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Pharmacokinetic Analysis of Dodecanedioic Acid in Humans From Bolus Data

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ABSTRACT. *Background:* Excretion and tissue uptake of dodecanedioic acid (C12), a proposed alternative fuel substrate, was investigated in humans by bolus experiments. *Methods:* Seven overnight-fasting healthy male volunteers received IV a bolus (1 g) of C12. Blood samples were collected after C12 administration at intervals of 15 minutes, and C12 serum concentration was measured by high-performance liquid chromatography. C12 excretion in 24-hour urine was measured. Binding of C12 in human serum was determined in separate equilibrium dialysis experiments by means of an isotopic compound (disodic salt of (1,12)^{14}C-dodecanedioic acid). A two-compartment model was used for describing C12 kinetics. *Results:* The excreted amount of C12 in 24-hour urine was found to be, on the average, 1.62% of administered dose. The apparent number of binding sites per albumin molecule was 3.1 ± 0.2 (estimate ± SE) with an affinity constant of 6.4 ± 1.8 mM^{-1}. The distribution volume of central compartment was 5.56 ± 3.13 L and that of peripheral compartment was 87.4 ± 30.4 L. The rate constant of exchange between compartments was 4.60 ± 3.50 L/min, that of urinary excretion 25.6 ± 15.5 mL/min, and that of tissue uptake 2.17 ± 0.86 L/min. *Conclusions:* These results are promising for C12 utilization in parenteral nutrition, because C12 elimination in urine is low whereas tissue uptake appears to be rather efficient. (*Journal of Parenteral and Enteral Nutrition* **19**:498–501, 1995)

Medium-chain dicarboxylic acids (MCDA) are characterized by the presence of two carboxylic groups at the ends of the molecule, which permits to these compounds to be water soluble, contrary to what occurs in the case of the corresponding monocarboxylic acids. Thus, the salts of MCDA can be administered without previous formation of emulsions, as it is necessary for both medium- and long-chain triglycerides (MCT or LCT), and they do not require any hydrolysis step prior to β-oxidation.[1,2]

In vivo and *in vitro* studies on the chain-length dependency of the β-oxidation of dicarboxylic acids showed an optimum for the dodecanedioic acid (C12-dicarboxylic acid), with decreasing β-oxidability of both shorter and longer dicarboxylic acids.[3] Also the amount of dicarboxylic acid lost with the urine is dependent on chain length: in fact, large amounts of azelaic acid (C9) were found in the urine after IV administration in healthy volunteers (45% to 75% of the given dose depending on the infusion rate[1,4,5]) while this amount decreased with the increase of the number of carbon atoms (16% to 42% excreted in the case of sebacic acid [C10][6–8]).

The pharmacokinetic analysis of plasma profile and of urine excretion suggested that azelaic acid is actively secreted in the kidney,[5] whereas sebacic acid is actively reabsorbed.[9] Preliminary studies on dodecanedioic acid kinetics in rats showed that C12 is eliminated with the urine in a very limited extent, ~3% of the administered dose.[10,11] This might represent a great advantage in the MCDA utilization as fuel substrate in humans during parenteral nutrition.

To explore the capability of humans to uptake and excrete dodecanedioic acid, we investigated the pharmacokinetics of C12 in humans after IV rapid bolus injection. The kinetics of C12 was analyzed by a two-compartment pharmacokinetic model with linear urinary excretion and tissue uptake, taking into account the binding of C12 to serum albumin. To this purpose, the binding parameters of C12 in human serum were estimated by means of independent equilibrium dialysis measurements. The present study indicates a better tissue utilization of dodecanedioic acid with respect to azelaic and sebacic acid.

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MATERIALS AND METHODS

Chemicals

Dodecanedioic acid and azelaic acid were from Sigma Chemical Co. (St Louis, MO). Dodecanedioic acid was purified by Real S.r.l. (Como,

Italy) and was free from pyrogens and contaminants with a degree of purification, ascertained by gas-liquid chromatography and mass spectrometry, of 99.8%. All other chemicals were purity available quality or of the highest purity available. A 0.4 mol/L solution of C12 salified with NaOH was used for the IV bolus. The solutions were sterilized by 0.25-μm Millipore filters (Molsheim, France) before administration.

