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Pharmacokinetic Study of Gadolinium-DOTA in Control and Streptozocin Diabetic Rats

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Abstract—The pharmacokinetics of gadolinium tetraazacyclododecanetetraacetic acid (Gd-DOTA), a contrast agent used in magnetic resonance imaging, have been evaluated in control and streptozocin-diabetic rats of different ages. In control rats, an age-related decrease in the Gd-DOTA elimination rate was noted, supported by a significantly lower apparent total body clearance and a significantly higher mean residence time. In diabetic rats, a similar but less important age-related change in the mean residence time and the apparent total body clearance was observed. Regardless of age-related differences in the pharmacokinetic parameters, a diabetic state induced several alterations in the Gd-DOTA pharmacokinetic parameters. The apparent total body clearance was significantly higher and the mean residence time significantly lower in diabetic rats indicating a higher elimination rate of Gd-DOTA. An important age-related increase in the volume of distribution at steady-state was noted in diabetic rats.

Magnetic resonance imaging (MRI) studies in streptozocin-diabetic rats showed a prolongation of the contrast visualization in the renal cavities, after an intravenous injection of MRI contrast media such as paramagnetic complexes (gadolinium tetraazacyclododecanetetraacetic acid, Gd-DOTA, and gadolinium-diethylenetriaminepentaacetic acid, Gd-DTPA) or piperidinic nitroxide spin label derivative (tempo carboxylic acid) (Michel et al 1986; Chapat et al 1987). A previous study (Michel et al 1989) indicated that diabetes induced only slight alterations in the pharmacokinetic parameters of tempo carboxylic acid. A significant decrease in the apparent total body clearance and in the volume of distribution of tempo carboxylic acid was observed while the area under the curve was increased. However, the increase in the concentration of free radical in the blood was not sufficient to explain the MRI abnormalities observed, since the elimination rate was rapid and similar both in control and diabetic rats. The prolongation of tempo carboxylic acid contrast visualization in the kidney might be explained by a blockage of contrast media in the kidney secondary to the tubular nephropathy or a decrease in renal metabolism leading to a persistance of paramagnetism. The purpose of this study was to evaluate, in control and diabetic rats, the pharmacokinetic parameters of a non-metabolized, paramagnetic complex, Gd-DOTA, after intravenous administration and to determine the tissue concentration of Gd-DOTA in the kidney.

Materials and Methods

Induction of diabetes

Male Wistar rats, 210–230 g, aged 7 weeks, were randomly selected for this study and had free access to laboratory chow and water. Diabetes was induced, under light ether anaesthesia, by a single intravenous dose of streptozocin (Sigma, USA) 50 mg kg^{-1} administered in 1 mL kg^{-1} citrate buffer, 0·05 M pH 4·5, in which it was dissolved just before injection. This dose of streptozocin is known to produce a mild diabetic state with hyperglycaemia but without ketosis (Tancrede et al 1983). Control rats received an equivalent volume of citrate buffer. Seven days after the onset of diabetes, plasma glucose was determined in streptozocin-treated rats using Detrostix (Ames Co.) and those rats with glucose levels lower than 250 mg% were discarded. Diabetic state was controlled by taking account of the following parameters: water intake, food intake and body weight. Plasma glucose was measured at the end of the study.

Pharmacokinetic studies

Pharmacokinetic studies were undertaken in streptozocin-diabetic rats, 2, 4 and 7 months after diabetes induction (group D1, D2 and D3) to evaluate the evolution of the pharmacokinetic parameters in relation to the pathologic state. These animals were respectively 15, 23 and 35 weeks old.

Pharmacokinetic studies were also performed in age-matched control rats (23 and 35 weeks old: groups C2 and C3) and, as the diabetic state was associated with a dramatic decrease in body weight gain, a pharmacokinetic study in 300 g body weight control rats (group C1, 10-week-old rats) was also undertaken. The animals were anaesthetized with intraperitoneal ethylcarbamate (1·2 g kg^{-1}). Gd-DOTA (Laboratoire Guerbet, France) as a meglumine salt (0·5 mmol kg^{-1}) was injected into a tail vein in a volume of 1 mL kg^{-1}. Blood samples (300 μL) were taken at 5, 10, 20, 40, 60, 120 and 180 min after injection via a carotidian heparinized catheter and centrifuged at 2000 g for 10 min. Plasma gadolinium concentrations were determined by atomic emission spectrophotometry (Spectrospan Beckman, DCP system, λ 342·247 nm). Plasma samples were assayed for gadolinium after half dilution in a 25 g L^{-1} $CsCl_3$ solution. Pharmacokinetic parameters were calculated from the plasma values using the computer program, IGPHARM (Gomeni & Gomeni 1978). The following abbreviations were used for selected pharmacokinetic parameters: MRT, mean

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residence time; Vd_{ss}, volume of distribution at steady-state; CL_T: apparent total body clearance; $t_{\frac{1}{2}\beta}$, apparent slow-phase blood half-life.

