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Pharmacokinetics of sebacic acid in rats

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Abstract. – The pharmacokinetics of disodium sebacate (Sb) was studied in Wistar rats of both sexes. Sebacate was administered either as intra-peritoneal (i.p.) bolus (six doses ranging from 10 mg to 320 mg) or as oral bolus (two doses: 80 and 160 mg). Plasma and urinary concentrations of Sb and urinary concentrations of Sb and its products of β-oxidation (suberic and adipic acids) were measured by an improved method using gas-liquid chromatography/mass-spectrometry.

A single compartment with two linear elimination routes was selected after no increase in significance was shown by an additional compartment and after a saturable mechanism was found to be unsuitable. Both renal and non-renal elimination parameters were obtained by Marquardt non linear fitting of plasma concentrations together with urinary elimination.

The data reported are calculated from the analysis on the whole population of rats and referred to an average body weight (bw) of 100 g. The Sb half-time was 31.5 min. The tissue elimination rate was 0.0122 min-1. The overall volume of distribution was found to be 26.817 ml/100 g bw. The renal clearance was 0.291 ml/min/100 g of bw, which is much less than the value of GFR reported in literature (about 1 ml/min/100 g bw), suggesting the presence of Sb reabsorption from the ultrafiltrate. The value of Sb renal clearance was found to be a concentration-independent function, suggesting the presence of a passive back-diffusion.

The relative bioavailability of the oral form compared to the i.p. form was 69.09%, showing a good absorption of the drug.

Key Words:

Sebacic acid, Dicarboxylic acids, Pharmacokinetics.

Introduction

In the last years we proposed the use of the salts of dicarboxylic acids (DA), in total parenteral nutrition (TPN) as an alternate energy source[1-3]. The advantage of these diacids over conventional lipid substrates (both long and medium chain triglycerides) is related to the immediate availability of their compounds. The salts of dicarboxylic acids are highly water soluble and thus can be directly administered through a peripheral venous route. Unlike long chain triglycerides (LCT), or medium chain triglycerides (MCT), which are available under emulsion form for clinical use, they do not require complex and expensive production procedures. In addition, DA are readily beta-oxidized at the level of mithocondria, through a carnitine independent mechanism, and peroxisomes.

DA provide a means of increasing the net oxidation of nutritional fuel without increasing the carbon dioxide production. This could be a significant advantage in critically ill patients, where different degrees of pulmonary insufficiency are usually present. However, odd carbon atom DA (e.g. azelaic acid) (Az), are incompletely oxidized since the β-oxidation is terminated at the level of malonic acid. Malonic acid is then the starting substrate in the synthesis of free fatty acids, thus creating the so-called futile metabolic circles. Azelate oxidation is associated with a low cost of ATP synthesis in terms of CO_2 production; however, the major problem is that more than 50% of the administered dose of this diacid is excreted in the urine[4].

Thus, we turned our attention to sebacic acid (Sb), the superior homologous, with 10 carbon atoms, which in preliminary studies[3], appeared to be excreted in the urine at low levels (only 16% vs 50% of Az). Like Az[5], Sb has been safely used in laboratory animals, and did not show any toxic effect during

chronic administration[6]. Similarly, no teratogenic effect was observed, and the development of the fetuses appeared to be normal[6].

The present study was undertaken to obtain a pharmacokinetics basis for pharmacodynamic studies of disodium sebacate in the rats. The disposition of sebacate was studied after three different doses (40, 80 and 160 mg) were administered, either as i.p. or as oral bolus. The urinary excretion modality was evaluated giving i.p. different doses (10, 40, 80, 160, 240, 320 mg) of sebacate.

Materials and Methods

Animals

Wistar rats of both sexes, weighing 130-250 g, were used throughout the study. They had free access to water and a standard pellet diet containing 20% protein, 4% fat and 50% carbohydrate. The rats which received oral doses of sebacate, were fasted previously for 18 hours but provided with water "ad libitum". Single i.p doses of DA salts (DAS), were administered to 8 groups of 5-11 rats (about half males and half females) and the animals were kept in a metabolism cage. The doses ranged from 10 to 320 mg (10, 40, 80, 160, 240, 320 mg). Urine was collected in containers with 1 ml 0.1% sodium azide to prevent bacterial growth and cooled in solid CO_2 during three different periods: 0-4, 4-24 and 24-48 hours.

