Disposition of metformin (N,N-dimethylbiguanide) in man*

Kinetic parameters of metformin (N,N-dimethylbiguanide), an anti-diabetic reported to be associated with a lower number of episodes of lactic acidosis than phenformin, were determined in volunteers with normal renal function and in patients with different degrees of renal impairment. Drug in body fluids was measured by a highly specific and sensitive mass fragmentographic method, after the formation of a triazine derivative, obtained with heptafluorobutyric anhydride. The half-life (t½) for the elimination of drug from plasma after intravenous injection in 5 normal subjects (1.52 ± 0.3 hr) (mean ± SD) was shorter than that reported for phenformin by a similar assay method (7 to 15 hr). The mean t½ in 5 renal patients was 4.94 ± 1.11 hr, and a correlation was observed between t½ of drug from plasma and creatinine clearance. After oral administration of metformin tablets, drug recovery in urines was only 37.6%, possibly not as a consequence of low bioavailability (a similar low recovery was found after oral administration of the metformin solution used for the intravenous studies), but of binding to the intestinal wall, as shown in animal and clinical studies with metformin and other biguanides. Metformin is rapidly eliminated through active secretion by the kidney (mean renal clearance, 440.8 ml/min)—it is neither metabolized nor protein-bound in plasma. The very brief plasma t½ makes significant cumulation, with a standard tid regimen, unlikely. These findings may help explain the lower incidence of toxic effects, particularly lactic acidosis, than after phenformin.

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The kinetics of biguanide anti-diabetic drugs has received little attention, possibly because of the lack of adequate specific and sensitive assay procedures.[5] Recently developed new methods for the specific determination of biguanides[20] in biologic fluids have made possible more accurate definition of kinetic parameters. By the use of these methods, it has been shown that significant toxic effects of biguanides, in particular lactic acidosis (LA), are often correlated with very high plasma drug levels.[4, 9, 28]

Metformin (N,N-dimethylbiguanide) is presently the most widely used drug of this type in Western Europe.[15] Interest in the use of metformin has recently been stimulated by the observation of significant hypolipidemic effects, particularly in hypertriglyceridemic pa-

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Table I. *Participating subjects with normal and altered renal excretory functions*

Subject	*Sex*	*Age (yr)*	*Weight (kg)*	*Creatinine clearance (ml/min)*
Normals				
1	M	36	70	98
2	M	39	64	108
3	F	46	68	105
4	M	60	81	92
5	M	43	77	94
Renal patients				
1	F	40	70	48
2	M	72	67	45
3	M	72	53	34
4	M	59	64	22
5	M	29	96	20

tients,[10, 23] and of changes in lipoprotein structure and metabolism in both experimental animals and in man.[24]

In spite of the very wide use of metformin in diabetes and in hyperlipidemia, a considerably lower incidence of LA has been reported after the use of this drug than after phenformin.[1] In a recent review of 330 cases of LA in biguanide-treated diabetics,[19] 12 were associated with metformin and 281 with phenformin. A detailed clinical analysis of this large patient sample showed that control of diabetes did not appear to be a significant factor in the development of LA, whereas impairment of kidney function was a frequent finding, particularly in the case of metformin, all 12 toxic patients exhibiting creatinine plasma levels above 3 mg/100 ml. Recent surveys in France and Switzerland comparing relative uses of biguanide drugs and market distribution indicated an incidence of LA with phenformin 20 and 40 times that with metformin.[6, 15]

In the present report, analysis of the most relevant kinetic parameters of metformin was carried out in volunteers with normal and altered renal excretory functions. Comparison of these data with findings previously reported for phenformin and buformin[2, 17] may help explain differences in the incidence of LA in patients treated with various biguanides.

Methods

Patients. The oral and intravenous kinetic studies were carried out in 5 patients (4 male and 1 female) with normal blood urea nitrogen, plasma creatinine (PCr), and creatinine clearance (CrCl). All the five patients had elevated plasma lipid levels, 2 of them also having a mild chemical diabetes. The kinetic studies were performed prior to the beginning of a study on the effects of metformin on plasma lipoproteins. An intravenous test was carried out in 5 additional patients (4 male and 1 female) with significantly impaired renal excretory function, as determined by CrCl and para-aminohippurate clearances (Table I).

