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Biological Disposition of Sodium Dichloroacetate in Animals and Humans after Intravenous Administration

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Abstract □ Sodium dichloroacetate, a potential antidote for lactic acidosis, was administered intravenously to rats, dogs, and four humans. In three rats, maximum plasma sodium dichloroacetate concentrations were 120–164 μg/ml after a 100-mg/kg dose and declined with half-lives of 2.1–4.4 hr. In two dogs, maximal concentrations of 447 and 508 μg/ml were attained after a 100-mg/kg dose. The subsequent decline was relatively slow with approximate half-lives of 17.1 and 24.6 hr. An intravenous infusion of 10 mg/kg was administered over 20 min to two human subjects. Two other subjects received 20 mg/kg. After the infusion, maximum plasma concentrations of 19.9 and 24.7 μg/ml were seen with the lower dose and 57.3 and 74.9 μg/ml were achieved with the higher dose. Thereafter, concentrations declined rapidly with half-lives of 20–36 min. The observed large interspecies differences in half-lives could be explained in terms of differences in the apparent volume of distribution and/or clearance.

Keyphrases □ Pharmacokinetics—sodium dichloroacetate, plasma concentration level-time curve, rats, dogs, humans □ Sodium dichloroacetate—pharmacokinetics, plasma concentration levels, rats, dogs, humans □ Clearance, intrinsic—plasma sodium dichloroacetate concentration, pharmacokinetics, rats, dogs, humans

Sodium dichloroacetate (I) has been reported to reduce normal or elevated blood lactate and pyruvate levels in animals by activating the pyruvate dehydrogenase enzyme complex (1–3). Oral administration of I to patients having nonketotic diabetes mellitus also led to an appreciable lowering of plasma lactate concentrations (4). The compound has been of possible interest as an intravenous antidote in patients suffering from lactic acidosis. This study was undertaken to provide information on the biological disposition of I in animals and humans since no data are available.

EXPERIMENTAL

Materials—Labeled[1] and unlabeled[2] sodium dichloroacetate were used. The material labeled in both carbon atom positions had a specific activity of 23.4 μCi/mg. Radiochemical purity, determined by TLC on silica gel plates in ethanol–chloroform–concentrated ammonium hydroxide–water (53:30:15:1.5), was >98%.

Animal Experiments—Each of three male Sprague–Dawley rats, 160–170 g, was given an intravenous injection of ^{14}C-I, diluted 1:20 with nonlabeled I, in a tail vein. The compound (100 mg/kg) was administered as a 10% aqueous solution. At specified times, animals were subjected to light ether anesthesia, and a 0.2–0.3-ml blood sample was collected in a heparinized centrifuge tube through a glass capillary by puncture of the suborbital sinus. Animals then were permitted to recover until the next sampling time.

Each of two male beagles, 9.0 and 10.5 kg, was given 100 mg of I/kg as a 20% aqueous solution intravenously in the cephalic vein. Animals were placed in individual metabolism cages. At specified times, blood was collected from a jugular vein without anesthesia.

Human Study—Four normal subjects, having given informed consent, participated after an overnight fast. Subjects 1 and 2, ages 42 and 38 years, respectively, each weighed ~70 kg. Subjects 3 and 4, ages 52 and 26 years, respectively, weighed 80 and 83 kg. In each case, the history, physical examination, ECG, and all laboratory tests were within normal limits.

Following the collection of the control urine sample and the drawing of a control blood specimen from Subjects 1 and 2, a 10-mg/kg dose of I dissolved in 100 ml of saline was infused over 20 min. Blood specimens were obtained from the contralateral antecubital vein at the time the infusion was completed. Specimens were drawn subsequently at 1 (40 min after termination of the infusion), 2, 3, 4, 6, 8, 10, 12, 24, 32, and 48 hr. All urine was collected for 96 hr with collection periods ending at 1, 2, 4, 8, 12, 24, 48, 72, and 96 hr.

