



Clinical trial results:

Pharmacokinetics and safety of treatment with paracetamol in children and adults with spinal muscular atrophy and cerebral palsy

Summary

EudraCT number	2018-002295-40
Trial protocol	DK
Global end of trial date	09 May 2024

Results information

Result version number	v1 (current)
This version publication date	21 June 2025
First version publication date	21 June 2025
Summary attachment (see zip file)	Medical journal publication of the results_publication 1 (Naume et al. Neuromuscular disorders.pdf) Medical journal publication of the results_publication 2 (Br J Clin Pharmacol - 2025 - Zhao - Paracetamol and its metabolites in children and adults with spinal muscular atrophy a.pdf)

Trial information

Trial identification

Sponsor protocol code	08-06-2018-paracet
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Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	-
WHO universal trial number (UTN)	-

Notes:

Sponsors

Sponsor organisation name	Rigshospitalet, Copenhagen University Hospital
Sponsor organisation address	Blegdamsvej 9 , København Ø, Denmark, 2100
Public contact	Copenhagen Neuromuscular Center, Rigshospitalet, marie.mostue.naume.01@regionh.dk
Scientific contact	Copenhagen Neuromuscular Center, Rigshospitalet, marie.mostue.naume.01@regionh.dk

Notes:

Paediatric regulatory details

Is trial part of an agreed paediatric investigation plan (PIP)	No
Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?	No
Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Notes:

Results analysis stage

Analysis stage	Final
Date of interim/final analysis	09 May 2024
Is this the analysis of the primary completion data?	No
Global end of trial reached?	Yes
Global end of trial date	09 May 2024
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

The main objective of the trial was to explore pharmacokinetics, pharmacogenetics and safety of treatment with paracetamol in children and adults with spinal muscular atrophy type II (SMA II) and cerebral palsy (CP).

Protection of trial subjects:

We measured liver biomarkers three times on study day 1 and day 3. If the liver biomarkers were markedly elevated (judged by the treating physician), the paracetamol intake was stopped, and the patient was excluded from the rest of the study. Before inserting venous catheters and blood samples from the children, EMLA cream, a local anaesthetic, was applied to minimise any discomfort. Furthermore, these procedures were performed only by experienced staff.

Background therapy: -

Evidence for comparator:

As a part of postoperative mild-to-moderate pain treatment and antipyretic treatment, patients with SMA often receive paracetamol for several days at same dose levels as healthy subjects without considering the patient's lower muscle mass. Thus, several individual case reports involving children and adults with SMA, limb-girdle muscular dystrophy, Duchenne muscular dystrophy (DMD) and congenital muscular dystrophy with low skeletal muscle mass have reported hepatotoxic side effects of paracetamol administered in therapeutic doses after surgery, infections, or critical illness. In line with this, we have experienced one adult patient with SMA II that developed fatal acute liver failure following abdominal surgery, suspected to be caused by paracetamol toxicity (unpublished data). Furthermore, a DMD boy in our clinic recently developed acute liver failure after intake of therapeutic doses of paracetamol during hospitalization (unpublished data).

The majority of paracetamol is conjugated to sulfate and glucuronide to form nontoxic metabolites. A small portion undergoes CYP-mediated metabolism, forming the reactive and potentially toxic metabolite N-acetyl-p-benzo-quinone imine (NAPQI). NAPQI is conjugated by glutathione (GSH) to the nontoxic oxidative metabolites cysteine and mercapturic acid. In toxic doses, the usual metabolic pathways are overloaded, and paracetamol is shunted to the oxidative pathway, leading to the depletion of GSH stores. Hepatic cellular injury and necrosis occur as NAPQI accumulates.

Glutathione is a tripeptide consisting of glutamate, cysteine, and glycine. However, GSH synthesis depends on glutamine from the skeletal muscle to form glutamate. Thus, patients with SMA may have a lower concentration of GSH compared to healthy due to their altered body composition, i.e., low skeletal muscle mass. This may increase the risk of paracetamol-induced hepatotoxicity in the patients, even when treated with therapeutic doses.

Actual start date of recruitment	01 August 2018
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	No

Notes:

Population of trial subjects

Subjects enrolled per country

Country: Number of subjects enrolled	Denmark: 24
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Worldwide total number of subjects	24
EEA total number of subjects	24

Notes:

Subjects enrolled per age group	
In utero	0
Preterm newborn - gestational age < 37 wk	0
Newborns (0-27 days)	0
Infants and toddlers (28 days-23 months)	0
Children (2-11 years)	5
Adolescents (12-17 years)	1
Adults (18-64 years)	18
From 65 to 84 years	0
85 years and over	0

Subject disposition

Recruitment

Recruitment details:

Patients of SMA II were eligible for inclusion if genetically verified. The patients with SMA II were in the age group of 6-45 years. We recruited healthy adults from official recruitment sites for healthy controls and Facebook.com. The healthy controls should be in the age group of 18-45 years.