(1,12)^{14}C-dodecanedioic acid (specific activity 117 mCi/mmol), purchased from Amersham (Amersham, England), was used for equilibrium dialysis experiments.

Equilibrium Dialysis

The binding of dodecanedioic acid in normal human serum was studied as previously described for binding of sebacic acid to defatted serum albumin and in serum.[12] Nine samples of human serum were obtained from healthy volunteers. The albumin concentration was determined in each sample used and was found to range from 0.57 to 0.71 mmol/L. For each sample, the binding was assessed at five different concentrations of C12. Concentration of total C12 added to samples ranged from 5.10^{-4} to $2.5.10^{-2}$ mol/L. Binding was measured in Sörensen's phosphate buffer at pH 7.4, using the above described method. Amounts of ~20,000 dpm of (1,12)^{14}C-dodecanedioic acid were added to the unlabeled C12.

Experimental Procedure

One gram of C12 was administered as an IV bolus in seven overnight-fasting healthy male volunteers aged 43.5 ± 9.5 years (mean ± SD) and with an average body mass index of 22.2 ± 2.7 (kg/m^2). Heparinized blood samples (8 mL) were taken at 5, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 minutes after the bolus and immediately centrifuged. Plasma samples were frozen at -20°C until analysis. Each subject voided before bolus administration and the 24-hour urine was collected in a container with 0.1% sodium azide to prevent bacterial growth.

The protocol was in conformity to the directives given by the Ethical Committee of the Institutional Health Review Board of the Catholic University, School of Medicine, Rome, Italy. Informed consent was obtained in all cases.

Dicarboxylic Acid Analysis

Serum samples. Azelaic acid (100 μg) was added to 1 mL of each serum sample as an internal standard. Proteins were precipitated with 0.1 mL of 4 N HCl and dicarboxylic acids extracted twice with 8 volumes of ethylacetate maintaining the solution at 60°C for 15 minutes. The combined extracts were dried in a GyroVap apparatus (Howe, mod. GV1, Gio. De Vita, Rome, Italy) operating at 60°C, coupled with a vacuum pump and a gas trap FTS system (Stone Ridge, New York).

Urine samples. Samples (0.5 mL) from 24-hour urine were added with 50 μg azelaic acid as internal standard and then were treated with cation-exchange resin (Dowex 50 W-X4, 100 to 200 μm mesh, H^+) to remove salts, concentrated under reduced pressure, and filtered through a Millipore HV (0.45 μm) filter. The samples were acidified to pH 1–2 with 4 N HCl, extracted twice with ethylacetate, and evaporated in the GyroVap as previously described.

High-performance liquid chromatography (HPLC) analysis. The HPLC of dicarboxylic acids was performed according to a previously described method.[7]

Binding Model

To describe the binding of C12 in serum, we assumed that all the binding sites on albumin and on other serum proteins have the same affinity and that the ratio between the number of total sites and the number of albumin sites remains constant in all subjects examined. Thus the binding data of C12 in serum, with the concentration of bound C12 in each sample normalized to the albumin concentration of the sample were analyzed by means of the following model:

$$\bar{\nu} = n \frac{Kc}{1 + Kc} \tag{1}$$

where $\bar{\nu}$ is the molar ratio of bound C12 to albumin; c is the concentration of free C12; n is the apparent number of binding sites per albumin molecule, and K is the association constant. The binding data were fitted by Eq 1 in the plane $\bar{\nu}$ *vs* c_u, c_u being the initial concentration of C12 in the nonalbumin side of the dialysis chamber. The free ligand concentration c was found for each c_u value by solving numerically the equation $c_u = 2c + A\bar{\nu}$, where was the albumin concentration and $\bar{\nu}$ is given by Eq 1. The binding parameters were estimated according to the method of maximum likelihood,[13] assuming the measured $\bar{\nu}$ normally distributed with unknown coefficient of variation, and neglecting the experimental uncertainty on c_u. The negative log-likelihood was minimized by a quasi-Newton algorithm. Standard errors of the estimates of unknown parameters were evaluated from the inverse Hessian matrix of the negative log-likelihood at the optimum.[13]

Pharmacokinetic Model

The C12 renal clearances of all subjects were computed determining the AUC of free C12 serum concentration, c_1, from the measured total concentration, c_{1t}, by means of the equation:

$$c_{1t} = c_1 + nA \frac{Kc_1}{1 + Kc_1} \tag{2}$$

where A was the serum albumin concentration of each subject and n and K were set to the values estimated from the equilibrium dialysis experiments.

