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Impact of chemical modification of sulfamidase on distribution to brain interstitial fluid and to CSF after an intravenous administration in awake, freely-moving rats

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ABSTRACT

Mucopolysaccharidosis III A (MPS IIIA) is an autosomal recessive lysosomal storage disorder caused by deficiency of the enzyme sulfamidase. The disorder results in accumulation of heparan sulfate, lysosomal enlargement and cellular and organ dysfunction. Patients exhibit progressive neurodegeneration and behavioral problems and no treatment is currently available. Enzyme replacement therapy is explored as potential treatment strategy for MPS IIIA patients and to modify the disease, sulfamidase must reach the brain. The glycans of recombinant human sulfamidase (rhSulfamidase) can be chemically modified to generate CM-rhSulfamidase. The chemical modification reduced the affinity to the cation-independent mannose-6-phosphate receptor with the aim a prolonged higher concentration in circulation and thus at the blood brain barrier. The pharmacokinetic properties in serum and the distribution to brain and to cerebrospinal fluid (CSF) of chemically modified recombinant human sulfamidase (CM-rhSulfamidase) were studied and compared to those of rhSulfamidase, after a single intravenous (i.v.) 30 mg/kg dose in awake, freely-moving male Sprague Dawley rats. Distribution to brain was studied by microdialysis of the interstitial fluid in prefrontal cortex and by repeated intra-individual CSF sampling from the cisterna magna. Push-pull microdialysis facilitated sampling of brain interstitial fluid to determine large molecule concentrations in awake, freely-moving male Sprague Dawley rats. Together with repeated serum and CSF sampling, push-pull microdialysis facilitated determination of CM-rhSulfamidase and rhSulfamidase kinetics after i.v. administration by non-compartments analysis and by a population modelling approach. Chemical modification increased the area under the concentration versus time in serum, CSF and brain interstitial fluid at least 7-fold. The results and the outcome of a population modelling approach of the concentration versus time data indicated that both compounds pass the BBB with an equilibrium established fairly rapid after administration. We suggest that prolonged high serum concentrations facilitated high brain interstitial fluid concentrations, which could be favorable to reach various target cells in the brain.

1. Introduction

Mucopolysaccharidosis III A (MPS IIIA), or Sanfillipo Type A, is an autosomal recessive lysosomal storage disorder. The disease is characterized by severe and progressive loss of cognitive and motor functions, behavioral difficulties and eventually death in the second decade of life, although the severity and progression of the disease varies widely [1,18]. MPS IIIA is caused by mutations in the *SGSH* gene that results in deficiency of the N-sulfoglucosamine sulfohydrolase enzyme sulfamidase (EC 3.10.1.1) and subsequent accumulation of undegraded heparan sulfate (HS), lysosomal enlargement and cellular and organ dysfunction. Patients exhibit progressive neurodegeneration and

Abbreviations: aCSF, artificial cerebrospinal fluid; AUC_{∞}, area under the concentration-time curve from $t = 0$ to infinity; AUC_{last}, area under the concentration-time curve from $t = 0$ to the last observed concentration; IF, interstitial fluid; CL, clearance; C_{max}, maximum concentration; CM-rhSulfamidase, chemically modified recombinant human sulfamidase; CNS, central nervous system; CSF, cerebrospinal fluid; h.a.d., hours after dose; HS, heparan sulfate; ID, identifier; i.v., intravenous; LLOQ, lower limit of quantification; M6PR, mannose-6-phosphate receptor; MPS IIIA, mucopolysaccharidosis type III A; MSD-ECL, meso scale discovery electrochemiluminescence; PBS, phosphate buffered saline; PK, pharmacokinetics; rhSulfamidase, recombinant human sulfamidase; SD, standard deviation; SGSH, N-sulfoglucosamine sulfohydrolase; $t_{½}$, terminal half-life; V, volume of distribution

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behavioral problems including hyperactivity, a reduced sense of danger, aggression and sleep disturbances [1,18].

Enzyme replacement therapy as potential treatment strategy for MPS IIIA patients has been explored by intravenous (i.v.) administration of recombinant human sulfamidase (rhSulfamidase) in MPS IIIA mice [5] or via the intra-cerebrospinal fluid (CSF) route in MPS IIIA patients [11]. Repeated i.v. administration of rhSulfamidase to a MPS IIIA mouse model did not have an effect on a lysosomal storage biomarker, the disaccharide GlcNS-UA, in brain [15]. However, when combined with intra-CSF or after intra-CSF administration alone, repeated intra-CSF rhSulfamidase administration reduces HS storage and neuropathology in brain [7,8]. Passage of the blood brain barrier (BBB) is thus important for rhSulfamidase to have effect in brain.

