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METHOD OF ANALYSIS OF THE NEW CARDIOTONIC AGENT, MDL 19,205, IN PLASMA AND URINE AND ITS APPLICATION IN A DOG PHARMACOKINETIC STUDY

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SUMMARY

A high-performance liquid chromatographic method has been developed for the analysis of plasma and urine concentrations of a new cardiotonic agent, MDL 19,205 (I). This procedure was utilized to study the pharmacokinetics of I in beagle dogs. The results of the dog study show that the compound is completely and rapidly absorbed. Plasma concentrations fell in a monoexponential manner with a half-life of about 1.3 h which was unaffected by dose in the range 3—30 mg/kg. Urinary excretion of unchanged I accounts for about one-half of the dose and is essentially complete in 24—48 h.

INTRODUCTION

MDL 19,205 (I) 4-ethyl-1,3-dihydro-5-(4-pyridinyl-carbonyl)-2H-imidazol-2-one, is a new non-catechol, non-glycoside cardiotonic agent which is currently undergoing clinical evaluation at Merrell Dow Pharmaceuticals for the treatment of congestive heart failure. The structure of the compound is shown in Fig. 1. Pharmacological studies in both anesthetized and conscious dogs have shown that it produces a dose-dependent increase in cardiac contractile force which was accompanied by relatively small increases in heart rate and minor decreases in blood pressure [1, 2]. These effects were not altered by

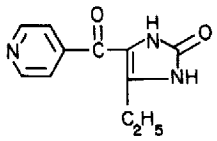

Fig. 1. Structure of MDL 19,205 (I).

α- or β-adrenergic receptor blockade, catecholamine depletion produced by reserpine or by bilateral carotid sinus denervation [2].

In order to facilitate the studies of bioavailability and pharmacokinetics in man of this compound, a sensitive and reliable analytical procedure was needed to measure plasma and urine concentrations of I. This communication describes a reversed-phase high-performance liquid chromatographic (HPLC) method developed for this purpose and the application of the assay to a pharmacokinetic study in the beagle dog.

EXPERIMENTAL

Materials

Methanol and acetonitrile were HPLC grade (J.T. Baker), ethyl acetate was glass-distilled (Burdick and Jackson Labs.) and the water was glass-distilled. I and the internal standard (MDL 82,261, 4-(3,4-dimethoxybenzoyl)-1,3-dihydro-5-methyl-2H-imidazol-2-one) were supplied by the Merrell Research Center. Standard solutions of I and the internal standard (I.S.) were prepared in methanol.

Sample preparation

Plasma samples (0.5 ml) were analyzed in duplicate by adding 1 μg of I.S., diluting to 2 ml with water and precipitating the proteins with 2 ml of acetonitrile. This mixture was then mixed, centrifuged and the supernatant decanted into an extraction tube containing 9 ml of water-saturated ethyl acetate. Ten ml of the organic layer was then transferred and evaporated to dryness under a stream of nitrogen. The residue was redissolved in 750 μl of methanol, diluted with 1.25 ml of distilled water and 400 μl injected into the liquid chromatograph.

Internal standard (10 μg) was added to 40-μl aliquots of urine samples and 50 μl of methanol. This solution was then diluted with 1.9 ml of mobile phase and 400 μl injected into the liquid chromatograph.

Chromatography was performed with a Waters Assoc. Model 6000A pump and Model 440 UV detector (340 nm wavelength) and a WISP 710B injector. The column was a DuPont Zorbax C-8 (25 cm × 4.6 mm I.D., 6 μm particle size) with a mobile phase of 37.5% methanol in distilled water (degassed) at a flow-rate of 1.5 ml/min. The retention time of I was ca. 5.4 min and that of I.S. ca. 7.4 min. Peak areas of I and I.S. were calculated using the CALS chromatographic data system (Computer Inquiry Systems). The peak area of I expressed as a percent of the I.S. peak area, was plotted against the I standard concentrations. The equation of the power-fitted linear regression line, determined from the standards, was then used to calculate the concentration of I in the unknown samples.

Animal experiment

Six male beagle dogs (three groups of two dogs) were dosed with 3 mg/kg intravenously, 3 mg/kg orally by gavage and 30 mg/kg orally by gavage in a three-way crossover. Blood samples were taken periodically, plasma obtained and frozen until assayed. Periodic urine samples were also obtained and frozen

until assayed. One animal which was difficult to handle was dropped from the study and not replaced.