The pharmacokinetics of dodecanedioic acid was described by means of the two-compartment model shown in Fig. 1. Urinary excretion rate was assumed to be proportional to free C12 serum concentration and the flow of free C12 between the compartments to be due to passive diffusion. Taking into account the binding of C12 to serum albumin, we obtained the following equation for the central compartment (see Bertuzzi et al[9]):

$$V_1 \frac{dc_1}{dt} = \frac{-\gamma_u c_1 - kc_1 + kc_2}{1 + nAK/(1 + Kc_1)^2} \tag{3}$$

where V_1 is the volume of the central compartment; γ_u the urinary excretion rate constant; k the rate constant of exchange between compartments; and c_2 the free C12 concentration in peripheral compartment. The binding of C12 with proteins in the peripheral compartment, assumed to be linear, will affect the value of the apparent distribution volume, V_2, of this compartment.[14] Taking the rate of tissue uptake proportional to c_2 with rate constant γ_t, we obtained for the second compartment the equation

$$V_2 \frac{dc_2}{dt} = kc_1 - kc_2 - \gamma_t c_2 \tag{4}$$

Initial conditions for Eqs 3 and 4 were as follows: $c_1(0)$ was the concentration of free C12 corresponding to a total concentration equal to the ratio dose/V_1, and $c_2(0) = 0$. The amount, Q, of C12 excreted in the 24-hour urine was expressed according to the following:

$$Q = \gamma_u \int_0^{24h} c_1(t)\,dt \tag{5}$$

The outputs of the model were the concentration of total C12 in serum, $c_{1t}(t)$, as given by Eq 2 and the excreted amount, Q. The identifiability of the unknown parameters V_1, V_2, k, γ_u, and γ_t was verified following the approach in Wajda et al.[15]

The unknown parameters of the pharmacokinetic model of Eqs 3 through 5 were estimated as population mean values, using the data obtained from all subjects and setting the value of A in Eqs 2 and 3 to the average albumin concentration of the subjects (0.61 mmol/L). The estimates were obtained by weighted least squares method, including in the index the excreted C12 amount. The weights, both for C12 serum concentrations and for the urinary amount, were the inverses of the sample estimates of the variances of individual data. The standard errors of the estimates were evaluated from the inverse Hessian matrix of the index at the optimum.

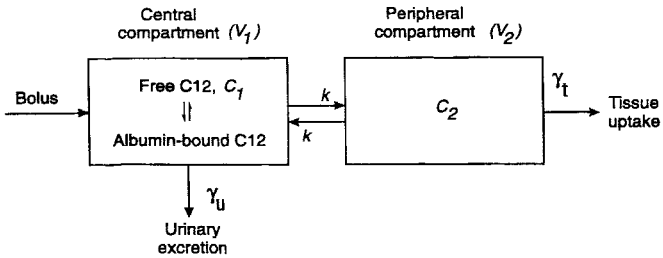

Fig. 1. Block diagram of the two-compartment model used for the pharmacokinetic analysis of dodecanedioic acid.

RESULTS

Figure 2 shows the binding data of C12 in normal human serum at 37°C, together with the best fitting curve of the experimental data according to Eq 1. The apparent number of binding sites per albumin molecule was 3.1 ± 0.2 with association constant 6.4 ± 1.8 mM^{-1}. Preliminary fitting of the data by a model with two classes of independent sites with different affinity, did not evidence a heterogeneity of the binding sites.

The experimental data of C12 total concentration in serum following the IV bolus are reported in Figure 3. The continuous line represents the optimal fitting curve given by the model of Eqs 2 through 4, with the values of C12 binding parameters set to the estimates given above. Table I reports the estimates (±SE) of the population mean of the pharmacokinetic parameters. Figure 4 shows the predicted profiles of free C12 concentration in the central and peripheral compartments.

The amount of C12 excreted in the 24-hour urine, as predicted by the model, was in good agreement with the average measured value of 16.2 mg (corresponding to 1.62% of the administered dose). The estimate of the rate constant was also in agreement with the measured renal clearance of the subjects examined (mean ± SE: 18.5 ± 7.0 mL/min).