CM-rhSulfamidase is a chemically modified variant of recombinant human sulfamidase intended for treatment of patients suffering from MPS IIIA. rhSulfamidase has an unglycosylated calculated molecular weight of 55 kDa, has four N-glycosylation sites and forms dimers in solution [16]. The chemical modification procedure used to generate CM-rhSulfamidase results in a modification of its glycans, which strongly reduces uptake of the enzyme into peripheral tissues by reducing the affinity of CM-rhSulfamidase to the cation-independent mannose-6-phosphate receptor [6]. The modification conditions are mild to maintain the structure and activity of the enzyme in the resulting drug substance. Using this chemical modification protocol, repeated i.v. administration of CM-rhSulfamidase reduced biomarkers HS, the disaccharide GlcNS-UA and the tetrasaccharide, GlcNS-UA-GlcNAc-UA (+1S) in brain of MPS IIIA mice [6,12].

The objectives of this study were to quantify the impact of chemical modification on the pharmacokinetics (PK) of rhSulfamidase in serum, in CSF and in brain interstitial fluid (IF). Brain IF was obtained by microdialysis in the prefrontal cortex from awake, freely-moving male Sprague Dawley rats, after a single intravenous (i.v.) dose of CM-rhSulfamidase or rhSulfamidase. The concentration versus time profiles of CM-rhSulfamidase were compared to those obtained with unmodified rhSulfamidase to explore the impact of chemical modification on distribution to the central nervous system (CNS).

2. Methods

Thirty-one male Sprague Dawley rats (308–510 g in three separate experiments; Envigo, The Netherlands and Charles River Laboratories, Germany) were used for the experiments. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals [13], with European Union directive 2010/63 and the Dutch law. The *in vivo* studies were carried out under a license issued by the national committee for licensing of animal experiments (Centrale Commissie Dierproeven) and were approved by the Animal Care and Use Committee (Instantie voor Dierenwelzijn) of Charles River Laboratories, Groningen, The Netherlands.

2.1. In vitro recovery

In vitro experiments were performed to determine the recovery over the push-pull PP-PES 200–6/4 probes (CNS probes with polyethersulfone (PES200) membrane with a cut-off of 2000 kDa from Charles River Laboratories, Groningen, The Netherlands). To this end, probes were placed in a beaker containing 10 nM of (CM-) rhSulfamidase diluted in artificial CSF (aCSF) to which 0.2% (*w/v*) bovine serum albumin (BSA) was added. aCSF was composed of 147 mM NaCl, 3.0 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$ in ultrapurified water. The probes (PES200 membranes) were perfused with 0.2% BSA/aCSF at 37 °C and a flow rate of 0.25 μL/min. After pre-stabilization, four microdialysis samples were collected in 60-min intervals. In addition, samples were taken from the beaker content. All samples were collected into polypropylene microvials (Microbiotech/se AB, Sweden; Cat. No. 4001048) and stored at −80 °C. Recovery was calculated as the mean concentration in dialysate divided by the mean observed concentration in the beaker samples. Beaker sample concentrations were found to be consistent over time, indicating that the compound did not degrade over time under the experimental conditions.

2.2. In vivo - surgery

Rats were anesthetized using isoflurane (2.0–2.5% and 800 mL/min O_2). Before surgery, Finadyne (1 mg/kg, s.c.) was administered for analgesia during surgery and the post-surgical recovery period. A mixture of bupivacaine and epinephrine was used for local analgesia of the incision site and periost of the skull.

The animals were placed in a stereotaxic frame (Kopf Instruments, USA). Guides (Charles River Laboratories, Groningen, The Netherlands) for push-pull microdialysis probes were implanted bilaterally into the prefrontal cortex (coordinates for the tip of the probes: AP = +3.4 mm from bregma, lateral = ± 3.0 mm from midline, ventral = −5.0 mm from dura). The incisor bar was set at −3.3 mm. All coordinates were based on "The rat brain in stereotaxic coordinates" by Paxinos and Watson [14]. The guides were attached to the skull with stainless steel screws and dental cement.

In the same surgical procedure, an indwelling cannula was inserted into the cisterna magna. The cannula was fixed in position with dental cement and attached to the skull with stainless steel screws. A catheter was also placed into the jugular vein in the same surgical procedure to accommodate blood sampling. The jugular vein catheter was inserted into the vein and held in place using a retention bead. The other end was exteriorized through an incision on top of the skull and fixed in position with dental cement and attached to the skull with stainless steel screws. Animals were allowed at least three days recovery from surgery before sample collection was initiated.

2.3. In vivo - compound administration and sampling

The cloning, expression and purification of rhSulfamidase and for CM-rhSulfamidase the additional chemical modification are described in Gustavsson et al. [6]. Per compound, 7 male Sprague Dawley rats received a single 30 mg/kg i.v. dose administered through the tail vein on Day 1 at $t = 0$. The compounds were formulated as 6 mg/mL in 50 mM arginine, 75 mM NaCl, 20 mg/mL sucrose, 0.01% Tween 20, pH 7.8.

