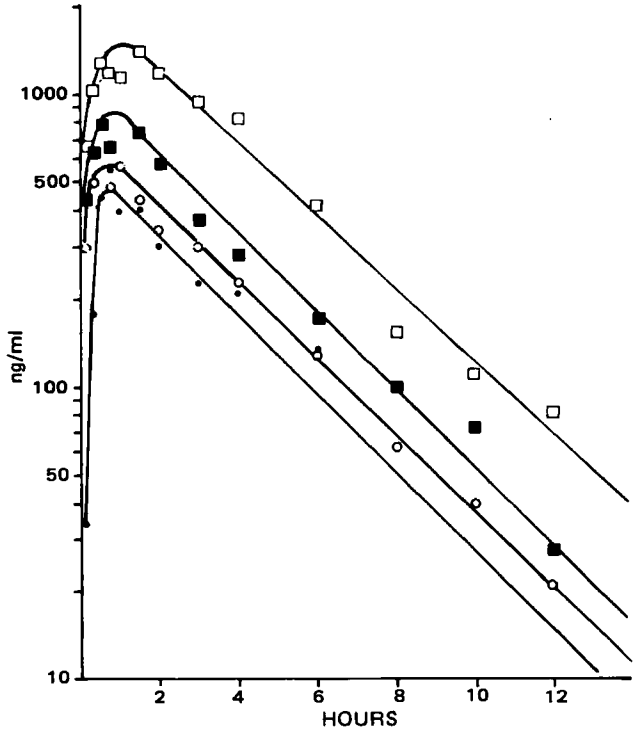

Figure 3—*Mean alizapride plasma concentration curves obtained in three subjects for each orally administered dose. Key: (●) 50 mg; (○) 100 mg; (■) 150 mg; (□) 200 mg.*

[5] Model 4051, Tektronix, Paris, France.

Table II—Mean Pharmacokinetic Parameters Obtained After Intravenous Administration of Alizapride

Parameter	Dose, mg			
	50	100	150	200
$T_{1/2\alpha}$, hr	0.135 ± 0.065	0.149 ± 0.109	0.092 ± 0.029	0.111 ± 0.055
$T_{1/2\beta}$, hr	3.453 ± 2.919	2.046 ± 0.698	1.810 ± 0.056	2.497 ± 0.081
K_{1-3}, hr^{-1}	0.915 ± 0.449	0.843 ± 0.375	1.177 ± 0.087	2.275 ± 2.458
K_{1-2}, hr^{-1}	3.291 ± 1.299	3.037 ± 4.526	4.544 ± 1.669	2.047 ± 1.071
K_{2-1}, hr^{-1}	0.978 ± 0.842	1.362 ± 1.396	2.606 ± 0.929	1.382 ± 0.428
V_1, liter	36.473 ± 8.745	29.760 ± 7.757	27.895 ± 0.177	27.486 ± 21.059
V_2, liter	73.073 ± 39.400	50.023 ± 11.548	48.540 ± 0.877	58.270 ± 10.784
AUC, mg hr/liter	1.977 ± 0.729	3.533 ± 0.899	4.660 ± 0.198	7.587 ± 2.693
Cl, ml/min	457.5 ± 148.3	495.2 ± 141.03	537.0 ± 22.2	472.6 ± 141.1
U_∞, mg	36.8 ± 5.77	61.2 ± 8.6	117.7 ± 4.95	157.6 ± 7.2
U_∞, %	73.6 ± 11.5	61.2 ± 8.6	78.5 ± 3.3	78.8 ± 3.3
$T_{1/2el}$, hr	1.98 ± 0.58	3.20 ± 0.45	2.51 ± 0.36	2.52 ± 0.14

RESULTS AND DISCUSSIONS

Figure 1 shows examples of chromatographic tracings. No peak corresponding to endogenous compounds interferes with the drug. A standard curve obtained with eight points had a mean slope of 0.0528 (1.12% *SD*), a mean correlation coefficient of 0.9998, and a mean *y*-intercept of 0.0146 (0.1% *SD*). These data indicate that the peak height at the origin is not significantly different from zero, which correlates with the absence of endogenous interfering peaks, and a linearity between 5 and 2000 ng of alizapride/ml of plasma.

Recovery of the drug from spiked plasma samples (n = 5) was determined (Table I) at several concentrations. Mean results appear to be very close to the theoretical concentrations, which show reasonable recovery and accuracy. The standard deviation decreased to a minimum of 1.5% obtained when the analyzed concentration was ~1 μg/ml. Under these conditions, assay sensitivity of alizapride in plasma was 5 ng/ml.