Pharmacokinetics

Area under the plasma concentration curve (AUC) was calculated by trapezoidal rule. Half-life of elimination ($t_{1/2}$) was calculated as

$$t_{1/2} = \frac{0.693}{-\beta \times 2.303}$$

where β was the terminal slope of the $\log_{10}$ plasma concentration vs. time curve.

Total body clearance was calculated as the ratio of the administered dose (adjusted for bioavailability) to AUC. Renal clearance was determined as the ratio of the cumulative amount excreted to AUC. Apparent volume of distribution, V_d, was calculated as the ratio of total body clearance to the elimination rate [3].

RESULTS AND DISCUSSION

The recovery of I from plasma was determined by comparing the peak areas of extracted standards to the peak areas of pure standards injected. The mean recovery was 61.6% with a range of 57.9 to 70.4% over the concentration of 0.2 to 30 μg/ml.

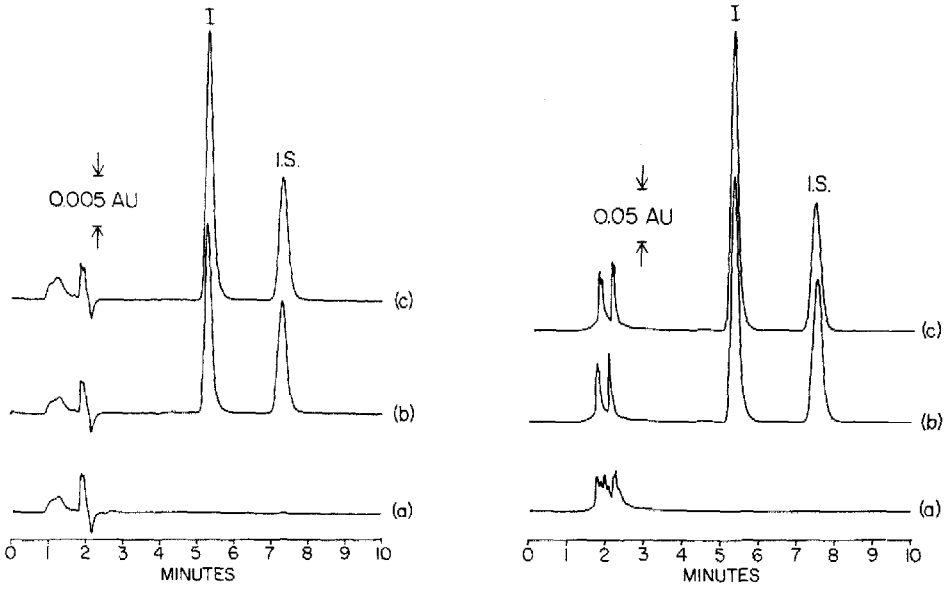

Fig. 2. Typical chromatograms in plasma: (a) extracted blank plasma; (b) extracted plasma from a dog dosed with I; (c) 5 μg/ml standards extracted from plasma.

Fig. 3. Typical chromatograms in urine: (a) blank urine; (b) urine from a dog dosed with I; (c) 250 μg/ml standards in urine.

TABLE I

SUMMARY OF WITHIN DAY ACCURACY AND PRECISION DATA WITH I (n = 6) IN PLASMA

Unknown concentration* (μg/ml)	Concentration found (μg/ml)	Standard deviation	Standard error	Coefficient of variation (%)	Percent of expected
0.25	0.255	0.028	0.011	10.71	102.0
1.00	1.073	0.094	0.038	8.72	107.3
5.00	4.573	0.274	0.112	5.98	91.5
8.75	9.100	0.494	0.202	5.43	104.0
15.00**	15.607	1.092	0.488	7.00	104.0
23.75	23.814	0.638	0.260	2.68	100.3

*Zero values were always zero.
**n = 5.

TABLE II

SUMMARY OF WITHIN-DAY ACCURACY AND PRECISION DATA WITH I (n = 6) IN URINE

Unknown concentration* (μg/ml)	Concentration found (μg/ml)	Standard deviation	Standard error	Coefficient of variation (%)	Percent of expected
9.43	10.459	0.638	0.261	6.10	110.9
33.00	37.257	0.702	0.287	1.88	112.9
94.28	102.431	4.239	1.731	4.14	108.6
330.00	346.300	11.655	4.758	3.37	104.9
1178.00	1112.942	45.031	18.384	4.05	94.5
3300.00	3128.501	199.195	81.321	6.37	94.8

*Zero values were always zero.