Serum exposure was determined after a single i.v. administration in the tail vein at 5 mL/kg at predose, 0.083, 0.5, 1, 2, 6, 24, 30, 48, 72, 96 and 100 (terminal) hours after dose (h.a.d.). Blood samples were collected into polypropylene serum collection tubes (BD, The Netherlands) without anticoagulant, and were permitted to clot for 30–60 min at room temperature. The blood samples were centrifuged at 1500 ×*g* for 10 min at 4 °C. Serum was stored at −80 °C.

CSF samples were taken from the cisterna magna through the cannula at 1 day before and at 0.5, 1, 2, 6, 24, 48, 72, 96 and 100 (terminal) hours after i.v. administration. Basal and experimental CSF samples had a volume of approximately 25 μL, and terminal CSF samples had a volume of > 25 μL. Samples were collected into polypropylene microvials (Microbiotech/se AB, Sweden) and stored at −80 °C. Prior to bioanalysis CSF was scored for hemolysis by visual inspection.

Push-pull microdialysis probes with 4 mm open membrane surfaces (PP-PES 200–6/4; Charles River Laboratories, Groningen, the Netherlands) were inserted into the guides of the animals the day before microdialysis sampling. On experimental days, the push-pull microdialysis probes were connected with flexible PEEK tubing (Western Analytical Products Inc., USA) to a microperfusion pump (CMA Microdialysis, Sweden) and perfused with 0.2% BSA/aCSF at a flow rate of 0.25 μL/min. After a minimum of 1 h of pre-stabilization, with at least 4 stable flow check samples (10 min each), microdialysis samples

were collected in 60-min intervals on Day 1 (15 μL/sample to which 15 μL aCSF was added) and 120-min intervals on Days 2–4 (30 μL/sample), with on Day 1 from 1 h before until 6 h post administration and on Days 2, 3 and 4 for 6 h per day, 24–30 h.a.d., 48–54 h.a.d. and 72–78 h.a.d.. Samples were collected into polypropylene microvials (Microbiotech/se AB, Sweden) using an automated fraction collector (UV 8301501, TSE, Univentor, Malta). Microdialysate samples were stored at −80 °C.

A total of three independent experiments were performed with CM-rhSulfamidase, one included rhSulfamidase as comparator, and representative data are presented in this paper.

2.4. Bioanalysis

The concentrations of rhSulfamidase or CM-rhSulfamidase were determined by a Meso Scale Discovery electrochemiluminescence (MSD-ECL) based method [6]. In short, MSD multi-array streptavidin coated plates were blocked with 5% Blocker A in Phosphate-Buffered Saline (PBS) for > 1 h on a shaker at 500 rpm. After blocking 25 μL of the sample dilutions (standard, control and unknowns respectively) and 25 μL mixed conjugates (capture mouse anti-human sulfamidase mAb 25-5-5-(PEG4)-biotin and detection rabbit 7383 anti-human sulfamidase-Sulfo-Ru-conjugate, both custom-made by Biogenes, Germany) were added to the MSD plates. The plates were incubated for 60 min at room temperature on a plate shaker at 500 rpm or overnight at 4 °C without shaking. The plate was washed with PBS-Tween and 4× MSD Read buffer (diluted 1:1 with Milli-Q water) was added before the plates were read in an MSD QuickPlex SQ120 instrument. Standard curves were fitted using a four-parameter curve fit. The final CM-rhSulfamidase or rhSulfamidase concentrations were calculated relative to this curve fit and the dilution factor of the sample.

For determination of compound concentrations in serum, CSF and brain microdialysate samples, 1% MSD blocker A in PBS-T was used for diluting samples and standards. For brain microdialysate the lower limit of quantification (LLOQ) for the assay with CM-rhSulfamidase as standard was 26 pM, for rhSulfamidase it was 4 pM, but values above the LOQ of individual plates were accepted. The concentrations as determined in the brain microdialysate samples were adjusted for the *in vitro* recovery value to obtain the brain IF concentration. In addition, the small dialysate volumes at Day 1 were diluted 1:1 with aCSF and compensation for this is also included in the values for brain IF concentrations as presented here.

2.5. Evaluation of the PK parameters

Individual and mean ± standard deviation (SD) compound concentrations in serum, CSF and brain microdialysis samples were plotted against nominal time. The values obtained in dialysates were appointed to the mid time-point of the collection period, i.e. the first sample after the dose at 0.5 h. The maximum compound concentration in serum, CSF or brain IF, C_{max}, and the time at which C_{max} was reached, t_{max}, were obtained directly from the concentration versus time data per animal identifier (ID). To obtain PK parameters, concentration versus time data were analyzed per ID by Non-Compartmental Analysis (NCA) using Phoenix 64 WinNonlin version 8.0 NMLE (Phoenix, Pharsight Corp., USA) using linear up-log down extrapolation and the best fit option for the determination of the terminal half-life. Per compound and serum, CSF or brain IF compartment, the values for each PK parameter estimate as obtained per ID were averaged to obtain the mean, as presented below. Concentrations below LOQ were not included in the analysis.