Kidney tissue studies

Gadolinium kidney concentration was studied after intravenous administration of Gd-DOTA at a dose of 0·01 mmol kg^{-1}, for in-vivo experimental MRI studies in the rat. Two groups of 12 diabetic rats (aged two and four months) and two groups of 12 age-matched control rats were anaesthetized with intraperitoneal ethylcarbamate (1·2 g kg^{-1}) and 10, 20 and 45 min after intravenous Gd-DOTA administration, the kidneys of 4 rats were removed. Kidneys were first dissolved in nitric acid and the renal gadolinium concentrations were then determined by atomic emission spectrophotometry.

Renal excretion

The excretion of gadolinium in the urine was evaluated in 4 four-month diabetic and 4 age-matched control rats. Access to the bladder was gained through a midline abdominal incision. The bladder was then pulled out and clamped just below the urethra and ureter. Urine was collected via a urethra catheter at 15, 30, 60, 90, 120, 150 and 180 min after a Gd-DOTA intravenous injection at a dose of 0·5 mM kg^{-1}. Results were expressed, for each time point, as the cumulated percentages of gadolinium recovery.

Statistics

Comparison between control and diabetic groups were made using the nonparametric Mann-Whitney test. The level of significance was set at $P < 0·05$.

Results

Diabetes study

The plasma glucose levels and the body weight of the animals used in the study are given in Table 1. The data indicate a significant increase in plasma glucose associated with a decreased body weight gain for diabetic rats. It should also be noted that the fluid and food intake of diabetic rats was greater than those of control rats. Sixty to seventy days after the injection of streptozocin, diabetic rats began developing cataracts which became bilateral after 3 to 4 months in a diabetic state. It must be noted that the 7-month diabetic rats were in poor condition with clinical signs of severe dehydration.

Table 1. Effect of diabetes on body weight and plasma glucose.

	Body weight (g)	Plasma glucose (g L^{-1})
Control rats		
≈300 g	299·7 ± 7·3	1·3 ± 0·1
4 months	616·6 ± 21·6	1·1 ± 0·1
7 months	767·5 ± 28·1	1·4 ± 0·1
Diabetic rats		
2 months	283·0 ± 23·1	3·6 ± 0·4[b]
4 months	369·0 ± 17·9[a]	3·2 ± 0·6[b]
7 months	320·7 ± 19·6[a]	4·1 ± 0·5[b]

Values are mean ± s.e.m. for 6 animals in each group. Mann-Whitney test: [a] values significantly different from respective control group ($P < 0·05$); [b] values significantly different from control values.

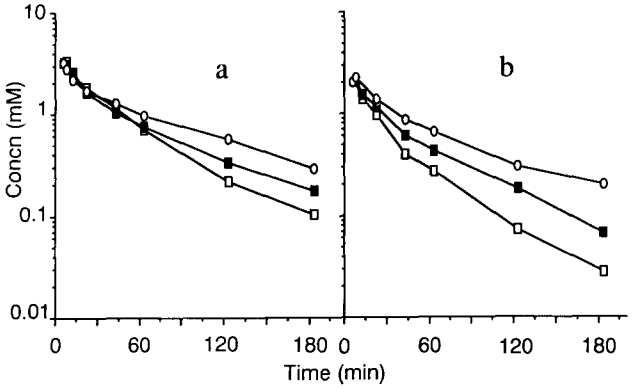

FIG. 1. Semi-logarithmic plot of blood Gd-DOTA concentration against time in control (a) and diabetic rats (b). For a: C1 □, C2 ■, C3 ○. For b: D1 □, D2 ■, D3 ○.