Urine samples were stored at – 20° C prior to analysis.

Fourteen rats (7 males, 7 females), were injected i.p. with three different doses of sebacate, i.e. 40, 80, 160 mg. Heparinized blood samples were taken pre-dose and at 5, 10, 20, 40, 80, 160, 320 minutes post-dose. Optimal blood sampling times were suggested by a pilot study in 2 rats (1 male, 1 female). Plasma was separated from the blood cells.

Separation of Samples

Samples of plasma (500 µl) were added to azelaic acid (10 µg), which acted as an internal standard. The samples were then treated with 10% trichloroacetic acid solution (50 µl) and thoroughly mixed. Within 1 our after sample collection the mixture was then centrifuged and the supernatant extracted three times with 0.5 ml ethylacetate. The residue was washed twice with 0.25 ml ethylacetate. The extracts were combined together and dried under nitrogen stream until a final volume of 200 ml was reached. The extracts were cromatographed on silica gel thin-layer plates (20 × 20) cm, 0.25 mm thickness, (Carlo Erba, Italy) in exan-diethylether-acetic acid (6:4:0.4, v:v:v). The area of silica gel which corresponded to the area of dicarboxylic acids (a mixture of adipic, suberic, azelaic and sebacic acids were used as a standard) was scraped of the plates and extracted three times with ethylacetate. The combined extracts were dried over anhydrous Na_2SO_4. The extracted solutes were recovered in conical tubes after evaporation of the solvent by N_2 stream and methylated with diazomethane in diethylether. The dicarboxylic acids derivatives were dissolved in 500 µl exane and 2 ml of the solution were injected in to the gas chromatograph.

Urine samples (0.5-1 ml) were treated with cation-exchange resin (Dowex 50 W-X4, 100-200 µ M mesh. H^+) to remove salts, concentrated under reduced pressure and finally filtered trough and a Millipore HV (0.45 µm). The samples were exstracted three times with 0.5 volumes of ethylacetate. The combined exstracts were dried under N_2 and thin layer chromatograph (TLC) was performed to purify the dicarboxylic acids prior to gas liquid chromatograph (GLC). The GLC analysis was performed as described below.

Gas Liquid Chromatography-Mass Spectrometry

Verification of the identity of the dimethylesters DAS was carried out by mass spectrometry (MS). A Hewlett-Packard 589A GLC, equipped with a split/splitless capillary injector and supplied with a 5970 MS detector was used. The GLC inlet system was equipped with a 30 m fused silica capillary column Supelcowax 10 (0.32 mm I.D., 0.25 µ film thickness from Supelco, Bellefonte, PA, USA). Helium was used as a carrier gas and maintained at a flow rate of 2 ml/min (column pressure 40 psi). A flow of 30 ml/min of helium was used as make-up gas. The injection and interface temperatures were maintained at 240°C. For the analysis of DA the column temperature was maintained initially at 50°C. Immediately after injection the temperature was programmed to increase at a rate of 10°C to a final temperature of 240°C.

A repetitive scanning mode (300 AMU/sec) was used for compound identification. The eluent from the capillary column was repetitively scanned from 49 to 550 m/z.

Calibration Curve

100 µg of the following standard dicarboxylic acids were esterified with diazomethane: adipic (C6), suberic (C8), azelaic (C9), sebacic (C10) and dodecanedioic (C12). The solvent was then evaporated by N2 stream and the residue dissolved in 1 ml hexane. Two ml of each solution at different concentrations were injected into GLC.

Recovery of Standard DA

10 to 100 µg of C6, C8, C9, C10 and C12 standard DA were added to 1 ml urine of an untreated rat. DA were extracted, esterified and analyzed as described above.

Pharmacokinetic Analysis

The first procedure was to normalize the plasma concentrations by multiplying them by the rat body weight and dividing them by the value of 100g. This allowed the different rat weights to be taken into account.

In order to choose the pharmacokinetic model to be used in the interpretation of the plasma level and urinary excretion data obtained, three steps were followed.