The serum concentration versus time profiles showed distribution and elimination phases. To facilitate estimation of volumes and clearances for each phase and distribution to CSF and brain IF, a compartmental analysis was performed using the software package described above and a population modelling approach. Model alternatives tested were compartment number, though not < 2 for the serum compartment, residual error models, number of transit compartments to brain IF, introduction and estimation of CSF turnover, clearance from CSF and from brain IF compartments. Alternatives were evaluated based on successful convergence, objective function value and visual evaluation of goodness-of-fit. The *in vivo* phase of the experiments presented here included CM-rhSulfamidase or rhSulfamidase concentrations in a total of 207 (98 and 109) microdialysis, 100 (49 and 51) CSF, and 149 (72 and 77) serum samples.

3. Results

3.1. In vitro and in vivo phases

Push-pull microdialysis of brain IF in prefrontal cortex and repeated intra-individual CSF sampling from the cisterna magna was performed in awake, freely-moving male Sprague Dawley rats. The described methods facilitated the study of the distribution to brain of the large molecules CM-rhSulfamidase and rhSulfamidase, which in solution form a dimer with a total unglycosylated molecular weight of 110 kDa, after a single intravenous (i.v.) 30 mg/kg dose in awake, freely-moving male Sprague Dawley rats. The microdialysis probes had been targeted towards the correct brain area as determined by post-mortem visual verification. *In vitro* recovery over the probe was 22% for CM-rhSulfamidase and 33% for rhSulfamidase. CSF samples were visually scored for blood contamination, but high CM-rhSulfamidase or rhSulfamidase values were not always correlated to visible blood contamination. No values were excluded based on hemolysis. As expected, no blood contaminations were found in microdialysis samples.

3.2. PK as quantified by NCA

Per individual rat a concentration versus time relationship was obtained in serum, CSF and brain IF. The PK and distribution profiles of CM-rhSulfamidase (Fig. 1) and rhSulfamidase (Fig. 2) had some common traits. The maximum concentration was reached first in serum, then in CSF and t_{max} was most delayed in brain IF (Table 1). C_{max} was at least 400-fold higher in serum relative to the C_{max} observed in CSF and in brain IF. AUC_{last} and AUC_{∞} were also highest for exposure in serum. For CM-rhSulfamidase and rhSulfamidase, AUC_{∞} in CSF were 0.3 and 0.8% of their respective AUC_{∞} in serum. For AUC_{∞} of brain IF, this was 0.3% for CM-rhSulfamidase and 0.2% for rhSulfamidase. Mean terminal half-life, $t_{½}$, was slightly shorter in brain IF relative to those observed in serum and CSF (Table 1).

There were also differences in PK profile between CM-rhSulfamidase and rhSulfamidase. In rats administered rhSulfamidase serum concentrations declined rapidly shortly after the dose, before stabilizing at a low level (Fig. 2), while CM-rhSulfamidase

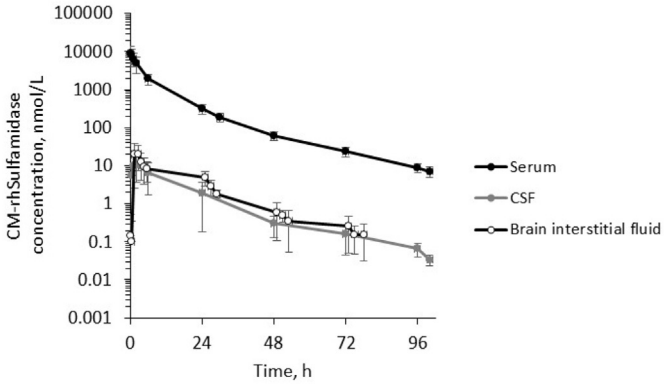

Fig. 1. Mean ± standard deviation concentration of CM-rhSulfamidase in serum, CSF and brain IF after a 30 mg/kg i.v. administration to male Sprague Dawley rats (n = 7).

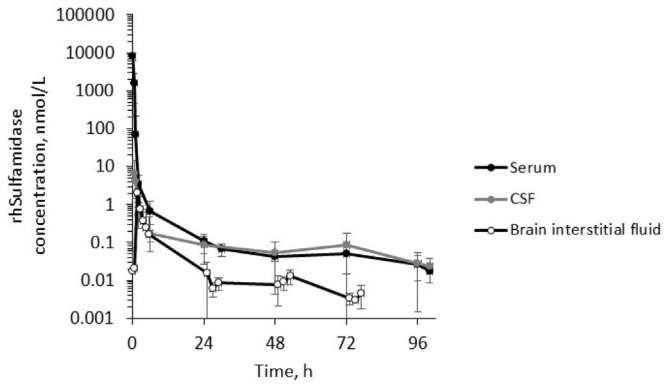

Fig. 2. Mean ± standard deviation concentration of rhSulfamidase in serum, CSF and brain interstitial fluid after a 30 mg/kg i.v. administration to male Sprague Dawley rats (n = 7).

