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Pharmacokinetics of GM1 Ganglioside Following Parenteral Administration

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Abstract—The pharmacokinetic parameters of monosialotetrahexosylganglioside (GM1) have been determined in healthy volunteers at 3 dose levels: 100, 200, 300 mg. Each dose was administered to separate groups of 12 volunteers. GM1 levels were determined in plasma, urine, and faeces by a method based on the property of the cholera toxin β subunit to react specifically with GM1 ganglioside. A non-compartmental model was applied to determine standard pharmacokinetic parameters. The average AUC increased with dose ($1002 \pm 121{\cdot}2$, $1306 \pm 146{\cdot}1$, $3155 \pm 121{\cdot}6$ $\mu g\ mL^{-1}\ h$ after 100, 200, 300 mg, respectively). Plasma clearance was less than 3 mL min^{-1} and the distribution volume was close to the plasma volume (on average between 4·3 and 7·2 L). Mean residence time was about 43 h for all doses. GM1 was not detected in urine, while in faeces the amount of GM1 determined was similar to the baseline values obtained before dosing.

Gangliosides play a role in neuronal differentiation processes (Facci et al 1984; Katoh-Semba et al 1984; Leon et al 1984; Skaper et al 1985). Gangliosides decrease degenerative processes and positively influence the neuronal reparative phenomena after nervous system injuries (Leon et al 1981; Toffano et al 1983a; Tettamanti et al 1984; Agnati et al 1985).

Gangliosides have also been shown to promote and improve functional recovery after brain (Agnati et al 1983; Toffano et al 1983b, 1984a, b, c) and spinal cord lesions (Gorio et al 1986; Commissiong & Toffano 1986).

Monosialotetrahexosylganglioside (GM1) in particular, has beneficial haemodynamic and metabolic effects in the acute phase of ischaemia in cats (Reivich et al 1984; Tanaka et al 1986; Urbanics et al 1989), and was chosen as a promising drug for treating central nervous system injuries due to cerebrovascular accidents (Karpiak & Mahadik 1984; Cahn et al 1986).

In the rat, GM1 exhibited first-order, monoexponential kinetics after intravenous administration, with a plasma half-life of 2·5–3 h and an apparent volume of distribution of 5% body weight (equal to the volume of total plasma). It was preferentially distributed in the liver, spleen, lungs, and kidneys. Biliary excretion is minimal, and GM1 could always be detected in the brain after administration (Lang 1981; Bellato et al 1989).

A study carried out in 502 patients with first hemispheric cerebral infarct showed a significant neurologic improvement in GM1 treated patients as compared with non-GM1 treated patients (Argentino et al 1989). In another study of 119 patients with evidence of subarachnoid haemorrhage, the GM1 treated patients showed an improvement in their clinical level of consciousness (Papo et al 1990).

The aim of the present investigation was the pharmacokinetic evaluation of GM1, following intravenous administration to healthy volunteers.

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Materials and Methods

The investigation was carried out in 36 healthy males (21–35 years, 53–90 kg) and with no major organ disease as documented by medical history and physical examination. Vital signs and laboratory profiles were normal during the three days before entry into the study. No subject smoked more than 10 cigarettes per day, none was a drug abuser or was taking any chronic medication, experimental drugs or drugs with a long half-life during the month before the start of the study. None took any medication (including alcohol) in the 72 h before the study. No subject reported fever.

All volunteers gave their informed consent for the study.

The subjects in groups of 12 were given 100, 200, or 300 mg GM1. All doses were given as a single intravenous bolus, which was approved by the hospital ethics committee.

GM1 basal plasma concentrations were measured in two samples taken the day before drug administration at −18 and −12 h (corresponding to 14.00 h and 20.00 h, respectively, after lunch and before dinner), and in a sample drawn at time 0 (08.00 h after overnight fasting). GM1 plasma concentrations were also determined in samples taken after 0·5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120 and 144 h following GM1 administration.

GM1 concentrations were also measured in urine collected on the day before drug administration (basal) and in samples collected over the time intervals 0–24, 24–48, 48–72, 72–96, 96–120, 120–144 h following drug administration. Urine volume was measured and recorded in the individual case report forms. GM1 in faeces was determined in samples collected on the day before drug administration (basal) and in samples collected over the time intervals 0–24, 24–48, and 48–72 h following drug administration. Faeces were weighed and the amount recorded in the individual case report forms. Immediately after GM1 administration radial blood pressure, heart rate and respiration were measured and recorded in individual case report forms.