The first step was to ascertain whether a non-linear overall elimination of the drug from plasma was present. The i.p. administered doses were divided into a low dose group (90 mg, 80 mg, HI = 0), and a high dose group (160 mg, HI = 1). Multiple linear regression of the log normalized plasma concentration values [log (concentration/administered dose)] was performed on time, HI and time × HI. The covariance analysis thus obtained failed to show any significance of the HI term, either singly or in conjunction with time. This means that no slope or intercept difference between low and high doses was detected and the same log-linear relationship between concentration and time existed at low and high doses. Therefore, non-linear rate of disappearance was excluded and only linear models needed to be taken into consideration.

The second objective was to ascertain whether the urinary data supported a mechanism of non-linear excretion (due to carrier mediated secretion or reabsorption) at the level of the nephron. To do so, stepwise regression of total drug recovered in the urine versus administered dose was performed, in which polynomials up to fifth degree in the independent variable (administered dose) were tested. Both forward and backward algorithms yielded significance for the linear term only (Table I). This was taken as an indication that a single linear relationship existed and that any active reabsorption or secretion processes were not operating in this experimental model.

In the third step a choice was made between a one-compartiment (single exponential) and a two-compartment (double exponential) model by comparing the increase in explained variance due to the double exponential model to the error variance by means of an F-test. This was not supported by the collected data. A single exponential linear model was thereby used, with two linear eliminations (one to the kidney and one to the tissues) differentiated on the basis of the urinary data[7].

Results

The plasma DAS extraction phase with ethylacetate involves a minimal simultaneous extraction of free fatty acid whose GLC peaks can be superimposed on those of some DAS. In particular, the retention time (RT) of the peak of palmitic acid is very close (in the order of some seconds) to that of adipic acid. Thus, in plasma samples (but not in urine which does not contain FFA) the peak assigned to adipic acid might be due to the presence of small amounts of palmitic acid. Therefore, definitive identification and quantification of DAS await completion of MS studies.

The calibration curve for each dimethylester DA showed a linear plot in a range between 10 to 1000 ng.

A value of 5 ng, which corresponds to the value of a peak with an amplitude twice that of the noise, was taken as the detectability limit of this analysis method.

The recoveries of each dicarboxylic acid standard are reported in Table II.

Table I. Summary of backward stepwise regression. It excludes significance of quadratic and higher administered dose terms to predict amount of drug, retrieved from urine. Forward mechanism selects the same model.

STEPWISE SELECTION FOR TOTAL URINE AMOUNT						
SELECTION: Backward R-squared: .97041 Variables in Model	Coeff.	Step 4 F-Remove	F-to-enter 4.00 MSE: 215.148 Variables	F-to remove: 4.00 d.f.:35 Not in Model	P Corr	F-enter
1. dose	0.50317	1147.7966	2.	dose^2	.1359	.6402
			3	dose^3	.1330	.6121
			4.	dose^4	.1246	.5363
			5.	dose^5	.1157	.4609

Model fitting results

Independent variable	coefficient	std. Error	t-value	
Dose	0.503707	0.0414868	33.8791	p<0.0001
Source	Sum of squares	DF	Mean square	F-Ratio
Model	246946.	1	246946.	1147.80
Error	7530.17	35	215.148	
Total	254476.17	36		
R-squared = 0.970409		SE of estimates = 14.6679		

The difference in abundance of fragment ions derived from individual dimethylesters derivatized DA is reported in Table III.

Excretion of Sebacate and Its Metabolites

After i.p. administration of Sb, about 50% of the dose was recovered in urine over 98 hours (Table IV). The metabolites excreted, suberic (C8) and adipic (C6) acids, accounted for approximately 3% and 1% respectively, of the dicarboxylic acid.

The majority of the drug was excreted in the first 9-6 hours. Gradually the urinary sebacate concentration decreased with time and in the urine samples collected 29 to 98 hours after bolus administration only traces of the diacid were detected.

Table II. Recovery of standard dicarboxylic acids (DA) from urine samples.