Several weeks later, the procedure was repeated on Subjects 3 and 4, the sole change being that each received 20 mg/kg. All subjects were given a standard breakfast after the 4th hr. They remained under clinical observation for 8 hr.

Blood specimens were centrifuged as soon as they were collected. Plasma was separated and stored frozen until analysis. The urinary volume was measured and recorded. Aliquots of 20 ml from each urine sample were labeled and frozen for analysis.

Analysis of Unchanged I—Plasma and urine I concentrations were analyzed by a reported GLC method (5) as modified (6). Briefly, the method involved addition of trichloroacetic acid as an internal standard and 14% boron trifluoride in methanol as the derivatizing reagent. The resulting methyl esters of I and the internal standard were extracted with benzene and analyzed on a Chromosorb 101 column using an electron-capture detector.

Preliminary analyses of plasma samples obtained from human subjects indicated that many specimens contained very low I concentrations. Therefore, method sensitivity was improved from 300 ng/ml as reported previously (6) to 40–50 ng/ml by three minor modifications: (*a*) the volume of solvent used in the extraction was reduced from 2 to 0.5 ml; (*b*) between the extraction and centrifugation steps, the entire contents of the vial were transferred to a 5-ml centrifuge tube to facilitate aliquot removal; and (*c*) the volume of extract analyzed was changed from 2 to 3 μl. The linearity of standard curves obtained during analysis demonstrated that these changes had no effect on the accuracy and precision of the results.

Analysis of Total Radioactivity—A 10-μl aliquot of plasma or urine was dissolved in 15 ml of scintillation cocktail and counted[3]. External standardization was used to correct for quenching. The cocktail had the following composition: naphthalene, 400 g; 2,5-diphenyloxazole, 20 g; 2,2′-*p*-phenylenebis(4-methyl-5-phenyloxazole), 1.2 g; dioxane, 2920 ml; toluene, 540 ml; and methanol, 140 ml.

[1] New England Nuclear Corp., Boston, Mass.
[2] Synthesized in these laboratories.
[3] Intertechnique model SL-40 liquid scintillation spectrometer.

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Table I—Daily Standard Curves (Peak Height Ratios) Generated during Analyses in Human Plasma

Day	Sodium Dichloroacetate				
	0.05 μg/ml	0.2 μg/ml	1 μg/ml	5 μg/ml	10 μg/ml
1	0.267	0.967	3.807	16.45	20.17
	0.282	1.027	3.809	16.81	22.07
2	0.376	1.178	3.684	15.57	22.42
	0.197	—	—	17.30	23.82
3	0.261	0.976	3.613	17.90	21.71
	0.303	1.023	3.473	17.17	22.08
4	0.226	1.187	3.636	18.58	25.30
	0.277	1.184	4.135	20.58	22.95
5	0.265	0.937	2.833	12.21	17.12
	0.255	—	3.622	13.96	20.36
Mean	0.271	1.060	3.623	16.65	21.80
CV[a]	17.45	10.00	9.66	14.10	10.24

[a] Overall coefficient of variation is 12.66%.

Table II—Plasma Sodium Dichloroacetate Levels (Micrograms per Milliliter) in Rats after a 100-mg/kg iv Dose [a]

Time	Rat A		Rat B		Rat C	
	^{14}C	GLC	^{14}C	GLC	^{14}C	GLC
10 min	195.0	150.0	126.5	85.7	209.0	163.5
20 min	178.0	122.0	165.9	120.0	191.8	149.0
30 min	171.8	117.5	160.8	100.5	181.0	131.8
1 hr	161.8	83.7	157.4	74.3	175.3	118.8
2 hr	153.6	50.0	153.9	47.5	171.4	87.5
3 hr	134.7	33.3	125.9	33.3	146.0	67.2
4 hr	116.9	22.0	120.2	19	142.7	52.5
6 hr	115.3	20.3	115.5	12	127.8	43.2
24 hr	28.5	NS[b]	51.2	ND[c]	48.8	3.8
28 hr	46.8	ND	44.6	ND	45.3	1
48 hr	26.7	ND	25.3	ND	23.7	0
52 hr	31.0	ND	26.1	ND	27.1	0.05
72 hr	17.4	ND	NS	NS	16.7	0
96 hr	16.6	ND	NS	NS	16.5	1

[a] Results are given in terms of both total radioactivity and GLC analysis. [b] NS = no sample. [c] ND = not detectable.