DISCUSSION

The analysis by equilibrium dialysis of dodecanedioic acid binding in human serum showed the presence of a single class of binding sites with an equilibrium association constant of 6.4×10^3 M^{-1}. Taking into account the possible presence of C12 competitors for albumin binding in serum, our estimate of K agrees with the value of 1.3×10^4 M^{-1}, reported by Tonsgard et al[16] for C12 binding to defatted bovine serum albumin at pH 7.4 and 37°C. These authors revealed only one C12 binding site on bovine serum albumin. The discrepancy with the results of the present experiments ($n = 3.1$) might indicate a substantial binding of C12 to sites on other plasma proteins, which were considered as albumin binding sites in our analysis. Our findings show also that C12 binds to serum proteins with lower affinity than monocarboxylic acids of similar chain length, as lauric acid (12 carbon atoms) and myristic acid (14 carbon atoms).[17] This suggests that C12 may be largely free in serum, and thus more promptly available for tissue uptake.

For the analysis of bolus data we used a two-compartment model, as suggested by the profile of the measured curve of C12 serum concentration *vs* time. The renal clearance found on the subjects examined was markedly low, thus indicating the presence of tubular reabsorption, which could derive from passive back-diffusion or from an active carrier-mediated reabsorption with high maximal transport rate. The urinary excretion rate was assumed to be proportional to the concentration of free C12 in the

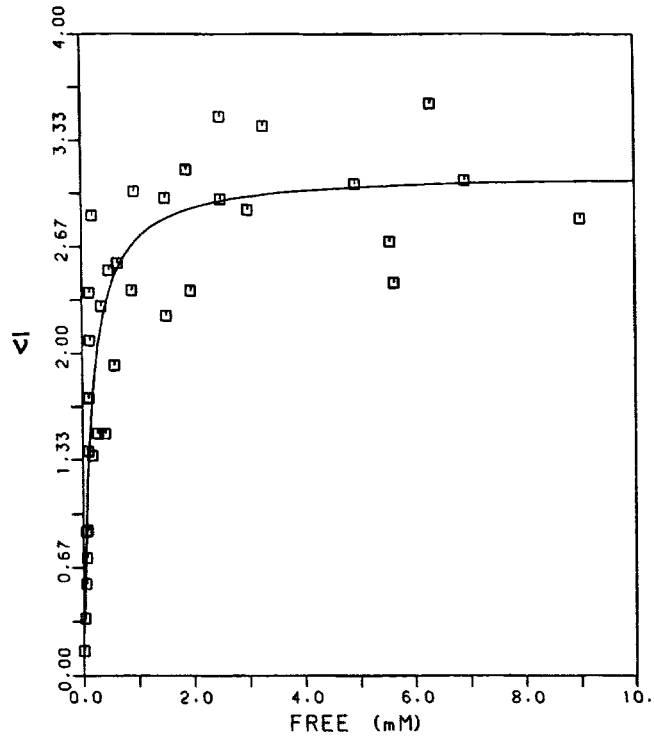

Fig. 2. Binding data of dodecanedioic acid in normal human serum at 37°C (□). The continuous line represents the optimal fitting curve given by the model of Eq 1.

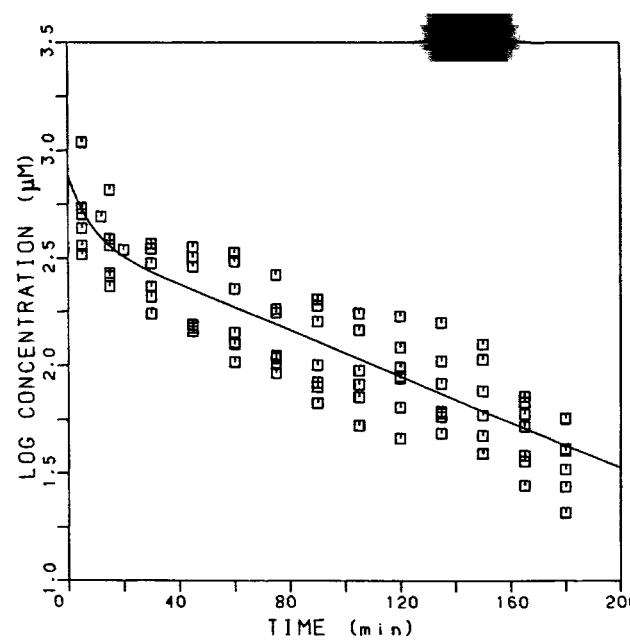

Fig. 3. Total plasma dodecanedioic acid concentration *vs* time after bolus dose of 1 g C12. Experimental data from 7 subjects (□); prediction by the model of Eqs 2 through 4 (——).