Pre-assignment

Screening details:

Exclusion criteria were intake of medications (that may interfere with the results and that may affect gastric emptying), failure to obtain consent, or the participant being considered unsuitable by the treating physician.

Pre-assignment period milestones

Number of subjects started	24
Number of subjects completed	24

Period 1

Period 1 title	Overall trial (overall period)
Is this the baseline period?	Yes
Allocation method	Non-randomised - controlled
Blinding used	Not blinded

Arms

Are arms mutually exclusive?	Yes
Arm title	Adult participants with the disease, SMA

Arm description:

The adult participants with the disease who received paracetamol, the study drug.

Arm type	Experimental
Investigational medicinal product name	Paracetamol
Investigational medicinal product code	
Other name	Acetaminophen
Pharmaceutical forms	Oral solution in single-dose container
Routes of administration	Oral use

Dosage and administration details:

All participants were treated with an oral liquid formulation of paracetamol in therapeutic doses, 15 mg/kg/dose every six hours, with a maximum of 1 gram x 4 per day, for three consecutive days.

Arm title	The children with the disease, SMA
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Arm description:

The participants being children with the disease who received paracetamol, the study drug.

Arm type	Experimental
Investigational medicinal product name	Paracetamol
Investigational medicinal product code	
Other name	Acetaminophen
Pharmaceutical forms	Oral solution in single-dose container
Routes of administration	Oral use

Dosage and administration details:

All participants were treated with an oral liquid formulation of paracetamol in therapeutic doses, 15 mg/kg/dose every six hours, with a maximum of 1 gram x 4 per day, for three consecutive days.

Arm title	Healthy controls
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Arm description:

The participants being healthy controls receiving paracetamol, the study drug.

Arm type	Experimental
Investigational medicinal product name	Paracetamol
Investigational medicinal product code	
Other name	Acetaminophen
Pharmaceutical forms	Oral solution in single-dose container
Routes of administration	Oral use

Dosage and administration details:

All participants were treated with an oral liquid formulation of paracetamol in therapeutic doses, 15 mg/kg/dose every six hours, with a maximum of 1 gram x 4 per day, for three consecutive days.

Number of subjects in period 1	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls
Started	6	6	12
Completed	6	6	12

Baseline characteristics

Reporting groups

Reporting group title	Overall trial
Reporting group description: -	

Reporting group values	Overall trial	Total	
Number of subjects	24	24	
Age categorical			
Units: Subjects			
Children (2-11 years)	5	5	
Adolescents (12-17 years)	1	1	
Adults (18-64 years)	18	18	
Gender categorical			
Units: Subjects			
Female	11	11	
Male	13	13	

Subject analysis sets

Subject analysis set title	Participants with SMA combined
Subject analysis set type	Full analysis

Subject analysis set description:

Participants with the disease SMA combined. This category is used when analysing the frequency of pharmacogenetic polymorphisms in the group.

Subject analysis set title	North-western European dataset
Subject analysis set type	Sub-group analysis

Subject analysis set description:

The prevalence of pharmacogenetics variants in healthy controls was assumed to be similar to the North-western European population, which had already been studied in a large sample of 4294 individuals, and thus not investigated in the healthy controls in this study. The pharmacogenetic profiles in the SMA patients were compared to the prevalence of homozygous carriers of the alternative alleles in the specific SNPs found in the North-western European population of 4294 individuals (1).

The system will not accept the number of subjects being 4294 since that is not the overall number in the study, 12 is therefore registered.

1. A genome-wide mutational constraint map quantified from variation in 76,156 human genomes | bioRxiv [Internet]. [cited 2023 Jun 13]. Available from: <https://www.biorxiv.org/content/10.1101/2022.03.20.485034v2>

Reporting group values	Participants with SMA combined	North-western European dataset	
Number of subjects	12	12	
Age categorical			
Units: Subjects			
Children (2-11 years)	5		
Adolescents (12-17 years)	1		
Adults (18-64 years)	6		
Gender categorical			
Units: Subjects			
Female	5		
Male	7		

End points

End points reporting groups

Reporting group title	Adult participants with the disease, SMA
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Reporting group description:

The adult participants with the disease who received paracetamol, the study drug.