Blood Sample Analysis—*Intravenous Administration*—Mean alizapride concentrations obtained from the three subjects receiving intravenous doses are shown in Fig. 2. Individual concentrations have been systematically interpreted according to three different pharmacokinetic models, *i.e.*, one-, two-, or three-compartment open models. At each step, a statistical Fischer test using the least-squares criterion was performed to evaluate the benefit of increasing the number of compartments. After intravenous administration a two-compartment open model was chosen. Mean pharmacokinetic parameters are presented in Table II. It shows a very rapid distribution phase corresponding to a 0.125 ± 0.07-hr half-life (range: 0.057–0.210 hr) and an elimination phase with a 2.51 ± 1.49-hr mean half-life (range: 1.25–6.79 hr). Volumes of distribution are large: 3.9–44.5 liters (mean: 30.4 ± 4.2 liters) for the central compartment and 37.0–115.1 liters (mean: 57.5 ± 11.2 liters) for the peripheral compartment.

Oral Administration—Mean alizapride concentrations obtained from the three subjects receiving oral doses are shown in Fig. 3. With this route of administration, the better fit was obtained with the one-compartment open model with first-order absorption. However, the two curves observed after the 200-mg administration in two subjects were better described with a two-compartment open model with first-order absorption. Results are presented in Table III, and it can be seen that the apparent elimination half-life is in the same range as that obtained after intravenous administration. The area under the plasma concentration curves are lower, with the systemic availability calculated in this preliminary study being 72.5%.

Urine Sample Analysis—*Intravenous Administration*—Sigma-minus plots of the urinary excretion of unchanged alizapride is shown in Fig. 4. Corresponding pharmacokinetic parameters are presented in Table II, and it can be seen that they are remarkably constant. Two parameters are of particular interest. The first is the urinary elimination half-life of alizapride (2.60 ± 0.57 hr) compared with the terminal half-life of plasma concentration decay $t_{1/2}$ = 2.51 ± 1.49 hr. The paired t test failed to show differences in these values. The overall urinary elimination of unchanged drug, 73.0 ± 9.9% of the administered dose, shows that urinary excretion is the mean phenomenon responsible for plasma alizapride decay. About 30% of the administered dose was not recovered

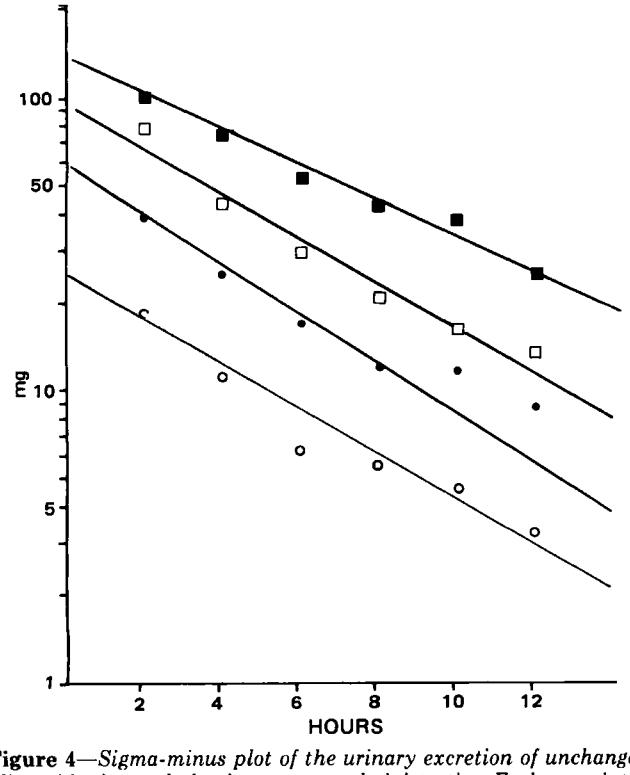

Figure 4—*Sigma-minus plot of the urinary excretion of unchanged alizapride observed after intravenous administration. Each curve is the mean of three subjects (○) 50 mg; (●) 100 mg; (□) 150 mg; (■) 200 mg.*