Pharmacokinetic studies

The decline of gadolinium in the blood after intravenous administration was adequately described by a single exponential curve and analysed as an open one compartment model. In some cases, we obtained a biexponential decay in the gadolinium concentration over time. This was analysed as an open two-compartment model.

Analysis of data in control rats. A typical example of the semi-logarithmic plot of gadolinium blood concentration against time is shown in Fig. 1a based on one rat from each control group. The curves showed a similar pattern of gadolinium blood levels during the first 30 min following intravenous administration. Subsequently gadolinium blood levels increased with the age of the animal, suggesting a decrease in gadolinium elimination.

The pharmacokinetic parameters of Gd-DOTA are shown in Table 2. These data confirm the decrease in gadolinium elimination rate with the increase in age of the animals, as shown by the MRT and the half-time increase, and CL_T decrease with age (Fig. 2). Table 2 indicates a lower Vd_{ss} (mL kg^{-1}) in the C2 control group. In a similar study undertaken in the same conditions using Gd-DTPA as a dimeglumine salt, no age-related difference in the Vd_{ss} was noted ($Vd_{ss} = 243·3 \pm 14·5$ and $209·0 \pm 13·7$ mL kg^{-1} in 10- and 23-week-old control rats, respectively, data not published). When the Vd_{ss} was expressed in mL, however, an age-dependent increase is noted which is most probably linked with body weight gain.

Analysis of data in diabetic rats. A typical example of the semi-logarithmic plot of the gadolinium blood concentration against time is shown in Fig. 1b based on one rat from each diabetic group. As with the control rats, gadolinium blood levels increased with the age of the animals. The analysis of pharmacokinetic parameters (Table 2) confirm the age-dependent decrease in the gadolinium elimination rate; $t_{\frac{1}{2}\beta}$ and the MRT were significantly greater in older rats, whilst the CL_T was significantly lower. Thus, a similar age-related

Table 2. Pharmacokinetic parameters of Gd-DOTA after intravenous administration in control and diabetic rats.

	$t_{\frac{1}{2}\beta}$ (min)	MRT (min)	Vd_{ss} (mL kg^{-1})	Vd_{ss} (mL)	CL_T (mL kg^{-1} min^{-1})
Control rats					
C1 ~300 g	42·8±8·4	47·6±5·9	231·8±23·3	69·4±6·8	4·67±0·36
C2 4 months	36·8±2·9	71·1±6·5[a]	158·3±8·3[a]	98·2±7·8[a]	2·58±0·25[a]
C3 7 months	119·7±12·5[a]	164·4±21·2[a]	255·5±6·7	195·8±8·2[a]	1·88±0·37[a]
Diabetic rats					
D1 2 months	12·6±1·4	34·9±4·7	202·5±11·7	58·1±6·9	8·80±2·41
D2 4 months	25·6±4·2[bc]	52·9±7·8[bc]	291±23·3[bc]	107·3±9·7[b]	7·58±1·11[c]
D3 7 months	101·9±15·1[b]	114·0±11·9[bc]	401·5±25·9[bc]	128·1±10·8[bc]	3·40±0·29[bc]

Values are mean±s.e.m. for 6 animals in each group. Mann Whitney test: [a] values significantly different from the control group C1 ($P<0·05$); [b] values significantly different from the diabetic group D1 ($P<0·05$); [c] values significantly different from the respective control group (D2 vs C2, D3 vs C3) ($P<0·05$).

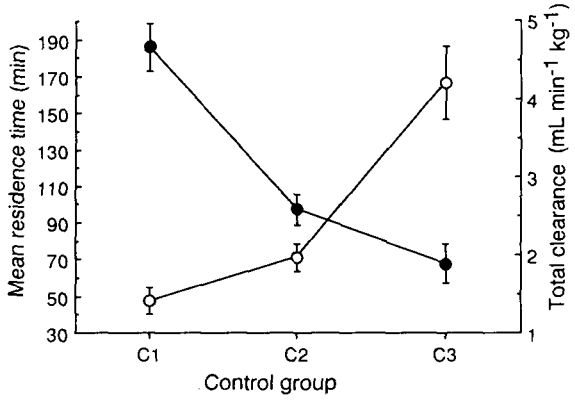

FIG. 2. Evolution with increasing age, of the mean residence time (○) and the apparent total body clearance (●) of Gd-DOTA in control rats. Data represent means±s.e.m. Values obtained from 6 animals in each group.

decrease in gadolinium elimination rate was found in both diabetic and control rats. However, significant differences in various parameters were found between control and diabetic rats at each time point; the apparent slow-phase half-time and the MRT were significantly lower, the CL_T significantly higher in diabetic rats. These results indicate that Gd-DOTA was more rapidly eliminated in diabetic rats. Table 2 also indicates, unlike the control results, an age-dependant increase in the Vd_{ss} which was related to body weight as it was not significantly different in the diabetic rats.