concentrations showed a more gradual decline. Mean CM-rhSulfamidase concentrations in serum were higher than the concentration in CSF and brain IF over the observation period (Fig. 1). But in rats administered rhSulfamidase, concentrations in serum were similar to compound levels in CSF from 24 h after the dose and onwards (Fig. 2). Chemical modification of rhSulfamidase resulted in a similar C_{max} in serum relative to rhSulfamidase, but thereafter a prolonged higher concentration was observed (Fig. 3A) resulting in an 18-fold higher AUC_{last} and AUC_{∞} relative to rhSulfamidase (Table 1). In CSF, AUC_{∞} was 7-fold higher for CM-rhSulfamidase relative to the AUC_{∞} of rhSulfamidase (Fig. 3B and Table 1). In brain IF, AUC_{last} and AUC_{∞} were 48-fold higher for CM-rhSulfamidase relative to rhSulfamidase (Fig. 3C and Table 1).

Compound levels above LOQ were measured in all individual rats in CSF sampled at 0.5 h after the dose, but in the microdialysis samples obtained from 0 to 1 h after the dose compound could only be quantified in 2 of 7 animals dosed with rhSulfamidase and in 2 of 7 rats administered CM-rhSulfamidase. In the animals dosed with CM-rhSulfamidase the maximum CSF and brain IF concentrations differed between individual rats, but after reaching the maximum level reduction in concentration occurred at a similar rate. CSF and brain IF concentrations declined fast shortly after administration in rhSulfamidase dosed rats (Fig. 3).

3.3. Determination of kinetics by a population modelling approach

Repeated serum and CSF sampling and push-pull microdialysis facilitated determination of CM-rhSulfamidase and rhSulfamidase kinetics after i.v. administration by a population modelling approach. Population modelling and simulation of serum, CSF and IF brain concentration versus time data resulted in two different models, one for CM-rhSulfamidase and one for rhSulfamidase (Fig. 4). The estimate for clearance from the central compartment was 12-fold higher for rhSulfamidase relative to CM-rhSulfamidase (Table 2). The estimated distribution clearances into and out of the CSF compartment were similar for the two compounds despite the large differences in serum concentration versus time profiles. Similar distribution clearances were also obtained for the distribution of CM-rhSulfamidase from serum into and out of brain IF. For rhSulfamidase the estimate of the distribution clearance into brain IF was uncertain and a high rate through the transit compartment was estimated (Table 2). The estimates of the model parameters are presented in Table 2 and a visual goodness-of-fit in Figs. 5 and 6.

4. Discussion

The results presented in this paper confirm the possibility to use push-pull microdialysis to sample brain IF and determine concentrations of large molecules in awake, freely-moving rats. Microdialysis has been used previously for protein and peptide biomarkers in mouse, rat and even human brain parenchyma [4,9,19]. Following i.v. administration microdialysis was used to study antibody kinetics in liver, skin, kidney, and muscle in mouse [10] and in brain IF and CSF of rats [2]. In this paper, push-pull microdialysis facilitated determination of CM-rhSulfamidase and rhSulfamidase kinetics in IF of brain and this was combined with repeated serum and CSF sampling, after i.v. administration.

The maximum CM-rhSulfamidase and rhSulfamidase concentrations were reached first in serum, followed by CSF and t_{max} was highest in brain IF. Because the t_{max} was derived directly from the concentration versus time data in each individual rat, the time-points at which samples were taken can have an impact on its value. No serum and CSF samples were obtained between 2 and 6 h after the dose, but mean t_{max} was below 2 h after the dose. Microdialysis samples were obtained at 1-h intervals up to 6 h after the dose on Day 1. The values of the brain IF concentrations were entered at the mid time point over the collection period. For CM-rhSulfamidase and rhSulfamidase, mean t_{max} in brain IF were 5.6 and 1.8 h, respectively. In all three compartments, t_{max} was smaller for rhSulfamidase relative to CM-rhSulfamidase. Sampling time-points might have had a minor impact on the mean t_{max}, but the rank order is not expected to change with different sampling times.

Another confounding factor in the determination of t_{max} was the void volume of the tubing from the probe to the sample collector, thereby creating a possible delay. On the other hand, both C_{max} was lower and t_{max} later in brain IF relative to the PK profile in CSF, which assuming minimal mixing in the tubing, would also point to a delay relative to CSF. This together with only a minor decline in CSF concentrations between 0.5 and 1 h after the dose pointed to a biological delay of distribution to brain IF.