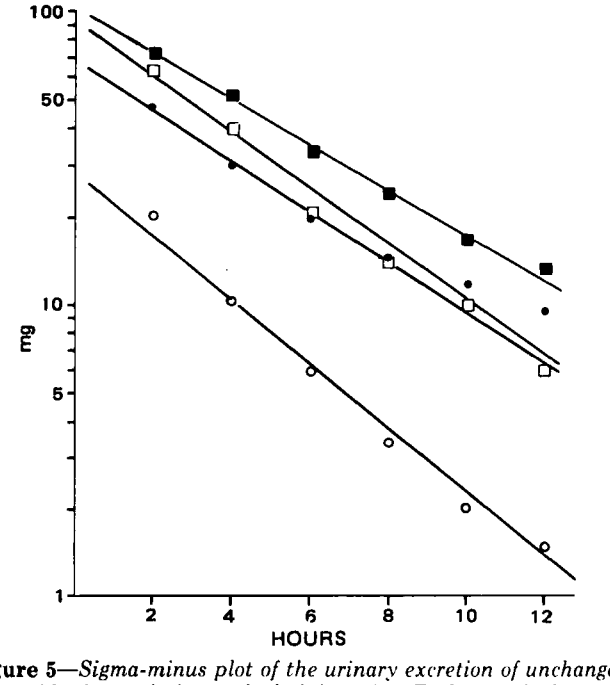

Figure 5—*Sigma-minus plot of the urinary excretion of unchanged alizapride observed after oral administration. Each curve is the mean of three subjects. Key: (○) 50 mg; (●) 100 mg; (□) 150 mg; (■) 200 mg.*

Table III—Mean Pharmacokinetic Parameters Obtained After Oral Administration of Alizapride

Parameter	Dose, mg			
	50	100	150	200
Lag time, hr	0.245 ± 0.021	0.285 ± 0.205	0.160 ± 0.169	0.513 ± 0.690
Kr, hr^{-1}	3.765 ± 1.859	6.495 ± 3.387	3.865 ± 2.567	6.060 ± 4.803
$T_{1/2r}$, hr	0.210 ± 0.099	0.125 ± 0.064	0.230 ± 0.155	1.133 ± 1.824
$T_{1/2\alpha}$, hr	—	—	—	2.240 ± 0.297
$T_{1/2\beta}$, hr	1.707 ± 0.962	2.750 ± 0.151	2.110 ± 0.532	2.817 ± 0.756
K_{1-3}, hr^{-1}	0.522 ± 0.326	0.982 ± 1.279	0.436 ± 0.091	0.303 ± 0.026
K_{1-2}, hr^{-1}	—	—	—	0.003 ± 1.10^{-5}
K_{2-1}, hr^{-1}	—	—	—	0.239 ± 0.061
V_1, liters	92.0 ± 56.1	217.3 ± 49.2	123.8 ± 21.4	101.2 ± 15.6
V_2, liters	—	—	—	1.33 ± 0.55
AUC, mghr/liter	1.706 ± 1.757	3.760 ± 3.166	3.170 ± 0.276	6.573 ± 0.464
Cl_T, ml/min	1099 ± 1006	895 ± 242	785 ± 107	472 ● 42
U_∞, mg	33.5 ± 47	80.3 ± 7.8	98.5 ± 16.5	144.9 ± 29.5
U_∞, %	67.0 ± 94	80.3 ± 78	65.7 ± 110	72.5 ± 14.8
$T_{1/2el}$, hr	2.17 ± 0.52	3.14 ± 1.56	2.26 ± 0.80	3.22 ± 1.25

during this study. Since urine was collected for 5 days and the elimination half-life is ~2.5 hr, a good approximation of the amount excreted in the urine at infinity exists. So the difference must be accounted for in the metabolism. The metabolic pathway of alizapride is unknown in humans. No extra peaks appeared on the chromatographic plots of the urine fractions collected. Furthermore, an attempt was made to see if one or more of the metabolites was eluted from the column simultaneously with the parent drug. For this reason, urine extracts have been chromatographed using different solvent systems, *i.e.*, methanol–pH 8.1 buffer from 20:80 (v/v) to 80:20 (v/v). No extra peak separated from the main peak. Either the metabolites are not extracted in chloroform or the solvent is unable to elute them from the HPLC column.

Oral Administration—Sigma-minus plots of the urinary excretion of unchanged alizapride are shown in Fig. 5, and corresponding pharmacokinetic parameters are presented in Table III. The urinary elimination half-life was 2.45 ± 1.38 hr, which is comparable to that observed after intravenous administration.