Kidney tissue studies. After an intravenous dose of 0·01 mmol kg^{-1} Gd-DOTA, kidney gadolinium levels were significantly higher in control rats at each time point (Table 3). This result is consistent with the higher blood gadolinium elimination rate found in control rats. There was no evidence of age dependency in the kidney gadolinium levels in either the control or the diabetic rats.

Renal excretion. The cumulated percentages of urine gadolinium recovery are shown in Fig. 3. The recovery of gadolinium, 3 h after intravenous administration reached 60–70% and no statistical differences were noted between control and diabetic rats.

Table 3. Evolution of kidney gadolinium concentrations over time in control and diabetic rats.

	Kidney gadolinium concn (μmol g^{-1})		
	10 min	20 min	45 min
Control rats			
2 months	0·22±0·022	0·418±0·068	0·395±0·042
4 months	0·235±0·011	0·385±0·026	0·343±0·017
Diabetic rats			
2 months	0·075±0·08*	0·047±0·003*	0·054±0·006*
4 months	0·089±0·003*	0·076±0·010*	0·099±0·016*

Values are mean±s.e.m for 4 animals in each group at each time. Mann Whitney test: * values significantly different from respective control group ($P<0·05$).

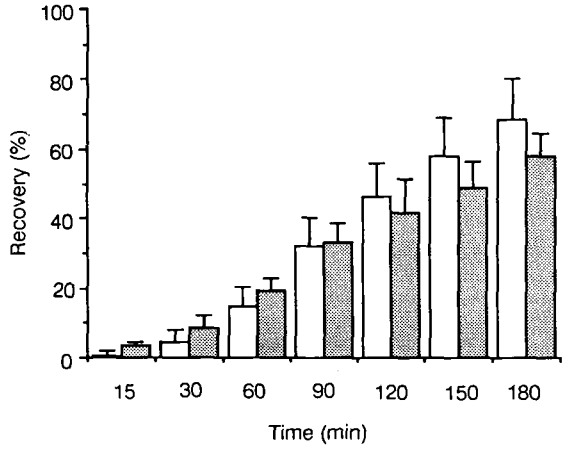

FIG. 3. Gd-DOTA urinary recovery after intravenous administration in control (■) and diabetic (□) rats. Data represents means ±s.e.m. Values obtained from 4 animals in each group at each time point.

Discussion

In control rats, a dramatic increase in body weight gain occurs and has required the use of an additional body-weight-matched control group. Spontaneous obesity in male Wistar rat has already been reported. Newby et al (1990) studied male Wistar rats between the age of 6 weeks and 2

years with body weight from 136 to 917 g. For Newby et al, this model of spontaneous obesity in the chow-fed male Wistar rat results from a combination of ageing, unrestricted access to food and limited spontaneous locomotor activity due to cage restriction. However, body weight gain was annihilated by the diabetic state.

The pharmacokinetic properties of Gd-DOTA after intravenous injection (0·5 mmol kg^{-1}) in the rat are consistent with previous results obtained using different animal species (Doucet et al 1989). In particular, a Vd of about 250 mL kg^{-1} indicates that Gd-DOTA is mainly confined to the vascular and other extracellular aqueous compartments without protein binding. Like iodinated contrast media, Gd-DOTA is rapidly distributed in the extracellular fluids and rapidly excreted by the kidney. The total body clearance, which was between 2 and 5 mL min^{-1} kg in control rats, must be mainly due to renal clearance. This observation is in agreement with those of Weinmann et al (1984) who reported equivalent renal and total body clearance in man after intravenous administration of Gd-DTPA dimeglumine, a similar MRI contrast medium.