Reporting group title	The children with the disease, SMA
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Reporting group description:

The participants being children with the disease who received paracetamol, the study drug.

Reporting group title	Healthy controls
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Reporting group description:

The participants being healthy controls receiving paracetamol, the study drug.

Subject analysis set title	Participants with SMA combined
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Subject analysis set type	Full analysis
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Subject analysis set description:

Participants with the disease SMA combined. This category is used when analysing the frequency of pharmacogenetic polymorphisms in the group.

Subject analysis set title	North-western European dataset
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Subject analysis set type	Sub-group analysis
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Subject analysis set description:

The prevalence of pharmacogenetics variants in healthy controls was assumed to be similar to the North-western European population, which had already been studied in a large sample of 4294 individuals, and thus not investigated in the healthy controls in this study. The pharmacogenetic profiles in the SMA patients were compared to the prevalence of homozygous carriers of the alternative alleles in the specific SNPs found in the North-western European population of 4294 individuals (1).

The system will not accept the number of subjects being 4294 since that is not the overall number in the study, 12 is therefore registered.

1. A genome-wide mutational constraint map quantified from variation in 76,156 human genomes | bioRxiv [Internet]. [cited 2023 Jun 13]. Available from: <https://www.biorxiv.org/content/10.1101/2022.03.20.485034v2>

Primary: Clearance of paracetamol in the participants

End point title	Clearance of paracetamol in the participants
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End point description:

Population pharmacokinetic parameters estimates were obtained using a nonlinear mixed effects model. The structure of the model involves single compartments of paracetamol and its metabolites in plasma. Since paracetamol-cysteine and paracetamol-mercapturic acid were derived from CYP-mediated oxidation, we summed them together into one compartment called oxidative metabolites. The estimated pharmacokinetic parameters in the model were total clearance of paracetamol, formation clearances of each metabolite, unmetabolized clearance of paracetamol, the volume of distribution of paracetamol and its metabolites, renal clearances of each metabolite, and half-life time of paracetamol and each metabolite. Unmetabolized clearance of paracetamol was the estimated paracetamol that the three metabolic pathways could not explain.

End point type	Primary
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End point timeframe:

The metabolites were measured every hour for 6 or 8 hours on day 1 and day 3 (last day of trial). The pharmacokinetic parameters are estimated with these numbers.

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[1]	
Units: L/h				
median (full range (min-max))	14.13 (10.53 to 20.7)	17.05 (9.07 to 26.60)	21.51 (17.58 to 37.68)	

Notes:

[1] - One was excluded from the analyses due to high levels of paracetamol in background sample

Statistical analyses

Statistical analysis title	Clearance of paracetamol 1
Statistical analysis description:	
Comparison of clearance of paracetamol in the adults participants with SMA and the healthy controls	
Comparison groups	Healthy controls v Adult participants with the disease, SMA
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Statistical analysis title	Clearance of paracetamol 2
Statistical analysis description:	
Comparison of clearance of paracetamol in the children participants and the healthy controls	
Comparison groups	The children with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Primary: Volume of distribution of paracetamol in the participants

End point title	Volume of distribution of paracetamol in the participants
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End point description:

Population pharmacokinetic parameters estimates were obtained using a nonlinear mixed effects model. The structure of the model involves single compartments of paracetamol and its metabolites in plasma. Since paracetamol-cysteine and paracetamol-mercaptopuric acid were derived from CYP-mediated oxidation, we summed them together into one compartment called oxidative metabolites. The estimated pharmacokinetic parameters in the model were total clearance of paracetamol, formation clearances of each metabolite, unmetabolized clearance of paracetamol, the volume of distribution of paracetamol and its metabolites, renal clearances of each metabolite, and half-life time of paracetamol and each metabolite. Unmetabolized clearance of paracetamol was the estimated paracetamol that the three metabolic pathways could not explain.

End point type	Primary
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End point timeframe:

3 days. The metabolites were measured every hour for 6 or 8 hours on day 1 and day 3 (last day of trial). The pharmacokinetic parameters are estimated with these numbers.