TABLE III

SUMMARY OF ACCURACY AND PRECISION DATA WITH I IN PLASMA OVER SIX DAYS

Unknown concentration* (μg/ml)	n	Concentration found (μg/ml)	Standard deviation	Standard error	Coefficient of variation (%)	Percent of expected
0.10	12	0.114	0.020	0.006	17.14	114.0
0.25	15	0.270	0.050	0.013	18.48	108.0
0.75	12	0.739	0.070	0.020	9.48	98.5
1.00	16	1.017	0.092	0.023	9.07	101.7
3.00	12	3.091	0.236	0.068	7.64	103.0
5.00	16	4.692	0.279	0.070	5.94	93.8
6.25	12	6.397	0.464	0.134	7.25	102.4
8.75	16	9.108	0.488	0.122	5.36	104.1
11.25	12	11.589	0.859	0.248	7.41	103.0
15.00	16	15.320	0.981	0.245	6.40	102.1
18.75	12	19.514	1.455	0.420	7.46	104.1
23.75	16	24.067	0.823	0.206	3.42	101.3

*Zero values were always zero.

TABLE IV

SUMMARY OF ACCURACY AND PRECISION DATA WITH I IN URINE OVER SIX DAYS

Unknown concentration* (μg/ml)	n	Concentration found (μg/ml)	Standard deviation	Standard error	Coefficient of variation (%)	Percent of expected
9.43	16	11.174	0.818	0.205	7.32	118.5
16.50	12	19.169	1.115	0.322	5.82	116.2
33.00	16	37.896	1.269	0.317	3.35	114.8
66.00	12	73.337	2.593	0.749	3.58	111.1
94.28	16	99.113	4.190	1.048	4.23	105.1
165.00	12	171.066	6.576	1.898	3.84	103.7
330.00	15	337.670	12.695	3.278	3.76	102.3
660.00	12	633.839	24.467	7.063	3.86	96.0
1178.00	16	1089.758	40.741	10.185	3.74	92.5
2640.00	12	2458.589	113.567	32.784	4.62	93.1
3300.00	16	3167.027	168.651	42.163	5.33	96.0

*Zero values were always zero.

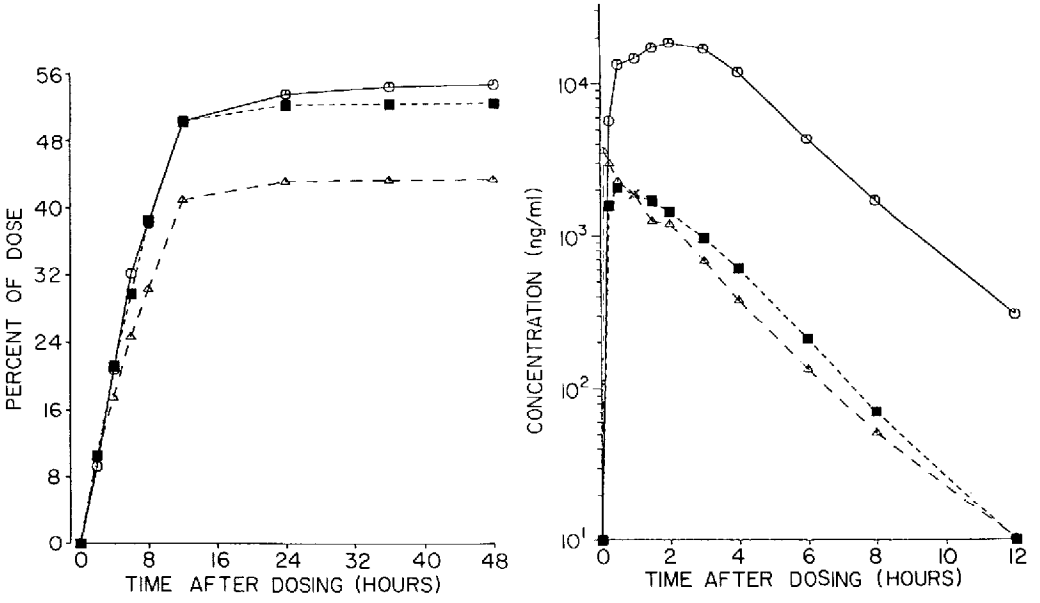

Fig. 4. Plasma concentrations in male beagle dogs following an acute dose of I: (△) 3 mg/kg intravenous; (■) 3 mg/kg oral solution; (⊙) 30 mg/kg oral solution. Points are mean of five determinations.

Fig. 5. Cumulative excretion of I in the urine of male beagle dogs following an acute dose of I: (△) 3 mg/kg intravenous; (■) 3 mg/kg oral solution; (⊙) 30 mg/kg oral solution.