The study was carried out in the Ornago Hospital, Dept. of Medicine, Milan (Italy). At each sampling, 5 mL of blood

was collected in heparinized tubes and centrifuged to obtain plasma, which was stored at −20°C.

Urine samples (5 mL) were also stored at −20°C until determination of GM1.

Samples of 0·5 g of faeces per evacuation were treated with 5 mL of buffered tetrahydrofuran before storage at −20°C.

GM1 determinations were performed in Fidia Research Laboratories by the method of Kirschner et al (1988) using the ability of the β subunit of cholera toxin to react specifically with GM1. Cross-reaction with GM1 metabolites and other gangliosides is low; in the range 50–1000 pmol L^{-1} the cross-reaction with GT1b is 1·1%, GD1a 1·6% and GD1b 2·4%.

Data registered on case report forms were collected on a Digital VAX 11/780 utilizing the CLINICS data base an in-house, clinical data management system. Data were processed using an SAS Statistical Package (SAS Institute Inc., Box 8000, Cary, North Carolina 2711-800). For each volunteer the distribution volume (V_d), distribution volume per kg of bodyweight (V_d kg^{-1}), area under curve (AUC), total clearance (CL_t) and mean residence time (MRT) were calculated according to a non-compartmental model (DiStefano 1982).

Mean, standard error of mean and standard deviation were calculated for each of the above parameters.

Results

All volunteers completed the study; but as a result of an error in technique the samples of volunteer n° 1 were rejected. GM1 plasma concentrations were, therefore, obtained in 35 subjects.

Table 1 illustrates mean GM1 plasma concentrations over 0–144 h after 100, 200 and 300 mg doses.

GM1 was not detected in any of the urine samples, and the concentrations of GM1 in faeces did not differ from the concentrations obtained the day before treatment (Table 2). Fig. 1 shows GM1 plasma concentration plotted vs time after 100, 200, 300 mg i.v. administration.

A summary of the pharmacokinetic results is given in Table 3.

Table 1. Mean GM1 plasma concentrations ($\mu g\ mL^{-1}$) from 0 to 144 h after 100, 200, 300 mg, i.v. single bolus dose.

Time (h)	Mean conc ± s.d. (GM1 100 mg)	Mean conc ± s.d. (GM1 200 mg)	Mean conc ± s.d. (GM1 300 mg)
−18·0	0·231 ± 0·105	0·199 ± 0·089	0·353 ± 0·121
−12·0	0·221 ± 0·111	0·183 ± 0·093	0·344 ± 0·125
0·0	0·368 ± 0·617	0·207 ± 0·106	0·338 ± 0·225
0·5	26·193 ± 10·195	39·307 ± 16·316	93·357 ± 11·718
1·0	22·045 ± 8·528	49·328 ± 14·628	94·851 ± 11·094
2·0	20·096 ± 8·613	37·867 ± 12·368	90·963 ± 12·601
4·0	19·205 ± 7·585	27·761 ± 7·867	81·012 ± 15·845
6·0	18·822 ± 8·858	27·019 ± 14·853	69·410 ± 14·074
8·0	16·038 ± 7·060	20·175 ± 9·783	45·906 ± 11·277
12·0	12·665 ± 5·636	19·098 ± 10·774	39·382 ± 9·889
24·0	10·846 ± 5·802	14·655 ± 5·238	36·121 ± 10·837
36·0	12·079 ± 5·328	12·304 ± 5·484	25·885 ± 7·646
48·0	8·130 ± 4·093	10·071 ± 4·969	21·080 ± 5·563
72·0	5·052 ± 2·591	6·673 ± 2·739	19·014 ± 5·351
96·0	4·215 ± 3·245	4·604 ± 2·476	12·373 ± 2·825
120·0	2·126 ± 1·331	3·046 ± 1·336	8·537 ± 2·395
144·0	1·573 ± 0·943	1·808 ± 0·661	4·688 ± 1·081

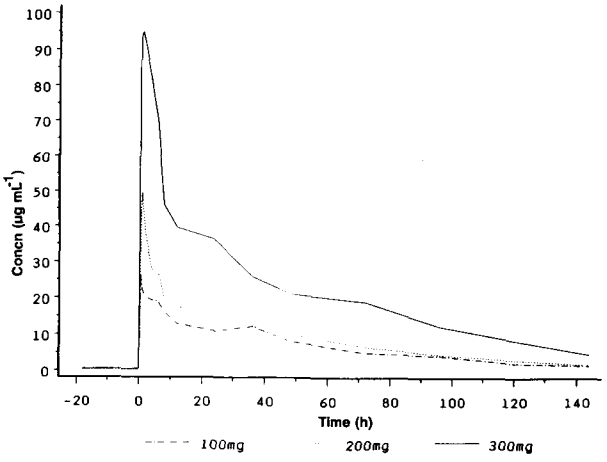

FIG. 1. Mean GM1 plasma concentration plotted vs time after 100 mg (–·–·–), 200 mg (·····), 300 mg (——) single i.v. bolus.