The unchanged urinary alizapride recovery amounted to 65.5 ± 22.7% of the administered dose. Compared with intravenous administration, a greater individual variation is observed after oral administration. This leads to a mean apparent systemic availability of 89.7%. This value is higher than the value found with corresponding AUC. However, the two values are not statistically different. This is probably due to the small number of subjects studied and the variations observed in urinary excretion after oral administration.

Dose-Dependency Analysis—From plasma results, dose-dependency has been checked by three methods. An example of the superposition method is shown in Fig. 6. Plasma concentrations divided by the administered dose as a function of time show reasonable superposition for the four doses tested. According to Dost's law (4), if pharmacokinetic parameters are independent of the dose, the area under the plasma concentration curve must be linearly related to the injected dose. The mean correlation coefficient was 0.9999, 0.9997, and 0.9928 for each subject. Despite the good correlation observed, there is little confidence in the results of this analysis because of the small amount of data used for the least-squares regression analysis. Accordingly, an ANOVA (5) was carried out for AUC, normalized to a 100-mg dose with the total amount of data available, obtained after either intravenous or oral administration. It failed to show a significant difference among the subjects ($^5_{16}F = 2.54$) and between administered doses ($^3_{16}B\ F = 0.74$), but, a significant difference at the 0.01 level was found between intravenous and oral administration. This must be related to the absolute availability of the oral form, which was calculated to be 72.5%.

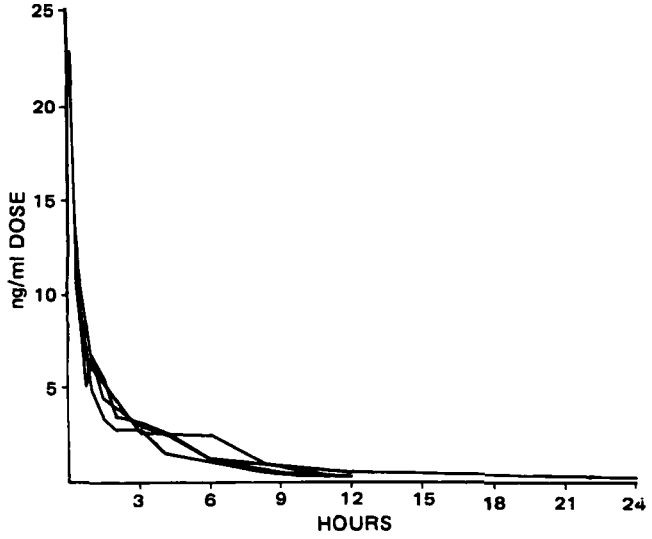

Figure 6—*Superposition of the plasma concentration curve divided by the injected dose in function of time, in subject C.*

Similarly, the same ANOVA analysis failed to show any difference between administered doses ($^3_{16}F = 0.61$) when considering urinary unchanged alizapride percentages. Nevertheless, it showed significant differences among individuals ($p < 0.05$) and between intravenous and oral administrations ($p < 0.01$).

From these three methods, it can be concluded that, in the range of administered doses, pharmacokinetic parameters of alizapride are not sensitive to dose variations.

Accordingly, variance analysis of the values of unchanged eliminated drug found for the different doses is unable to show any difference in drug elimination. The relationship between drug elimination and administered dose shows highly significant mean correlation coefficients in each subject.

The fluorescent HPLC method for biological sample analysis of alizapride has been shown to be sensitive enough to allow human pharmacokinetic studies of the drug. Preliminary results show a very rapid distribution phase with a mean half-life of 7.5 min followed by an elimination phase with a mean half-life of 2.5 hr. Urinary excretion represents the major phenomenon responsible for this elimination. In the range of tested doses, pharmacokinetic parameters of alizapride are linear.

REFERENCES

(1) P. Casanova, *Hépatol*, **16**, 381 (1980).

(2) J. M. Segrestaa, J. Gueris, D. Julien, and M. Tiar, *Thérapie*, **34**, 437 (1979).

(3) Ph. D'Athis, M. O. Richard, D. Delauture, E. Rey, M. Bouvier-D'Yvoire, E. Clement, and G. Olive, *ibid.*, **36**, 443 (1981).

(4) F. H. Dost, "Grundlagen der Pharmakokinetik," 2nd. ed., Stuttgart, Gearg Thieme Verlag, 1968, pp. 156–158.

(5) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, Ill, 1975.

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