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[2]	
Units: L/h				
median (full range (min-max))	53.02 (32.1 to 56.47)	41.50 (32.76 to 67.69)	69.46 (30.90 to 96.50)	

Notes:

[2] - One was excluded from the analyses due to high levels of paracetamol in background sample

Attachments (see zip file)	Table 2.docx
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Statistical analyses

Statistical analysis title	Volume of distribution of paracetamol 1
Statistical analysis description: Adults with SMA vs healthy controls	
Comparison groups	Adult participants with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[3]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[3] - To investigate if the pharmacokinetics of paracetamol are significantly different in SMA patients compared to people without the disease

Statistical analysis title	Volume of distribution of paracetamol 2
Statistical analysis description: Children with SMA vs healthy controls	
Comparison groups	The children with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[4]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[4] - To investigate if the pharmacokinetics of paracetamol are significantly different in SMA patients compared to people without the disease

Primary: Formation rate of glucuronide

End point title	Formation rate of glucuronide
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End point description:

Population pharmacokinetic parameters estimates were obtained using a nonlinear mixed effects model. The structure of the model involves single compartments of paracetamol and its metabolites in plasma. Since paracetamol-cysteine and paracetamol-mercapturic acid were derived from CYP-mediated oxidation, we summed them together into one compartment called oxidative metabolites. The estimated pharmacokinetic parameters in the model were total clearance of paracetamol, formation clearances of each metabolite, unmetabolized clearance of paracetamol, the volume of distribution of paracetamol and its metabolites, renal clearances of each metabolite, and half-life time of paracetamol and each metabolite. Unmetabolized clearance of paracetamol was the estimated paracetamol that the three metabolic pathways could not explain.

End point type	Primary
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End point timeframe:

The metabolites were measured every hour for 6 or 8 hours on day 1 and day 3 (last day of trial). The pharmacokinetic parameters are estimated with these numbers.

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[5]	
Units: L/h				
median (full range (min-max))	3.87 (2.02 to 4.32)	3.61 (1.95 to 6.58)	7.25 (4.17 to 13.85)	

Notes:

[5] - One was excluded from the analyses due to high levels of paracetamol in background sample

Attachments (see zip file)	Table 2.docx
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Statistical analyses

Statistical analysis title	Formation of glucuronide 1
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Statistical analysis description:

Adults SMA patients compared to healthy controls

Comparison groups	Adult participants with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[6]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[6] - To investigate if the pharmacokinetics of paracetamol is significantly different in SMA patients compared to people without the disease

Statistical analysis title	Formation of glucuronide 2
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Statistical analysis description:

To investigate if the pharmacokinetics of paracetamol is significantly different in SMA patients compared to people without the disease

Comparison groups	The children with the disease, SMA v Healthy controls
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Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Primary: Elimination rate of glucuronide

End point title	Elimination rate of glucuronide
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End point description:

Population pharmacokinetic parameters estimates were obtained using a nonlinear mixed effects model. The structure of the model involves single compartments of paracetamol and its metabolites in plasma. Since paracetamol-cysteine and paracetamol-mercapturic acid were derived from CYP-mediated oxidation, we summed them together into one compartment called oxidative metabolites. The estimated pharmacokinetic parameters in the model were total clearance of paracetamol, formation clearances of each metabolite, unmetabolized clearance of paracetamol, the volume of distribution of paracetamol and its metabolites, renal clearances of each metabolite, and half-life time of paracetamol and each metabolite. Unmetabolized clearance of paracetamol was the estimated paracetamol that the three metabolic pathways could not explain.

End point type	Primary
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End point timeframe:

The metabolites were measured every hour for 6 or 8 hours on day 1 and day 3 (last day of trial). The pharmacokinetic parameters are estimated with these numbers.

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[7]	
Units: L/h				
median (full range (min-max))	3.24 (2.63 to 4.42)	2.75 (2.39 to 4.88)	6.18 (4.49 to 7.60)	

Notes:

[7] - One was excluded from the analyses due to high levels of paracetamol in background sample

Attachments (see zip file)	Table 2.docx
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Statistical analyses

Statistical analysis title	Elimination rate of glucuronide 1
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Statistical analysis description:

Adults SMA patients vs healthy controls

Comparison groups	Adult participants with the disease, SMA v Healthy controls
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Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[8]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[8] - To investigate if the pharmacokinetics of paracetamol is significantly different in SMA patients compared to people without the disease

Statistical analysis title	Elimination rate of glucuronide 2
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Statistical analysis description:

SMA children vs healthy controls

Comparison groups	The children with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[9]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[9] - To investigate if the pharmacokinetics of paracetamol is significantly different in SMA patients compared to people without the disease

Primary: Formation rate of sulfate

End point title	Formation rate of sulfate
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End point description:

Population pharmacokinetic parameters estimates were obtained using a nonlinear mixed effects model. The structure of the model involves single compartments of paracetamol and its metabolites in plasma. Since paracetamol-cysteine and paracetamol-mercapturic acid were derived from CYP-mediated oxidation, we summed them together into one compartment called oxidative metabolites. The estimated pharmacokinetic parameters in the model were total clearance of paracetamol, formation clearances of each metabolite, unmetabolized clearance of paracetamol, the volume of distribution of paracetamol and its metabolites, renal clearances of each metabolite, and half-life time of paracetamol and each metabolite. Unmetabolized clearance of paracetamol was the estimated paracetamol that the three metabolic pathways could not explain.