Surprisingly, from 24 h after dose and onwards rhSulfamidase concentrations in CSF and serum were similar possibly due to local redistribution or compound retention. In adult rat the total CSF volume is about 400 µL. The CSF volume in three months old rats is about 270 µL. The turnover rate of CSF is 9–11 per day in rats between 3 and 20 months of age, which translates in a CSF production rate of 84 µL/h or 1.4 µL/min [3]. Inclusion of CSF turnover in the model was explored but did not improve the goodness-of-fit and was therefore rejected as modelling approach with this data-set. The AUC of CSF and brain IF is < 1% of the AUC in serum and C_{max} is much lower in CSF and brain

Table 1
Mean PK parameters as determined by Non-Compartmental Analysis of CM-rhSulfamidase and rhSulfamidase in serum, CSF and brain interstitial fluid after a 30 mg/kg i.v. administration to male Sprague Dawley rats.

Compartment	Compound	t_{max} (h)	C_{max} (nmol/L)	AUC_{last} (h*nmol/L)	AUC_{∞} (h*nmol/L)	$t_{½}$ (h)
Serum	CM-rhSulfamidase	0.27	$9.95*10^3$	$49.6*10^3$	$49.7*10^3$	16.1
	rhSulfamidase	0.08	$8.09*10^3$	$2.69*10^3$	$2.69*10^3$	29.3
CSF	CM-rhSulfamidase	1.7	13.8	145	146	12.4
	rhSulfamidase	0.7	7.08	17.6	21.3	28.6
Brain interstitial fluid	CM-rhSulfamidase	5.6	22.9	224	227	11.4
	rhSulfamidase	1.8	2.14	4.68	4.76	12.2

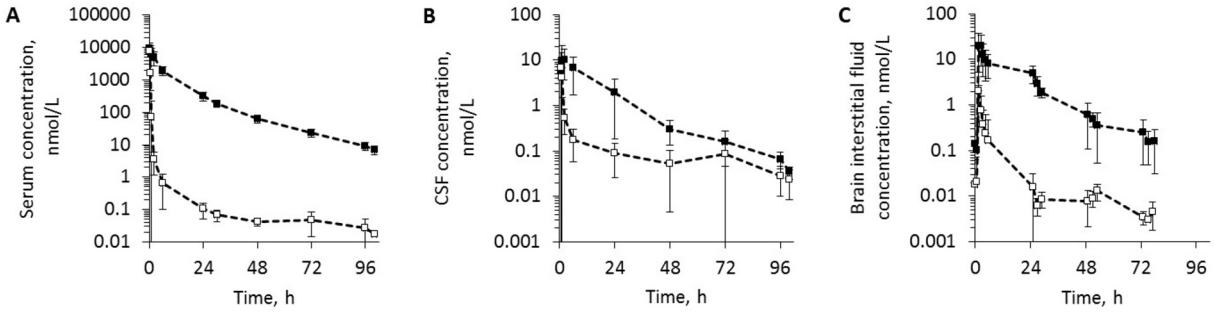

Fig. 3. Mean ± standard deviation serum (A), CSF (B) and brain interstitial fluid (C) concentration of CM-rhSulfamidase (filled symbols) or rhSulfamidase (open symbols) after a 30 mg/kg i.v. administration to male Sprague Dawley rats, as compiled from Figs. 1 and 2.

IF than in serum. rhSulfamidase and CM-rhSulfamidase have a low passage across the blood-CSF and blood brain barriers possibly aggravated by a high efflux to serum from these compartments. To maintain CSF concentrations at the level of serum concentrations as observed for rhSulfamidase from 24 h after the dose, a redistribution of compound had to take place to replenish and compensate for the constant production of CSF and efflux. This was further confirmed by the addition of a second distribution CSF compartment for rhSulfamidase to optimize the model fit.

The *in vitro* recovery differed between CM-rhSulfamidase and rhSulfamidase, 22% for CM-rhSulfamidase and 33% for rhSulfamidase. The impact of a high recovery is a smaller factor when adjusting the microdialysis concentrations. The AUC_{last} and AUC_{∞} for brain IF was 18-fold higher for CM-rhSulfamidase relative to that for rhSulfamidase, hence a difference in *in vitro* recovery cannot explain this change in PK profile. CSF was sampled directly, i.e. without a microdialysis probe, and the AUC_{last} and AUC_{∞} of CSF were 7-fold higher for CM-rhSulfamidase relative to rhSulfamidase.

Chemical modification of rhSulfamidase does not always result in a successful central effect as published by Rozaklis et al. [15]. Neither does repeated i.v. administration of unmodified rhSulfamidase [7,8,15]. In neonate mice, sulfamidase transported across the BBB at a rate higher than that of albumin and this transport was mediated by the

Table 2
Pharmacokinetic model parameters and their estimates.