Distribution of I between Plasma and Erythrocytes—To 2 ml of heparinized fresh human blood obtained from three healthy volunteers was added 0.2 ml of an aqueous solution of ^{14}C-I to provide concentrations of 0.5, 5, and 50 μg/ml. The mixtures were incubated for 1 hr at 37° in a shaking water bath and cooled. Triplicate 0.1-ml aliquots were combusted in a sample oxidizer[4]. The resulting $^{14}CO_2$ was trapped and counted for the estimation of radioactivity in whole blood. The remaining blood samples were centrifuged, and radioactivity in triplicate 0.1-ml plasma aliquots was analyzed similarly by combustion and counting. In a separate experiment, the stability of I in whole blood for 1 hr at 37° was ascertained.

Stability of I in Frozen Plasma—To 1-ml aliquots of human plasma obtained from healthy volunteers was added 1 μg of I in 50 μl of water. The aliquots were sealed, stored at −20°, and analyzed in triplicate after periods ranging from 1 week to 6 months.

Pharmacokinetic Calculations—Pharmacokinetic parameters were calculated using standard procedures and assuming a one-compartment open model with constant-rate intravenous infusion (7). Half-lives were obtained with the least-squares method after logarithmic transformation of concentrations. Plasma clearance (8) was calculated as the ratio of the dose and the area under the concentration–time curve. The apparent volume of distribution was obtained as the ratio of clearance and the elimination rate constant, the latter value having been derived from the corresponding half-life estimate.

The importance of using blood clearance rather than plasma clearance in the calculation of intrinsic clearance was demonstrated previously (8, 9). Blood clearance was obtained from plasma clearance using a constant correction factor of 0.688. The value was obtained assuming a mean hematocrit of 0.45 and a mean distribution ratio of 4:1 between plasma and the cellular fraction of blood for I (see *Results*):

$$F = \frac{B(1 - H)}{P} \qquad \text{(Eq. 1)}$$

[4] Packard model 306.

where F is the correction factor, H is the hematocrit, and B and P represent concentrations in equal-volume aliquots of whole blood and plasma, respectively.

Intrinsic clearance was obtained from the blood clearance as described (9) by:

$$Cl_{in} = \frac{QCl_{bl}}{Q - Cl_{bl}} \qquad \text{(Eq. 2)}$$

where Cl_{in} and Cl_{bl} refer to the intrinsic and blood clearance, respectively, and Q indicates the hepatic blood flow rate. An approximate mean value of 1500 ml/min was used for the calculations in conformity with literature reports (9).

RESULTS

Reproducibility of GLC Method—Standard curves generated daily in conjunction with the analyses of human plasma samples are summarized in Table I. Each of the five curves represents five concentrations of I, usually in duplicate determinations, ranging from 0.05 to 10 μg/ml. Daily variations in the individual standard curves were acceptable, with the relative standard deviations ranging from 9.66 to 17.45% and giving an overall value of 12.66% over 5 days.

Animal Studies—Plasma levels in three rats after 100 mg/kg iv are summarized in Table II. Maximal concentrations of unchanged I ranged between 120 and 164 μg/ml. Subsequent declines of plasma concentrations occurred with half-lives of 2.1–4.4 hr. Total radioactive materials attained maximal concentrations of 166–209 μg/ml (expressed as unchanged I). Thus, at the peak concentration, unchanged I in plasma represented the bulk of total radioactivity. However, the subsequent decline of the total radioactivity concentrations was considerably less rapid, and apparent half-lives of 21–36 hr could be estimated, indicating extensive metabolism and slow elimination of metabolites.