DA	Amount added (μg)	Amount recovered (μg)
C6	10.0	9.3 ± 5.1
C6	50.0	6.5 ± 2.8
C6	100.0	93.1 ± 5.7
C8	50.0	46.6 ± 1.9
C8	100.0	94.0 ± 4.8
C9	10.0	9.4 ± 2.3
C9	50.0	47.0 ± 4.1
C9	100.0	94.5 ± 4.7
C10	50.0	47.4 ± 3.0
C10	100.0	96.9 ± 4.2
C12	50.0	48.0 ± 2.5
C12	100.0	98.2 ± 2.9

Sebacate Pharmacokinetics

Plasma concentration curves were fitted minimizing with the Marquardt algorithm the unweighted squared error for the three i.p. doses separately and yielded the parameter estimates reported in Table V.

It can be observed how well the separate estimates agree. Since a linear model was supported by the data, pooled parameter estimates (K, C_o) were obtained by fitting all plasma concentrations together, normalized by dividing them by the administered dose and multiplying them for a common dose value of 80 mg (X_o). The global plasma half-life ($t_{1/2}$) of sebacate was found to be 31.5 min. The overall volume of distribution (V_d -

Table III. Significant ions (a, b, c, and d) for the identification and quantification of dimethylesters of dicarboxylic acids and their relative intensities (ri, %).

Dicarboxylic Acids	Molecular weight	Ions							
	M^+	a	ri	b	ri	c	ri	d	ri
C6	174	143	48.7	111	66.8	101	63.4	—	—
C8	202	171	60.1	138	76.3	129	74.6	97	58.3
C9	216	185	54.7	152	92.2	143	49.8	111	66.5
C10	230	199	28.8	166	19.1	157	29.2	125	55.4
C12	258	227	30.2	194	15.0	185	46.5	153	80.4

Ion a corresponds to a fragment M+ - OCH_3
Ion b corresponds to a fragment M+ - 2($HOCH_3$)
Ion c corresponds to a fragment M+ - CH_2COOCH_3
Ion d corresponds to a fragment M+ - $(CH_2)_4COOCH_3$
Fragments at m/e 74(CH_2=C-OCH_3) and at m/e 55 ($C_4H_7^+$) are common at all the dimethylesters ^+OH

Dose/ C_o) was found to be 26.817 ml/100 g body weight.

The apparent volume of distribution estimate thus obtained does not take into account sebacate binding to albumin. This stems from the necessity of dosing total instead of free diacid concentrations in plasma.

A pooled estimate (X_u) of total drug excreted in the urine was obtained by averaging the recovered amount for each rat divided by the dose given to that rat and multiplied by 80 mg. X_u was found to be 35.589 mg. The renal elimination constant (K_e) was then computed as follows:

$K_e = K\ X_u\ X_o^{-1} = 0.00977\ min^{-1}$

The tissue elimination rate was computed as follows:

$Km = K - K_e = 0.0122\ min^{-1}$

Renal clearance was evaluated by multiplying the value of the sebacate apparent volume of distribution (V_d) and the first order elimination constant (K_e) of the diacid urinary excretion.

Renal clearance was thus found to be 0.291 ml/min/100 g body weight, that is much less than the reported value of GFR in rats (1.012 ± 0.043 ml/min/100g body weight)[8]. This indicates the presence of sebacate reabsorption from the ultrafiltrate. The fact that renal clearance of sebacate is not a concentration-dependent function suggests the absence of a carrier mediated reabsorption and indicates that passive back-diffusion is present.

Plasma concentration curves were also fitted for 80 mg oral administration route. It is easy to see that the dispersion of the points is much greater, and that the R-squared is consequently lower.

We did use these data to effect a comparison between the bioavailability of the two routes of administration. The AUC's under i.p. and p.o. plasma concentration curves were computed (100 g bw) by solving the definite integral of the fitted curve between zero and infinity with a relative bioavailability (AUC_{PO}/AUC_{IP}) of the oral form with respect to the i.p. form of 69.09%.

Table IV. I.p. administered sebacate versus sebacate recovered from urine: averages for each administered dose.

No.rats	Administered dose (mg)	Total 48 hrs SB urine amount (mg)	% of administered dose
5	10	2.74 ± 1.35	27.40
8	40	20.43 ± 10.73	51.07
9	80	36.89 ± 7.2	46.11
4	160	77.02 ± 19.65	48.13
5	240	122.50 ± 11.77	51.04
5	320	169.55 ± 65.62	52.98

Table V. Parameter estimates computed for each i.p. dose separately.