In control rats, we observed an age-dependent diminution in the blood gadolinium elimination rate: an increase in the mean residence time associated with a decrease in the apparent total body clearance (mL kg^{-1} min^{-1}) without any evolution in the volume of distribution at steady state (mL kg^{-1}). These results may be consistent with an age-dependent alteration in the kidney function. Yagihashi & Kaseda (1978) described a gradual thickening of the glomerular basement membrane with increasing age in Wistar rats. They noted an even basement membrane in 12–16-week-old rats and a thickening from the age of 24 weeks. Thus, it is possible that the decrease in the apparent total body clearance (mL^{-1}) in control groups (23 and 35 weeks old) is related to a decrease in the glomerular filtration rate induced by the age-related alteration of the glomerular basement membrane. In fact, the effect of ageing on whole kidney glomerular filtration rate (GFR) is not as well documented as the effects of ageing on proteinuria and alterations of glomerular basement membranes which is obvious at 3 months of age (Goldstein et al 1988). Some studies indicate that GFR is not affected whereas others report a reduced GFR in ageing rats. An additional problem comes from the mode of expression of GFR. Goldstein et al (1988) reported a 60% decrease of GFR in 12-month-old male Sprague Dawley rats when expressed on a body weight basis (mL^{-1} kg^{-1}), and only a 12% decrease of total kidney GFR (mL^{-1}kg). Such a discrepancy is also present in our results since no statistical difference appears when apparent total body clearance is expressed in mL kg^{-1}. Goldstein et al also mentioned that the severity of progressive nephropathy in the rat is sex dependant. In general, male rats are more susceptible to age-related nephropathy than females, and these alterations are influenced by food intake. Gehrig et al (1988) reported that long term food restriction preserves GFR in ageing rats.

In control rats, we observed an age-dependent decrease in the blood gadolinium elimination rate: an increase in the MRT associated with a decrease in the CL_T without any effect on the Vd_{ss}. These results consistent with an age-dependent alteration in the kidney function. Yagihashi & Kaseda (1978) described a gradual thickening of the glomerular basement membrane with increasing age in Wistar rats. They noted an even basement membrane in 12 to 16 week-old rats and a thickening from the age of 24 weeks. Thus, it is possible that the decrease in the CL_T in control groups (23 and 35 weeks old) is related to a decrease in the glomerular filtration rate induced by the age-related alteration of the glomerular basement membrane.

So it is possible, that in our experimental conditions, male Wistar rats with free access to food, developed obesity, which, in turn, induced alterations of kidney functions.

Regardless of the age-related evolution in pharmacokinetic behaviour of Gd-DOTA, the diabetic state induced several alterations in the pharmacokinetic parameters studied. The CL_T was significantly higher and the MRT significantly lower in diabetic rats indicating a higher elimination rate of Gd-DOTA. A tubular reabsorption defect induced by the diabetic epithelial degenerative changes in the distal tubule could not be sustained, since Gd-DOTA, as for many iodinated contrast media, is mainly subject to glomerular filtration. On the other hand, an increase in the glomerular filtration rate in diabetic rats may be postulated. Jensen et al (1981) described a rise in the glomerular filtration rate associated with an increase in kidney size and in renal plasma flow in diabetic rats. Thus, the higher CL_T observed in 2- and 4-month-old diabetic rats could be attributed to the increase in the glomerular filtration rate. In 7 month-old diabetic rats, the decrease in the CL_T may be the result of either the age-related thickening of the glomerular basement membrane or a decrease in renal plasma flow induced by the severe dehydration.

The age-related increase in the Vd_{ss} in diabetic rats is not clearly understood, as the Gd-DOTA clearance was higher in these animals and the body weight gain suppressed. A defect in gadolinium protein binding could not be the cause because of the absence of protein binding in such substances. It is possible that this increase in the Vd_{ss} is secondary to a decrease in the tissue perfusion rate induced by diabetic microangiopathy. However, this hypothesis does not completely explain the age-related increase in the Vd_{ss}. Waber et al (1981) reported that the percentage of diabetic capillary basement membrane thickening compared with the control had reached its maximum 4 months after the induction of diabetes.

The gadolinium kidney studies confirm the higher gadolinium elimination rate, and the gadolinium urinary secretion indicate that the diabetic epithelial degenerative changes in the distal tubule have no influence on the amount of gadolinium excreted.

In conclusion, these studies indicate that the pharmacokinetic behaviour of Gd-DOTA (present study) and tempo carboxylic acid (Michel et al 1989) in diabetic rats are different. This may be explained by the fact that the gadolinium chelates, unlike nitroxide derivatives, are not subject to metabolic processes.

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