Steady-state plasma levels of metformin were determined in patients chronically treated with different doses of the drug for hyperlipidemia or diabetes. All these patients had a normal renal function as assessed from the PCr and CrCl tests.

Protocol of the pharmacokinetic studies. All the subjects were fasted for 12 hr before the oral or intravenous administration of metformin. In the intravenous test, 1 gm of metformin HCl (Spemsa) was rapidly injected intravenously and plasma samples were collected from an indwelling venous catheter into heparinized tubes. Collection of samples was at 5, 10, 30, 60, 90, 120, and 240 min, and 6, 8, 24, 36, and 48 hr. Urine was collected after 1, 3, and 6 hr and every 6 hr thereafter. Plasma samples and a small sample of metformin solution used for injection were kept frozen (−20° C) until analysis.

The oral test was carried out by administering

to 850 mg of metformin HCl. Samples were collected after 1, 2, 3, 4, 6, 10, 16, 24, 30, 36, 48, and 72 hr. Urine collections were at 2, 4, 12, 24, 30, 36, 48, 54, 60, and 72 hr. The schedule of plasma and urine collections was simplified and shortened after the first two patients, when it became clear that the drug was not detectable in blood or urine after 48 hr.

In the five patients with impaired renal function, only the intravenous kinetic study was carried out. A dose of metformin two thirds of that used for the normal subjects was injected (the correct concentration was later calculated by mass fragmentography). Plasma samples were collected after 1, 2, 4, 8, 12, and 24 hr. The steady-state plasma samples from 55 patients treated with metformin for hyperlipidemia or diabetes were collected after an overnight fast. The last drug administration had taken place the night before; i.e., 12 to 14 hr before sampling. Daily drug dosage and interval between drug intake and blood sampling were recorded for each patient.

Determination of metformin in biologic fluids. Concentrations of unchanged metformin in plasma and urinary samples were determined by mass fragmentography after the preparation of triazine derivatives essentially as described by Matin, Karam, and Forsham,[20] with deuterated metformin as the internal standard. Deuterated metformin was prepared by fusion of d_6-dimethylammonium chloride with 1-cyanoguanidine as described by Werner and Bell.[26] The obtained d_6-metformin analyzed by mass spectrometry using the direct inlet system showed 98% isotopic enrichment.

For the quantitative drug determination in biologic samples, an alkaline solution of d_6-metformin (between 1 and 10 μg of d_6-metformin based on the predicted drug concentrations) was added to the plasma or urine samples. After deproteinization with 10% trichloroacetic acid in 1 N HCl followed by alkalinization with 10 N NaOH, samples were extracted with methylene chloride.[20] Heptafluorobutyric anhydride (10 μl) was added to the extracts, and, after refluxing for 1 hr, the methylene chloride was washed with 1 N NaOH followed by water. The residue, obtained after evaporation of the solvent and containing the

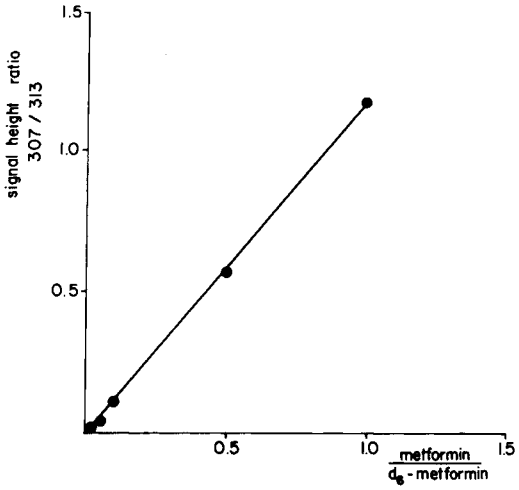

Fig. 1. Standard curves showing the ratio of signal intensities of the metformin derivative (m/e 307) and of the deuterated metformin derivative (m/e 313) plotted against the ratio of μg metformin to μg of deuterated metformin.