Administered dose (mg)	K (min^{-1})	Co (μg/ml)
40	-0.020 ± 0.0030	550 ± 20
80	-0.030 ± 0.0075	1906 ± 227
160	-0.016 ± 0.0016	2718 ± 101

Considering this parameter as a measure of the extent of gut absorption of the drug, we see that the route of administration plays a relatively minor role.

Analysis for Sb, C8 and C6 in faeces registered only traces of these compounds. The relatively low systemic availability of Sb in rats after p.o. administration, with respect to i.p. administration may be due to a high extraction by the liver. This might depend on the high peroxisome content of the hepatocyte, yielding a high rate of β-oxidation.

For a definite identification of a compound, comparison with a reference standard is essential. Using capillary column GLC and mass spectral fragmentation the accuracy and specificity of the analysis is very high.

Discussion

This very sensitive technique allows very small amounts of dicarboxylic acids, down to 1 ng, to be assayed. It can be utilized for the pharmacokinetic studies, yielding the same accuracy as radioactive labeled compounds. However, this technique does not allow for the determination of the oxidation rate of a substance, which is possible by using the labeled ^{14}C or ^{13}C compound.

The experimental data show a linear relationship between the administered doses and the amounts of sebacate excreted with the urine. These data suggest the presence of a non-saturable mechanism of urinary excretion of the diacid. Sebacate renal clearance (0.291 m1/min/100 g bw) is lower than the mean value of glomerular filtration rate (inulin clearance) reported in the literature (1.05 ml/min/100g b.w.) indicating that the GFR is reduced by passive sebacate back diffusion.

Bergseth et al[9] showed that the clearance of suberic acid (C8) in perfused rat kidney exceeded the glomerular filtration rate by a factor of 5, suggesting that C8 must be actively secreted, while the ratio of dodecanedioic acid (C12) clearance to inulin clearance was less than 1, meaning that C12 was reabsorbed. No data are available in the literature about C10 in perfused rat kidney, but our data suggest the presence of linear reabsorption. It is possible that the rate of urinary excretion of dicarboxylic acids depends upon their chain length.

The decrease in time of plasma sebacate concentration after i.p. injection can be approximated by an exponential function. In other words, a constant fraction of plasma Sb is removed per unit time, so that elimination is high when plasma concentrations are high but low when plasma concentrations are low. The average half-time sebacate elimination was 31.5 min, indicating the presence of rapid clearance from the plasma compartment. Brief tissue half-life (56.80 min, obtained dividing $\log^2$ by the tissue elimination rate constant k_m), suggests rapid cellular uptake. The fact that parameter estimates varied considerably between i.p. and p.o. models depends most probably on two factors. First of all, variability in individual rat absorption of sebacate from the stomach induced a high variability between individuals in measured plasma concentrations after p.o. administration. The error was therefore much higher and the corresponding parameter estimates less reliable.

Secondly, it is quite possible that the simple standard equation used to model gut absorption as a first-order process is not adequate. More complicated models could be set up. However, in the presence of a large amount of variability, fitting more complicated models by estimating more parameters gives rise to questionable model identification.

The mean apparent volume of distribution of sebacate was 26.817 ml/100 g bw. Generally two processes contribute to the

elimination of a drug: excretion and metabolism. The values of renal and tissue clearance showed a high tissue uptake. The fact that the major Sb urinary excretion occurred in the first few hours following the administration suggests that the sebacate remaining in the body was metabolized.

The disposition of sebacate after oral dose was similar to that found following i.p. bolus injection. This suggests a rapid absorption of the dicarboxylic salt, which probably takes place at the stomach level, having this diacid a pK similar to that of salicilate (sebacate: $K_1 = 2.6 \times 10^{-5}$, $K_2 = 2.6 \times 10^{-6}$, salicilate: $Ka = 1 \times 10^{-3}$)[10]. The systemic bioavailability of sebacate after oral administration was not much less than that obtained when the same doses were given i.p.. These data indicate a good absorption of sebacate by the oral route.

In conclusion, sebacate seems to be characterized by a brief half-life, high tissue uptake and passive renal reabsorption.

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