Compartmental and non-compartmental analyses were applied to determine the common pharmacokinetic parameters. The non-compartmental model was found to be the most suitable because: (a) the drug is injected directly into a "central" pool; (b) the drug is directly eliminated from this "central pool"; (c) vascular-extravascular exchanges are relatively rapid.

No significant change in vital signs (blood pressure, respiratory frequency and heart rate of volunteers) was observed after GM1 administration.

Discusssion and Conclusions

Although the time course of plasma GM1 in animals seems to require a one compartment model, in particular when the β phase is analysed (Bellato et al 1989), data obtained in this

Table 2. GM1 (μg) excreted in faeces (mean ± s.e.).

Dose (mg)	Time (h)	Faecal excretion $\mu g \pm$ s.e.
100	−24– 0	387·2 ± 121·53
	0–24	315·4 ± 115·98
	24–48	319·5 ± 89·84
	48–72	298·0 ± 114·43
200	−24– 0	142·5 ± 30·32
	0–24	151·8 ± 29·57
	24–48	243·0 ± 109·40
	48–72	443·4 ± 280·05
300	−24– 0	696·1 ± 169·42
	0–24	658·2 ± 206·30
	24–48	581·5 ± 103·04
	48–72	515·5 ± 195·09

Table 3. Pharmacokinetic paramaters (mean ± s.e.) after intravenous 100, 200, 300 mg GM1.

GM1 (mg)	AUC ($\mu g\ mL^{-1}\ h$)	CL_t ($mL\ min^{-1}$)	V_d (L)	MRT (h)
100	1002 ± 121·2	1·9 ± 0·23	4·9 ± 0·51	43·4 ± 2·14
200	1306 ± 146·1	2·8 ± 0·26	7·2 ± 0·66	42·1 ± 0·87
300	3155 ± 121·6	1·6 ± 0·07	4·3 ± 0·24	44·6 ± 1·19

study population cannot be fitted to a single compartmental model: at least two phases of elimination were seen when plasma concentrations were plotted against time.

Baseline concentrations of GM1 determined the day before the exogenous administration (−18 and −12 h) and at time 0 (the day of the administration) show that some of the fluctuation is physiological; a further confirmation of this is shown by the amount found in faeces. This is different in the three groups of volunteers showing possible large variations of the GM1 turnover in man.

The levels of "physiological" GM1 in plasma are far less than 1 $\mu g\ mL^{-1}$; the exogenous administration of GM1 increases the plasma concentration in such a way that the baseline values become irrelevant for a relatively long period (at least 48 h). However, in the pharmacokinetic model the baseline values were subtracted from the concentrations obtained after exogenous GM1 administration.

One of the main characteristics of GM1 is its relatively long MRT of around 43 h which leads to, on average, a period of about 7 days to reach steady-state, given single daily dosing.

With such a long MRT the plasma concentrations will be similar whether the drug is infused (5–10 min) or administered as a slow bolus (10–30 s); this is also evident from the analysis of the plasma concentration data which are similar for the first 2 h after the drug administration.

The distribution volume is similar to the plasma volume and it would be expected that the concentration in tissues would be low and in many tissues be lower than the plasma concentration. The plasma clearance is less than 3 $mL\ min^{-1}$ and this is consistent with the observation that GM1 was not detected in urine, while in faeces the amount determined after exogenous administration did not differ from baseline values.

The AUC is roughly proportional to the dosage although one might have expected the dose of 200 mg to give an average value higher than that observed ($1306 \pm 146{\cdot}1$); the variability in the AUC at this dosage was higher than at 100 and 300 mg and therefore it seems probable that this value is low. When normalized for body weight the relationship between AUC and the dosage has a correlation coefficient of 0·7479 ($P < 0{\cdot}05$).

Cellular catabolism probably determines the half-life of GM1. Renal excretion of unchanged GM1 is practically absent possibly due to reabsorption, and intestinal elimination is minimal. The GM1 found in faeces probably comes from food and represents ganglioside that cannot be absorbed from the gastrointestinal tract.

GM1 is involved in pathophysiologic processes, and its pharmacokinetics following administration are complex and worthy of further investigation.

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