2-amino-4-dimethylamino-6-heptafluoro-s-triazine, was dissolved in ethyl acetate and aliquots of the solution were injected for fragmentographic analysis into a Varian 112 S spectrometer connected to a Varian SS 100 data system.

Conditions of the analysis were the following:

Glass column packed with 1% SE 30 on 100 to 120 mesh Gaschrom P. The oven temperature was 160° C; injector and detector were at 250° C; the helium flow was 20 ml/min. The electron energy was set at 70 eV and other operating parameters were: emission current, 1.5 mA; electron multiplier, 2.5 kV; molecular separator and ion source were at 280° C. For the fragmentographic analysis, the molecular ion (M^+) at m/e 307 of the triazine, and the peak at m/e 313 corresponding to the molecular ion of the deuterated triazine, were focused.

A standard curve was prepared by adding to 1 ml of plasma or urine a standard amount of deuterated metformin HCl and increasing amounts of metformin HCl, and by treating samples as described for plasma analysis. The ratios of peak intensities were plotted against the theoretical values of μg metformin/μg d_6-metformin (Fig. 1). Concentrations of metformin in the analyzed fluids were calculated from the standard curve.

Table II. *Two-compartment disposition constants for metformin following intravenous administration in 5 normal and 5 renal patients*

	Subject	Dose (mg)	A (μg/ml)	α (hr^{-1})	t½ (min^{1})	β (μg/ml)	β (hr^{-1})
Normal	1	900	28.7	1.59	26.1	6.31	0.45
	2	960	44.7	1.80	23.1	10.80	0.55
	3	912	57.2	4.80	8.7	9.40	0.56
	4	912	45.5	2.53	16.4	4.32	0.35
	5	950	60.1	4.04	10.3	6.99	0.44
Mean ± SEM		927 ± 12	47.2 ± 5.6	2.95 ± 0.6	16.9 ± 3.4	7.56 ± 1.15	0.47 ± 0.04
Renal	1	680	158.5	4.22	9.8	14.56	0.25
	2	689	39.8	2.35	17.7	17.08	0.20
	3	680	33.9	1.29	32.2	17.07	0.10
	4	686	17.2	1.02	40.8	14.84	0.10
	5	684	15.8	0.26	160	10.95	0.15
Mean ± SEM		684 ± 2	53.0 ± 27	1.83 ± 0.7	52.1 ± 27	14.90 ± 1.12*	0.16 ± 0.03†

Significances of the differences between values of normal and renal patients:
*$p < 0.005$.
†$p < 0.001$.
‡$p < 0.02$.
§$p < 0.01$.

Analysis of data. Plasma concentration–time curves after intravenous administration of metformin were determined after calculating the log $C_p = f(t)$ regression lines of the β-phase (elimination phase) and of the residuals of the α-phase (distribution phase), according to a two-compartment open model as suggested by Lintz and co-workers.[17] From the curves of each studied subject, the hybrid constants A, α, B, and β were computed:

A = intercept at time zero of the first order plot of slope $-\alpha$ obtained from the "feathered" plot of ln C against time, where B and β are obtained and then the residuals plotted to obtain A and α.
α = apparent first order rate constant for distribution of the drug in the body.
B = intercept at time zero of the first order plot of slope $-\beta$ obtained from the plot of ln C against time.
β = apparent first order rate constant for elimination of the drug from the body.

From these constants pharmacokinetic parameters were then determined according to the following equations[17]:

Total area under plasma concentration curve:

$$AUC_{iv} = \int_0^\infty C_p dt = \frac{A}{\alpha} + \frac{B}{\beta}$$

Plasma concentration at zero time: $C_p^0 = A + B$.

Rate constant:

$$K_{el} = \frac{C_p^0}{AUC_{iv}}; \; K_{21} = \frac{\alpha\beta}{K_{el}}; \; K_{12} = \alpha + \beta + K_{el}.$$

Total clearance rate: $Dose/AUC_{iv} = Cl_{tot}$.

Renal clearance rate: $\frac{\text{Drug excreted in urine}}{\text{Dose}} \times Cl_{tot}$.

Volume of distribution: $V_{d\beta} = \frac{D}{C_p^0} \cdot \frac{K_{el}}{\beta}$.

Half life: $\frac{0.693}{\beta}$.