Compartment	Parameter	Unit	CM-rhSulfamidase	rhSulfamidase
Serum	V	mL/kg	53 (9.1)	21 (9.6)
	CL	mL/(kg*h)	11 (6.8)	136 (6.5)
	V2	mL/kg	27 (27)	13 (18)
	CL2	mL/(kg*h)	7.6 (32)	0.25 (12)
	V3	mL/kg	27 (9.7)	1.0 (27)
	CL3	mL/(kg*h)	1.2 (14)	0.40 (16)
CSF	CL to CSF	mL/(kg*h)	1.2E-3 (18)	1.8E-3 (26)
	V	mL/kg	1.0 (31)	0.56 (29)
	CL from CSF	mL/(kg*h)	0.36 (19)	0.43 (21)
	V4	mL/kg	N.A.	16 (18)
	CLCSF2	mL/(kg*h)	N.A.	0.59 (30)
Brain interstitial fluid	CL to BIF	mL/(kg*h)	2.1E-3 (25)	1.3E-2 (83)
	ktrans	1/h	0.15 (10)	1.2 (25)
	V	mL/kg	0.18 (32)	17 (21)
	CL from BIF	mL/(kg*h)	0.42 (22)	13 (42)
	V5	mL/kg	N.A.	312 (46)
	CLBIF2	mL/(kg*h)	N.A.	5.1 (22)

Abbreviations: N.A. not applicable. Values between brackets are the coefficient of variation in % and calculated as (standard deviation/mean) * 100.

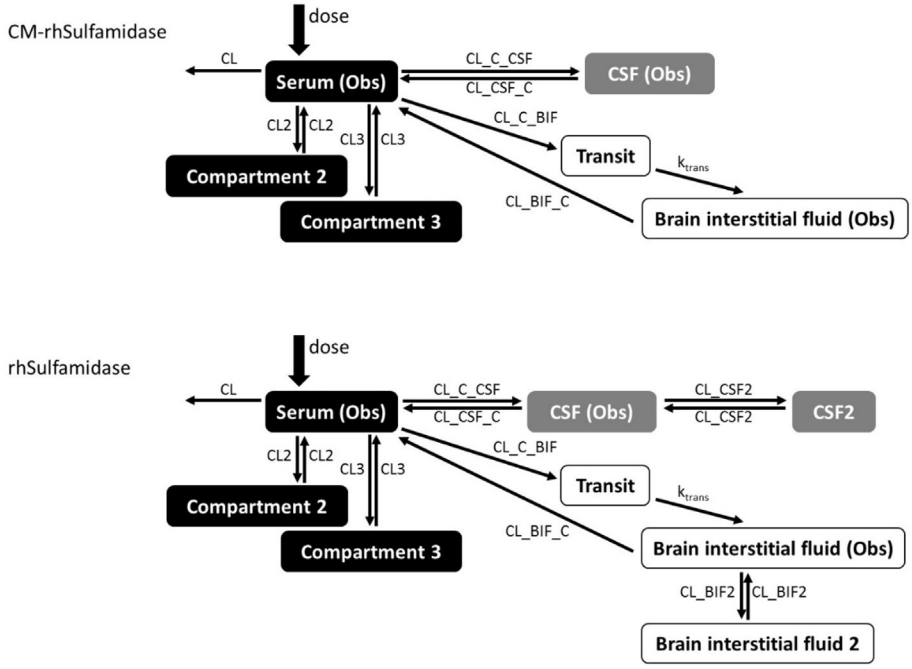

Fig. 4. Schematic overview of the final pharmacokinetic models. The models were optimized towards serum, CSF and brain interstitial fluid data. Abbreviations: BIF: brain interstitial fluid, C: concentration in serum, CL: clearance, CSF: cerebrospinal fluid, k_{trans}: transit compartment rate constant, Obs: observations.

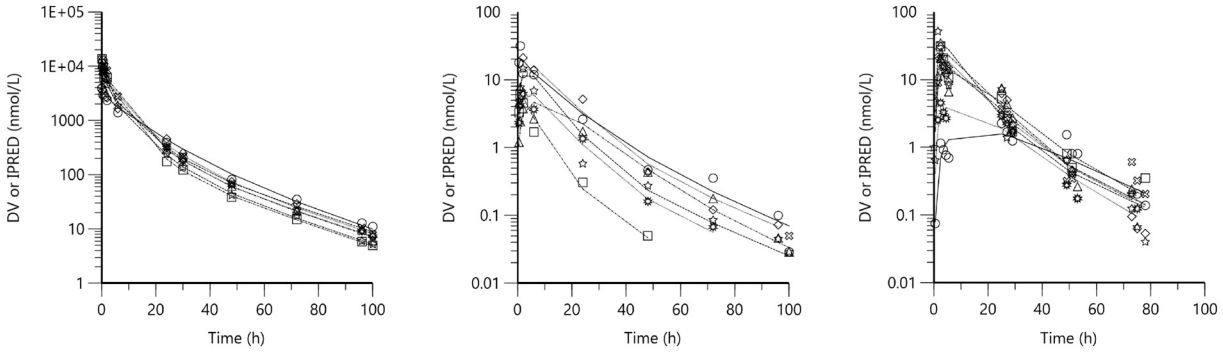

Fig. 5. Goodness-of-fit in serum, CSF and brain interstitial fluid respectively of CM-rhSulfamidase. Observations (DV; symbols) and individual predictions (IPRED; lines) of CM-rhSulfamidase concentrations in serum, CSF and brain interstitial fluid grouped per individual rat.