Plasma levels of unchanged I in two dogs after 100 mg/kg iv are summarized in Table III. Maximum concentrations of 447 and 508 μg/ml were measured in the first plasma samples at 5 min. The subsequent decline was slow. Sampling in one animal was continued for 48 hr. In the other animal, sampling had to be discontinued after 24 hr because of collapsing veins and the formation of hematomas. Nevertheless, approximate half-lives of 17.1 and 24.6 hr could be estimated in the two animals.

Human Studies—No subjective or objective changes or signs of clinical activity were noted in any subject upon intravenous infusion of I.

Plasma concentrations of unchanged I are summarized in Table IV.

Table III—Plasma Sodium Dichloroacetate Levels (Micrograms per Milliliter) in Dogs following a 100-mg/kg iv Dose

Time	Dog 1	Dog 2
5 min	447	508
15 min	418	502
30 min	412	439
45 min	412	410
1 hr	390	418
1.5 hr	298	206
2 hr	300	384
3 hr	290	314
4 hr	266	341
5 hr	290	—
6 hr	278	—
24 hr	155	192
28 hr	163	—
48 hr	50	—

Table IV—Plasma Sodium Dichloroacetate Concentrations (Micrograms per Milliliter) in Humans after an Intravenous Infusion

Hours[a]	10-mg/kg Dose		20-mg/kg Dose	
	Subject 1	Subject 2	Subject 3	Subject 4
0.33[b]	24.7	19.9	57.3	74.9
1	3.27	4.44	29.3	46.8
2	0.304	0.585	5.96	11.2
3	0.086	0.117	0.623	4.68
4	<0.04	<0.04	0.140	0.608
6	<0.04	<0.04	<0.04	0.175
8	<0.04	--[c]	<0.04	<0.04

[a] All samples collected at 10, 12, 24, 32, and 48 hr after dosing contained <0.04 μg of I/ml. [b] End point of 20-min infusion. [c] Sample lost during analysis.

Table V—Pharmacokinetic Parameters of Sodium Dichloroacetate in Humans

Parameter[a]	Subject 1	2	3	4
Dose, mg	700	700	1600	1650
Weight, kg	69.5	69.1	80.0	83.3
$t_{1/2}$, min	19.6	21.6	24.3	36.6
$AUC_{0-\infty}$, (hr μg)/ml	15.46	14.35	59.85	93.61
V_d, liters	21.4	25.3	15.6	15.5
Cl_{plasma}, ml/min	755	813	446	294
Cl_{bl}, ml/min	1100	1180	648	427
Cl_{in}, ml/min	4125	5530	1140	600

[a] AUC = area under the plasma level-time curve, V_d = apparent volume of distribution, and Cl = clearance.

Table VI—Average Values (and Ranges) of Pharmacokinetic Parameters of Sodium Dichloroacetate in Humans, Rats, and Dogs [a]

Parameter	Humans 10 mg/kg	Humans 20 mg/kg	Rats, 100 mg/kg	Dogs, 100 mg/kg
$t_{1/2}$, hr	0.34 (0.33–0.36)	0.51 (0.41–0.61)	2.97 (2.1–4.4)	20.8 (17.1–24.6)
V_d, ml/kg	337 (308–366)	190 (186–195)	932 (701–1080)	256 (249–262)
Cl_{plasma}, ml/(min kg)	11.31 (10.86–11.76)	4.55 (3.53–5.58)	4.22 (1.84–5.94)	0.146 (0.123–0.168)
Cl_{in}, ml/(min kg)	69.7 (59.3–80.0)	10.7 (7.2–14.2)	6.9 (2.8–9.9)	0.21 (0.18–0.24)

[a] Values of 20, 67, and 40 ml/(min kg) were used in humans (9), rats (12), and dogs (13), respectively, for hepatic blood flow.