End point type	Primary
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End point timeframe:

The metabolites were measured every hour for 6 or 8 hours on day 1 and day 3 (last day of trial). The pharmacokinetic parameters are estimated with these numbers.

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[10]	
Units: L/h				
median (full range (min-max))	4.70 (2.51 to 6.76)	4.58 (3.4 to 8.40)	9.50 (6.66 to 12.52)	

Notes:

[10] - One was excluded from the analyses due to high levels of paracetamol in background sample

Attachments (see zip file)	Table 2.docx
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Statistical analyses

Statistical analysis title	Formation rate of sulfate 1
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Statistical analysis description:

Adults with SMA vs healthy controls

Comparison groups	Adult participants with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[11]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[11] - To investigate if the pharmacokinetics of paracetamol is significantly different in SMA patients compared to people without the disease

Statistical analysis title	Formation rate of sulfate 2
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Statistical analysis description:

Children with SMA vs healthy controls

Comparison groups	The children with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[12]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[12] - To investigate if the pharmacokinetics of paracetamol is significantly different in SMA patients compared to people without the disease

Primary: Elimination rate of sulfate

End point title	Elimination rate of sulfate
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End point description:

Population pharmacokinetic parameters estimates were obtained using a nonlinear mixed effects model. The structure of the model involves single compartments of paracetamol and its metabolites in plasma. Since paracetamol-cysteine and paracetamol-mercapturic acid were derived from CYP-mediated oxidation, we summed them together into one compartment called oxidative metabolites. The estimated pharmacokinetic parameters in the model were total clearance of paracetamol, formation clearances of each metabolite, unmetabolized clearance of paracetamol, the volume of distribution of paracetamol and its metabolites, renal clearances of each metabolite, and half-life time of paracetamol and each metabolite. Unmetabolized clearance of paracetamol was the estimated paracetamol that the three metabolic pathways could not explain.

End point type	Primary
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End point timeframe:

The metabolites were measured every hour for 6 or 8 hours on day 1 and day 3 (last day of trial). The pharmacokinetic parameters are estimated with these numbers.

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[13]	
Units: L/h				
median (full range (min-max))	11.62 (9.44 to 15.88)	9.86 (8.58 to 17.52)	22.16 (16.12 to 27.30)	

Notes:

[13] - One was excluded from the analyses due to high levels of paracetamol in background sample

Attachments (see zip file)	Table 2.docx
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Statistical analyses

Statistical analysis title	Elimination rate of sulfate 1
Statistical analysis description: Adults with SMA vs healthy controls	
Comparison groups	Adult participants with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[14]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[14] - To investigate if the pharmacokinetics of paracetamol is significantly different in SMA patients compared to people without the disease

Statistical analysis title	Elimination rate of sulfate 2
Statistical analysis description: Children with SMA vs healthy controls	
Comparison groups	The children with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[15]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[15] - To investigate if the pharmacokinetics of paracetamol is significantly different in SMA patients compared to people without the disease

Primary: Formation rate of oxidative metabolites

End point title	Formation rate of oxidative metabolites
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End point description:

Population pharmacokinetic parameters estimates were obtained using a nonlinear mixed effects model. The structure of the model involves single compartments of paracetamol and its metabolites in plasma. Since paracetamol-cysteine and paracetamol-mercapturic acid were derived from CYP-mediated oxidation, we summed them together into one compartment called oxidative metabolites. The estimated pharmacokinetic parameters in the model were total clearance of paracetamol, formation clearances of each metabolite, unmetabolized clearance of paracetamol, the volume of distribution of paracetamol and its metabolites, renal clearances of each metabolite, and half-life time of paracetamol and each metabolite. Unmetabolized clearance of paracetamol was the estimated paracetamol that the three

metabolic pathways could not explain.

End point type	Primary
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End point timeframe:

The metabolites were measured every hour for 6 or 8 hours on day 1 and day 3 (last day of trial). The pharmacokinetic parameters are estimated with these numbers.