Typical chromatograms obtained from plasma and urine analyses are shown in Figs. 2 and 3, respectively. As the assay is presently configured, the limit on quantitation of I in plasma is ca. 40 ng/ml; in urine the limit appears to be ca. 1—5 μg/ml.

TABLE V

PHARMACOKINETIC SUMMARY OF I DATA IN THE BEAGLE DOG

Administration	Dog*	AUC (μg h/ml)	Peak concn. (μg/ml plasma)	Time to peak concn. (h)	$t_{1/2}$ (h)	F** Bioavailability (%)	Cumulative percent recovery in urine	Clearance (ml/min/kg)			V_d (l/kg)
								Total	Renal	Extrarenal	
3 mg/kg intravenously	81-106	8.80	—	—	1.46	—	40.7	5.68	2.31	3.37	0.72
	81-107	4.98	—	—	0.83	—	54.0	10.03	5.42	4.62	0.72
	81-108	5.56	—	—	1.21	—	24.0	8.99	2.15	6.83	0.94
	81-109***	—	—	—	—	—	—	—	—	—	—
	81-111	6.06	—	—	1.40	—	47.0	8.24	3.88	4.37	1.00
	Mean	6.35	—	—	1.23	—	41.4	8.24	3.44	4.80	0.85
	S.D.	1.69	—	—	0.28	—	12.8	1.85	1.53	1.46	0.15
3 mg/kg orally	81-106	8.08	2.52	0.25	1.47	99.3	44.1	6.14	2.37	3.41	0.78
	81-107	5.67	2.68	0.5	1.25	109.3	57.4	9.65	5.07	4.58	1.05
	81-108	5.86	1.60	1.5	1.20	94.3	44.9	8.04	3.83	4.21	0.84
	81-109	5.40	2.81	1.0	1.05	***	57.8	10.83	5.35	5.47	0.98
	81-111	7.77	2.56	0.5	1.58	130.0	56.0	8.37	3.60	4.77	1.14
	Mean	6.56	2.43	0.8	1.31	108.2	52.0	8.61	4.12	4.49	0.96
	S.D.	1.27	0.48	0.5	0.21	15.8	6.9	1.77	1.08	0.76	0.15
30 mg/kg orally	81-106	98.26	16.69	3.0	1.76	116.0	23.6	5.90	1.20	4.70	0.90
	81-107	89.79	29.92	1.0	1.23	179.9	64.7	10.02	3.60	6.42	1.07
	81-108	85.13	19.34	3.0	1.40	136.9	63.9	8.04	3.75	4.29	0.97
	81-109	71.52	17.82	2.0	1.12	***	58.8	10.68	4.11	6.57	1.04
	81-111	85.52	19.90	2.0	1.53	142.3	61.1	8.31	3.57	4.75	1.10
	Mean	86.04	20.73	2.2	1.41	143.8	54.4	8.59	3.25	5.35	1.02
	S.D.	9.69	5.29	0.8	0.25	26.6	17.4	1.87	1.16	1.07	0.08

*Individual animal number.
**Based on plasma AUC.
***Pharmacokinetics for intravenous data not computed for this dog due to unusual plasma level profile.

The composite power-fitted least-squares regression equation for standards in plasma was $Y^{1.0121} = 32.8259\ X—0.0008$, with a correlation coefficient (r) of 0.9979 over the range of the assay. In urine, the line was $Y^{0.9523} = 0.2608\ X—0.0001$, with $r = 0.9986$.

The within-day accuracy and precision data are shown in Tables I and II for plasma and urine, respectively. The corresponding day-to-day data are shown in Tables III and IV. These data indicate that the assay as described is sufficiently accurate and precise for use in pharmacokinetic studies.

This method was then utilized in a dog pharmacokinetic study. The mean plasma concentrations of I in male beagle dogs following single doses of either 3 mg/kg intravenously, 3 mg/kg orally or 30 mg/kg orally are shown in Fig. 4 and the cumulative urinary excretion data are shown in Fig. 5. The summary of the pharmacokinetic variables is found in Table V.

These data indicate that I undergoes complete and rapid absorption in the dog. Plasma levels decline in a monoexponential manner with a half-life of about 1.3 h which was unaffected by dose. The apparent volume of distribution of about 1 l/kg would suggest extensive distribution approaching that of body water.

The compound is rapidly excreted and approximately one-half of the dose is recovered in the urine as unchanged I within 48 h (the majority of which is recovered in the first 24 h).

In conclusion, an HPLC method of analysis of plasma and urine concentrations of MDL 19,205 has been developed and applied in a pharmacokinetic study in beagle dogs. This method will be utilized in a human pharmacokinetic study which will be published shortly.

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