Areas up to time t after intravenous injections were calculated from the infinite area according to the equations:

$$\int_0^\infty C_p dt = \int_0^\infty C_p dt + C_p^t/\beta$$

where

$$\int_0^\infty C_p dt = \frac{A}{\alpha} + \frac{B}{\beta} \text{ and } C_p^t = B\, e^{-\beta t}.$$

Bioavailability after oral administration of metformin was determined according to Equation 1:

$$F = \frac{AUC_{po}}{AUC_{iv}} \frac{D_{iv}}{D_{po}}, \qquad (1)$$

$t½$ (hr^{-1})	k_{el} (hr^{-1})	k_{21} (hr^{-1})	k_{12} (hr^{-1})	$V_{d\beta}$ (l)	Cl_{tot} (ml/min)	Cl_{ren} (ml/min)
1.54	1.09	0.66	0.29	62.0	467	255
1.26	1.25	0.79	0.31	39.3	359	304
1.24	2.30	1.17	1.88	56.3	530	513
1.98	1.64	0.54	0.70	85.8	334	286
1.58	2.18	0.82	1.48	70.1	514	317
1.52 ± 0.13	1.69 ± 0.24	0.80 ± 0.11	0.93 ± 0.32	62.7 ± 7.7	441 ± 40	335 ± 46
2.77	1.81	0.58	2.08	28.4	118	65.3
3.47	0.56	0.84	1.15	33.9	112	78.4
6.93	0.26	0.50	0.63	34.7	57.5	37.4
6.93	0.19	0.54	0.39	40.7	69.3	47.1
4.62	0.20	0.20	0.01	34.2	85.1	73.7
4.94 ± 0.86*	0.60 ± 0.31‡	0.53 ± 0.10	0.85 ± 0.36	34.4 ± 1.9§	88.4 ± 11.8†	60.4 ± 7.8†

the AUC being estimated by the trapezoidal rule.

All statistical and pharmacokinetic calculations were performed with the help of a Hewlett-Packard 65 calculator. Mean values and standard deviations were calculated according to standard formulas; significance of the differences was determined by Student's t test. Regression analysis was carried out by the least-squares method.

Protein binding study. Protein binding was estimated in vitro by equilibrium dialysis as described by Keen[14] using ^{14}C uniformly labeled metformin (Amersham-Searle). The radiochemical purity of the ^{14}C-metformin was checked by radio–gas chromatography of the triazine derivative, prepared as previously described. More than 98% of the radioactivity was shown to be associated with the derivative.

The percentage of binding was calculated after the serum samples, taken from 5 healthy laboratory co-workers and kept in dialysis tubings (Thomas), had been in contact for 18 hr at 4° C with a 0.15 M phosphate buffer solution containing the labeled metformin, at concentrations of 0.5, 0.05, and 5 μg/ml, i.e., approximately equivalent to steady-state plasma concentration of chronically treated patients, 1/10 to 10 times this value.

Protein concentrations[18] and radioactivity were estimated both inside and outside the dialysis bags and albumin content of each serum sample was determined by electroimmunodiffusion against anti-albumin serum.[16] For radioactivity determination, a 1:2 Lumasolve (Lumac GmbH-Essen)-isopropanol solution was added to plasma followed by bleaching with H_2O_2. Samples were then added with a 9:1 Lumagel-0.5 M HCl solution, and counted on a 3385 Packard B Counter. Values were corrected for efficiency and background.

Ancillary measurements. All subjects undergoing the oral and intravenous tests, in particular patients with renal excretory dysfunction, were carefully monitored for any changes in vital signs (EKG, blood pressure) during the experiments. In addition, plasma lactate levels were determined enzymatically (Boehringer-Mannheim kit) in all patients undergoing the intravenous kinetic studies, and in patients in chronic treatment with metformin.

Results

Kinetic parameters in subjects with normal renal excretory function. After intravenous administration of metformin (the injected dose was calculated to be 926 ± 26 mg), a biexponential plasma decay curve was determined in all patients. Correlation coefficients for both the α and β phase regression lines were always higher than 0.7, with a good fitting ($p < 0.001$) of the observed values with the calculated lines

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