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[16]	
Units: L/h				
median (full range (min-max))	0.12 (0.09 to 0.16)	0.10 (0.09 to 0.17)	0.22 (0.16 to 0.27)	

Notes:

[16] - One was excluded from the analyses due to high levels of paracetamol in background sample

Attachments (see zip file)	Table 2.docx
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Statistical analyses

Statistical analysis title	Formation rate of oxidative metabolites 1
Statistical analysis description: Adults with SMA vs healthy controls	
Comparison groups	Adult participants with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[17]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[17] - To investigate if the pharmacokinetics of paracetamol is significantly different in SMA patients compared to people without the disease

Statistical analysis title	Formation rate of oxidative metabolites 2
Statistical analysis description: Children with SMA vs healthy adults	
Comparison groups	The children with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[18]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[18] - To investigate if the pharmacokinetics of paracetamol is significantly different in SMA patients compared to people without the disease

Primary: Elimination rate of oxidative metabolites

End point title	Elimination rate of oxidative metabolites
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End point description:

Population pharmacokinetic parameters estimates were obtained using a nonlinear mixed effects model. The structure of the model involves single compartments of paracetamol and its metabolites in plasma. Since paracetamol-cysteine and paracetamol-mercapturic acid were derived from CYP-mediated oxidation, we summed them together into one compartment called oxidative metabolites. The estimated pharmacokinetic parameters in the model were total clearance of paracetamol, formation clearances of each metabolite, unmetabolized clearance of paracetamol, the volume of distribution of paracetamol and its metabolites, renal clearances of each metabolite, and half-life time of paracetamol and each metabolite. Unmetabolized clearance of paracetamol was the estimated paracetamol that the three metabolic pathways could not explain.

End point type	Primary
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End point timeframe:

The metabolites were measured every hour for 6 or 8 hours on day 1 and day 3 (last day of trial). The pharmacokinetic parameters are estimated with these numbers.

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[19]	
Units: L/h				
median (full range (min-max))	2.52 (1.45 to 4.51)	1.65 (1.30 to 2.69)	3.82 (2.43 to 4.95)	

Notes:

[19] - One was excluded from the analyses due to high levels of paracetamol in background sample

Attachments (see zip file)	Table 2.docx
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Statistical analyses

Statistical analysis title	Elimination rate of oxidative metabolites 1
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Statistical analysis description:

Adults with SMA vs healthy controls

Comparison groups	Adult participants with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[20]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[20] - To investigate if the pharmacokinetics of paracetamol are significantly different in SMA patients compared to people without the disease

Statistical analysis title	Elimination rate of oxidative metabolites
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Statistical analysis description:

Children with SMA vs healthy controls

Comparison groups	The children with the disease, SMA v Healthy controls
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Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[21]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[21] - To investigate if the pharmacokinetics of paracetamol are significantly different in SMA patients compared to people without the disease

Primary: Unmetabolised paracetamol clearance

End point title	Unmetabolised paracetamol clearance
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End point description:

Population pharmacokinetic parameters estimates were obtained using a nonlinear mixed effects model. The structure of the model involves single compartments of paracetamol and its metabolites in plasma. Since paracetamol-cysteine and paracetamol-mercapturic acid were derived from CYP-mediated oxidation, we summed them together into one compartment called oxidative metabolites. The estimated pharmacokinetic parameters in the model were total clearance of paracetamol, formation clearances of each metabolite, unmetabolized clearance of paracetamol, the volume of distribution of paracetamol and its metabolites, renal clearances of each metabolite, and half-life time of paracetamol and each metabolite. Unmetabolized clearance of paracetamol was the estimated paracetamol that the three metabolic pathways could not explain.

End point type	Primary
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End point timeframe:

The metabolites were measured every hour for 6 or 8 hours on day 1 and day 3 (last day of trial). The pharmacokinetic parameters are estimated with these numbers.

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[22]	
Units: L/h				
median (full range (min-max))	6.22 (1.99 to 10.85)	9.11 (2.72 to 13.01)	4.56 (1.19 to 14.64)	

Notes:

[22] - One was excluded from the analyses due to high levels of paracetamol in background sample

Attachments (see zip file)	Table 2.docx
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Statistical analyses

Statistical analysis title	Unmetabolised paracetamol clearance 1
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Statistical analysis description:

Adults with SMA vs healthy controls

Comparison groups	Adult participants with the disease, SMA v Healthy controls
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Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[23]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[23] - To investigate if the pharmacokinetics of paracetamol are significantly different in SMA patients compared to people without the disease