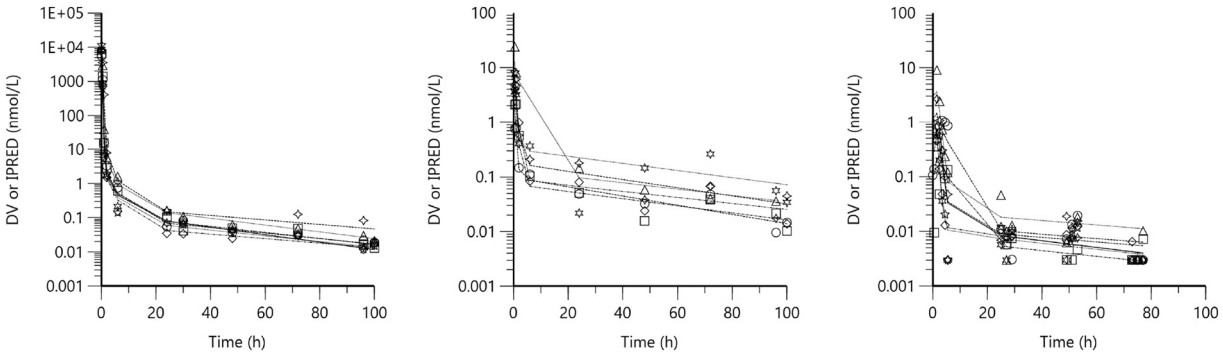

Fig. 6. Goodness-of-fit in serum, CSF and brain interstitial fluid respectively of rhSulfamidase. Observations (DV; symbols) and individual predictions (IPRED; lines) of rhSulfamidase concentrations in serum, CSF and brain interstitial fluid grouped per individual rat.

mannose 6-phosphate receptor (M6PR). However, transport of sulfamidase across the BBB was negligible in 8-week-old mice [17]. BBB transport via the M6PR is thus developmentally downregulated. Chemical modification, i.e. uncoupling of M6PR, is not expected to negatively impact brain exposure in the rats studied here (at least 8 weeks of age). Indeed, the AUC of IF is 0.2–0.3% of the AUC in serum for both compounds, while the AUC in serum is 18-fold higher for CM-rhSulfamidase. This suggests that serum concentrations drive the concentrations in brain interstial fluid. However, the population modelling approach estimated faster kinetics for rhSulfamidase into and out of brain IF relative to the estimates for CM-rhSulfamidase and needed an additional distribution compartment for the best fit. Residual M6PR on the BBB might still play a role, but also affinity to alternative receptors, intracellular distribution and trafficking through endothelium may differ between rhSulfamidase and CM-rhSulfamidase. It is of note that the AUC_{∞}s of CM-rhSulfamidase and rhSulfamidase concentrations in CSF were 0.3 and 0.8% of their AUC_{∞} in serum, respectively, and the clearances of serum to CSF and from CSF to serum and the estimated CSF volumes for the two compounds were similar. Interestingly, Chang et al. [2] reported similar AUC ratios for concentrations in CSF and brain IF relative to serum levels for an antibody against human epidermal growth factor receptor-2 dosed i.v. in male Sprague Dawley rats.

CM-rhSulfamidase was observed in brain homogenate of MPS IIIA mice after repeated i.v. administration [6]. Although the brains were perfused to remove blood prior to sampling, compound present in the capillary wall and in brain parenchyma was included in the homogenate. CM-rhSulfamidase presence in brain parenchyma after repeated i.v. administration in MPS IIIA mice was confirmed using immunofluorescent localization [6]. Here we show that chemical modification is beneficial for increasing brain IF AUC of rhSulfamidase. The impact of chemical modification on the ability of rhSulfamidase to reach various target cells in the brain is still unresearched but warrants further investigation.

5. Conclusion

After an i.v. administration, CM-rhSulfamidase had at least a 7-fold higher AUC in serum, CSF and brain IF as determined by repeated sampling in awake, freely moving Sprague Dawley rats. Serum concentrations appeared to drive CSF and brain IF concentrations, although for rhSulfamidase two additional compartments had to be added to the PK model, one for CSF and one for brain IF, to obtain a satisfying fit of the observed data. The data support the attempts of developing CM-rhSulfamidase as a treatment for MPS IIIA patients and clinical studies are currently ongoing (NCT03423186 & NCT03811028).

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Disclosures

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Ethical conduct of research

The authors state that studies were reviewed and approved by an

animal ethics committee.

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