The highest concentrations were obtained immediately after the end of the infusion. Subjects 1 and 2, receiving 10 mg/kg, had maximum values of 19.9 and 24.7 μg/ml, respectively. Maximum concentrations of 57.3 and 74.9 μg/ml were seen in Subjects 3 and 4 after 20 mg/kg.

Concentrations of I declined rapidly following monoexponential kinetics. The values decreased to <0.04 μg/ml, the sensitivity of the method, at 4 hr after the lower dose and at 6 or 8 hr after the higher dose.

Urinary excretion of unchanged I became negligible after the first 8 hr; in all subjects, cumulative excretion amounted to considerably less than 1% of the dose.

The pharmacokinetic parameters are summarized in Table V. Half-lives in the four subjects ranged from 20 to 36 min. Values for the apparent volume of distribution were 15.5–25.3 liters. Plasma clearance ranged from 294 to 813 ml/min, and the corresponding values for blood clearance were 427–1180 ml/min. Hematocrit in the four subjects had a mean value of 0.45 (range of 0.43–0.49).

The following values were obtained for intrinsic clearance: Subjects 1 and 2, 4125 and 5530 ml/min; and Subjects 3 and 4, 1140 and 600 ml/min. Expressed on the basis of unit body weight, these values were 59.3, 80.0, 14.2, and 7.2 ml/(min kg), respectively.

The stability of I stored in frozen human plasma for 6 months was demonstrated in separate experiments.

In an *in vitro* study using fresh blood from three subjects, an average of 80% (range of 72–94%) of I present in whole blood was in the plasma. The distribution between plasma and the cellular fraction was independent of the concentration in the 0.5–50-μg/ml range.

DISCUSSION

After an intravenous infusion, I in human plasma declined monoexponentially over a 200–500-fold concentration range, corresponding to a range of 7–9 half-lives. These findings permitted a relatively simple pharmacokinetic analysis. Examination of the individual pharmacokinetic parameters (Table V) suggests some dose dependence because the apparent volume of distribution and the plasma clearance decreased with a doubling of the dose. Although this observation is necessarily limited by the size of the study, it suggests capacity-limited elimination.

Intrinsic clearance refers to the maximum capacity of an organ to remove the drug irreversibly by all pathways when blood flow to the organ is not rate limiting (10). The metabolism of I has not been explored in detail, but Demaugre *et al.* (11) demonstrated its hepatic biotransformation to oxalate. The present results show that the intrinsic clearance of I was considerably greater than, and the elimination presumably limited by, blood flow in subjects receiving 10 mg/kg. In subjects receiving 20 mg/kg, intrinsic clearance was considerably lower, lending support to the hypothesis of capacity-limited metabolism. Corresponding intrinsic values expressed on the basis of unit body weight indicated even more clearly the dose-dependent pharmacokinetics of I. A doubling of the dose led to an approximately sevenfold decrease in intrinsic clearance, yet the systemic (plasma or blood) clearance values decreased only by a factor of two or three. Thus, when the elimination rate is limited by hepatic blood flow, systemic clearance appears to reflect only partially any large changes occurring in intrinsic clearance.

Pharmacokinetic parameters also were estimated for rats and dogs. Average values and ranges of the animal and human data are summarized in Table VI for comparison. The large difference in the half-lives of I between humans and dogs may reflect the difference in clearance. In contrast, the observed difference in half-lives between humans and rats is consistent with the relatively high value of the apparent volume of distribution in the latter species.

In the present study, the I elimination rate in humans was dose dependent and limited by hepatic blood flow. The role of blood flow appeared to diminish with increasing dose. Elimination of high doses was not flow limited in rats and, particularly, in dogs since hepatic blood flow substantially exceeded intrinsic clearance[5]. The large differences seen show the difficulty in predicting the biological disposition and toxicity of therapeutic doses of a drug in humans from animals given large doses.

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[5] The relative contributions of dose dependence and species difference were not determined. These factors could be assessed by additional studies wherein each subject or animal would receive both larger and smaller doses of I.