Statistical analysis title	Unmetabolised paracetamol clearance 2
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Statistical analysis description:

Children with SMA vs healthy controls

Comparison groups	The children with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other ^[24]
P-value	< 0.05
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Median difference (final values)

Notes:

[24] - To investigate if the pharmacokinetics of paracetamol are significantly different in SMA patients compared to people without the disease

Secondary: ALT during paracetamol treatment in the participants

End point title	ALT during paracetamol treatment in the participants
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End point description:

Paracetamol parent compound and its metabolite concentrations were measured every hour for six or eight hours after the initial dosing on study days 1 and 3. The participants were at home on day 2 without blood sampling. Liver plasma biomarkers (ALT, aspartate aminotransferase (AST), alkaline phosphatase, INR, lactate dehydrogenase (LDH), bilirubin, miRNA 122, and miRNA 192), kidney plasma biomarkers (creatinine, potassium, sodium, and urea) and creatinine kinase (CK) were collected at three different time points during study days 1 and 2.

The endpoint mean is the mean of the ALT measured in the afternoon on day 3.

End point type	Secondary
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End point timeframe:

3 days

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[25]	
Units: U/l				
arithmetic mean (standard deviation)	31.4 (± 34.18)	20.6 (± 8.79)	26.1 (± 11.84)	

Notes:

[25] - One was excluded from the analyses due to high levels of paracetamol in background sample

Statistical analyses

Statistical analysis title	Liver biomarker ALT after paracetamol treatment 1
Statistical analysis description: Adults with SMA vs healthy controls	
Comparison groups	Adult participants with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other
P-value	< 0.05
Method	Kruskal-wallis
Parameter estimate	Mean difference (final values)

Statistical analysis title	Liver biomarker ALT after paracetamol treatment
Statistical analysis description: Children with SMA vs healthy controls	
Comparison groups	The children with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other
P-value	< 0.05
Method	Kruskal-wallis
Parameter estimate	Mean difference (final values)

Secondary: pharmacogenetic screening of SNPs in paracetamol-metabolizing enzymes in SMA participants

End point title	pharmacogenetic screening of SNPs in paracetamol-metabolizing enzymes in SMA participants
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End point description:

DNA for investigation of pharmacogenetics was available in our biobank for the SMA patients. The prevalence of pharmacogenetics variants in healthy controls was assumed to be similar to the North-western European population, which had already been studied in a large sample of 4294 individuals, and thus not investigated in the healthy controls in this study. The investigated single-nucleotide polymorphisms (SNPs) were selected after a review of the literature, researching the reported SNPs with possible effects on acetaminophen metabolism.

The endpoint is the number of participants being homozygous carriers of the alternative allele in the UGT1A-3 gene and CYP1A. UGT1A is important for the glucuronide pathway, including the SNPs rs10929303 (C/C), rs1042640 (C/C) and rs8330 (C/C). CYP1A2 is important in the oxidation pathway with SNP rs762551 (A/A).

End point type	Secondary
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End point timeframe:

one time measurement

End point values	Participants with SMA combined	North-western European dataset		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	12	4294		
Units: percentage				
rs10929303	9	2662		
rs1042640	9	2722		
rs8330	9	2559		
rs1902023	5	988		
rs762551	8	2186		

Statistical analyses

Statistical analysis title	Pharmacogenetic polymorphisms in the participants
Statistical analysis description: The SMA participants vs North-Western European population	
Comparison groups	Participants with SMA combined v North-western European dataset
Number of subjects included in analysis	4306
Analysis specification	Pre-specified
Analysis type	other ^[26]
P-value	< 0.05
Method	The two-sample binomial test, Boschloo's
Parameter estimate	percentages

Notes:

[26] - The two-sample binomial test, Boschloo's test, was used to compare the percentages of homozygous carriers of the alternative alleles in the investigated SNPs between the patient group and the North-western European population.