TABLE I
Estimates of the population means of pharmacokinetic parameters for dodecanedioic acid

Parameter	Estimate ± SE
V_1 (L)	5.56 ± 3.13
V_2 (L)	87.4 ± 30.4
k (L/min)	4.60 ± 3.50
γ_u (mL/min)	25.6 ± 15.5
γ_t (L/min)	2.17 ± 0.86

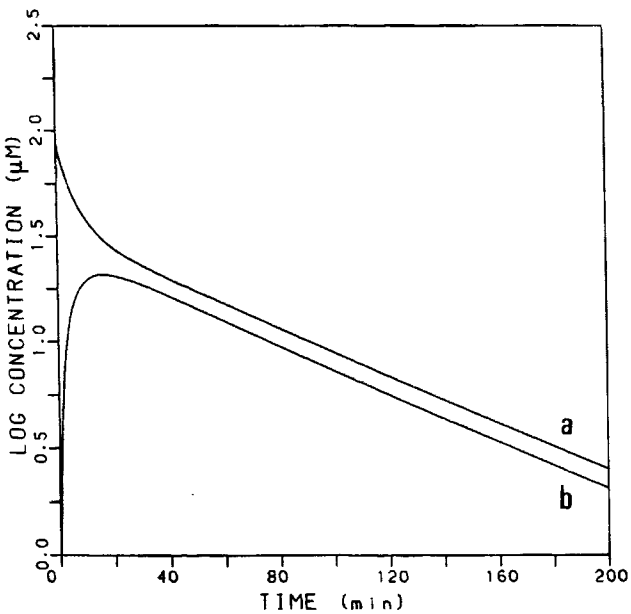

Fig. 4. Prediction by the model of Eqs 3 and 4 of the time course of free C12 concentration in central compartment (*A*) and in peripheral compartment (*B*).

central compartment. The estimated rate constant γ_u, normalized to 64.4 kg (average body weight of the subjects), gave 0.40 mL/min per kilogram, a value that is not far from the value of 0.63 mL/min per kilogram found in rat.[11]

The rate of tissue uptake of dodecanedioic acid was considered to be linearly related to the concentration of free C12 in peripheral compartment. In a preliminary analysis of the data, the assumption of a nonlinear saturable uptake did not significantly improve the fitting of the experimental values. In our bolus experiments, indeed, the maximal concentration of free C12 in the peripheral compartment was likely to be low (see Fig. 4), so that a possible saturation of tissue uptake would have been hardly detected. The tissue uptake of C12 in man, which was represented by a linear term in Eq 4, can be compared, at least when the concentration of dicarboxylic acid in the peripheral compartment is low, to the tissue uptake of azelaic and sebacic acids, represented by a nonlinear Michaelis-Menten term in Bertuzzi et al.[5,9] To this aim, the estimated γ_t of C12 has to be compared with the ratio of the maximal rate of tissue uptake, T_m, to the Michaelis-Menten constant, K_m; this ratio, in fact, is the initial slope of Michaelis-Menten curve. For both azelaic and sebacic acid T_m/K_m did not exceed 0.5 L/min, so that the rate of C12 tissue uptake at low concentrations is more than four times larger than that of both azelaic and sebacic acid. The value of γ_t of C12 normalized to the average body weight of the subjects examined (0.034 L/min per kilogram), is again not far from the normalized ratio found in rat (0.15 L/min per kilogram).[11]

In conclusion, from these preliminary kinetic data, the use of dodecanedioic acid in parenteral nutrition appears to be promising, because C12 elimination in urine is low, whereas the tissue uptake appears to be characterized by a considerable rate constant. Additional experiments of continuous infusion with C12 should allow us to estimate the maximal rate of tissue uptake and thus to confirm its suitability as a fuel substrate in parenteral nutrition.

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