Secondary: miRNA 122 during paracetamol treatment

End point title	miRNA 122 during paracetamol treatment
End point description: Liver plasma biomarkers (ALT, aspartate aminotransferase (AST), alkaline phosphatase, INR, lactate dehydrogenase (LDH), bilirubin, miRNA 122, and miRNA 192), kidney plasma bi-omarkers (creatinine, potassium, sodium, and urea) and creatinine kinase (CK) were collected at three different time points during study days 1 and 2. The miRNA results are registered from the afternoon day 3.	
End point type	Secondary
End point timeframe: 3 days	

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[27]	
Units: Relative expression				

arithmetic mean (standard deviation)	2.0 (\pm 1.8)	1.6 (\pm 1.4)	1.5 (\pm 1.2)	
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Notes:

[27] - One was excluded from the analyses due to high levels of paracetamol in background sample

Attachments (see zip file)	miRNA change /10.1016-j.nmd.2023.11.005Figure5.pptx
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Statistical analyses

Statistical analysis title	miRNA 122 during paracetamol treatment 1
Statistical analysis description:	
Adults with SMA vs healthy controls	
Comparison groups	Adult participants with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other
P-value	< 0.05
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)

Statistical analysis title	MiRNA 122 during paracetamol treatment 2
Statistical analysis description:	
Children with SMA vs healthy controls	
Comparison groups	The children with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other
P-value	< 0.05
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)

Secondary: miRNA 192 during paracetamol treatment

End point title	miRNA 192 during paracetamol treatment
End point description:	
Liver plasma biomarkers (ALT, aspartate aminotransferase (AST), alkaline phosphatase, INR, lactate dehydrogenase (LDH), bilirubin, miRNA 122, and miRNA 192), kidney plasma bi-omarkers (creatinine, potassium, sodium, and urea) and creatinine kinase (CK) were collected at three different time points during study days 1 and 2.	
Registered results from the afternoon on day 3	
End point type	Secondary
End point timeframe:	
3 days	

End point values	Adult participants with the disease, SMA	The children with the disease, SMA	Healthy controls	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	6	6	11 ^[28]	
Units: relative expression				
arithmetic mean (standard deviation)	1.3 (± 1.5)	1.3 (± 0.7)	1.2 (± 0.8)	

Notes:

[28] - One was excluded from the analyses due to high levels of paracetamol in background sample

Attachments (see zip file)	miRNA change /10.1016-j.nmd.2023.11.005Figure5.pptx
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Statistical analyses

Statistical analysis title	miRNA 192 during paracetamol treatment 1
Statistical analysis description: Adults with SMA vs healthy controls	
Comparison groups	Adult participants with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other
P-value	< 0.05
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)

Statistical analysis title	miRNA 192 during paracetamol treatment 2
Statistical analysis description: Children with SMA vs healthy controls	
Comparison groups	The children with the disease, SMA v Healthy controls
Number of subjects included in analysis	17
Analysis specification	Pre-specified
Analysis type	other
P-value	< 0.05
Method	t-test, 2-sided
Parameter estimate	Mean difference (final values)

Adverse events

Adverse events information

Timeframe for reporting adverse events:

3 days

Adverse event reporting additional description:

The treatment of paracetamol was stopped if the ALT and/or AST were elevated compared to the baseline sample during the study days judged by the investigator on a patient-by-patient basis or if any other adverse events occurred. All participants completed a journal during the study period to report and monitor compliance and side effects.

Assessment type	Systematic
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Dictionary used

Dictionary name	None
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Dictionary version	0
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Reporting groups

Reporting group title	Adult participants with SMA
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Reporting group description: -

Reporting group title	Children with SMA
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Reporting group description:

The participants being children with SMA

Reporting group title	Healthy controls
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Reporting group description:

Participants being healthy controls

Serious adverse events	Adult participants with SMA	Children with SMA	Healthy controls
Total subjects affected by serious adverse events			
subjects affected / exposed	0 / 6 (0.00%)	0 / 6 (0.00%)	0 / 11 (0.00%)
number of deaths (all causes)	0	0	0
number of deaths resulting from adverse events	0	0	0

Frequency threshold for reporting non-serious adverse events: 5 %

Non-serious adverse events	Adult participants with SMA	Children with SMA	Healthy controls
Total subjects affected by non-serious adverse events			
subjects affected / exposed	4 / 6 (66.67%)	3 / 6 (50.00%)	8 / 11 (72.73%)
Gastrointestinal disorders			
Diarrhoea			
subjects affected / exposed	3 / 6 (50.00%)	2 / 6 (33.33%)	4 / 11 (36.36%)
occurrences (all)	3	2	4
Abdominal pain			

subjects affected / exposed	2 / 6 (33.33%)	0 / 6 (0.00%)	4 / 11 (36.36%)
occurrences (all)	2	0	4
Poor appetite			
subjects affected / exposed	0 / 6 (0.00%)	1 / 6 (16.67%)	0 / 11 (0.00%)
occurrences (all)	0	1	0

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? No

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

None reported

Online references

<http://www.ncbi.nlm.nih.gov/pubmed/38052667>

<http://www.ncbi.nlm.nih.gov/pubmed/40040359>