European Review for Medical and Pharmacological Sciences 2023; 27: 12103-12111

Phase I study of the pharmacokinetics and safety of single and multiple doses of intravenous N-acetylcysteine in healthy Chinese subjects

J. SUN[1], X. ZHANG[2], L. WANG[2], A.F.D. DI STEFANO[3], V. ZANIN[4], P. MAGRONE[4], Y. YUAN[1]

[1]Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[2]Medical and Regulatory Affairs, Hainan Zambon Pharmaceutical Co., Ltd, Hainan, China
[3]CROSS Research SA, Mendrisio, Switzerland
[4]Medical Department, Zambon S.p.A., Bresso, Milan, Italy

Abstract. – OBJECTIVE: The aim of the study was to determine the pharmacokinetics (PK) and safety of single and repeat doses of intravenous (IV) N-Nacetylcysteine (NAC) in Chinese subjects.

PATIENTS AND METHODS: A total of 24 healthy male and female Chinese subjects aged 19-40 years were enrolled in this open-label phase I study. All subjects received a single dose of NAC 600 mg IV on day 1 and, after a 3-day washout, received repeat doses of NAC 600 mg IV (twice daily on days 4 and 5 and once on day 6).

RESULTS: Following a single dose, plasma NAC concentrations peaked rapidly, starting to fall at the end of the 5-minute infusion in a multiphasic manner. Mean C_{max} was 83.30 μg/mL (CV% 30.7%), median T_{max} was 0.083 h (range 0.08-0.25 h), and mean $AUC_{(0\text{-}12\ h)}$ was 81.87 h*μg/mL (CV 14.0%). Following repeat dosing, C_{max} was approximately 20% higher than after a single dose, with similar T_{max}. Total exposure $AUC_{(0\text{-}12)}$ was 13% higher at steady state than after single dosing. The accumulation ratio was approximately 1.13, indicating only a slight accumulation with multiple dosing. NAC was eliminated with $T_{1/2}$ of approximately 8 hours. Around 15% of the total NAC dose was excreted in the urine in the 32 hours post-dose, keeping with extensive NAC metabolism and transformation. Renal clearance of NAC was 995.2 mL/h (CV 50.2%). IV NAC was well tolerated after both single and multiple dosing.

CONCLUSIONS: This is the first robust study evaluating the PK and safety of IV NAC 600 mg in Chinese subjects and provides important data if this agent is to be used IV as a mucolytic in this population.

Key Words:
Pharmacokinetics, Safety, Intravenous, Single dose, Multiple-dose, N-acetylcysteine, Chinese subjects.

Introduction

N-acetylcysteine (NAC) was first introduced in the 1960s and has become an established mucolytic agent for chronic respiratory conditions. The efficacy and safety of oral NAC are well documented, and this route is commonly used to administer NAC as a mucolytic and expectorant[1]. Besides this, the use of NAC as an adjunctive treatment for respiratory viral infections, as a potential agent in preventing cytokine storm and prothrombotic state in COVID-19, as well as in multidrug-resistant infections, has been recently proposed[2-5]. Although the efficacy and safety of intravenous (IV) NAC given as a mucolytic is relatively limited, parenteral administration may be preferred in certain clinical situations, such as in hospitalized patients, as shown by Tang et al[6].

The pharmacokinetics (PK) of oral NAC in healthy subjects is well documented, and many studies are available in the literature[7-10]. One study[11] compared the PK of oral NAC in healthy Chinese and Caucasian subjects. It concluded that the PK characteristics, safety, and tolerability of oral NAC are similar in healthy Chinese and Caucasian individuals after single and multiple administration[11].

PK data following IV administration of NAC are more limited, with early studies using analytical methods that precipitate blood proteins before reducing NAC's intermolecular disulfide bonds, which would miss a large part of the analyte in the measurements[12,13]. Recently, more sensitive bioanalytical methods foresee bisulfide reduction before deproteinization. As far as the

Corresponding Author: Y. Yuan, MD; e-mail: yyz28@medmail.com.cn

authors are aware, there have been no studies to date on the PK and safety and tolerability of IV NAC in healthy Chinese subjects.

Establishing PK parameters and safety and tolerability of IV NAC in healthy Chinese subjects in a robust Phase I clinical trial carried out using modern bioanalytical methods is important to confirm the feasibility of using IV NAC as a mucolytic and expectorant in this population.

The current phase I study aimed to evaluate the pharmacokinetics, safety, and tolerability of single and repeat doses of IV NAC in healthy Chinese subjects.

Patients and Methods

Study Subjects

A total of 24 healthy male and female adult Chinese subjects with body weight ≥50 kg and body mass index (BMI) of 19-26 kg/m^2 were enrolled in the study at a single phase I center.

All subjects were required to have blood pressure and heart rate within the normal range at baseline and to be able to abstain from smoking for the study duration. Women of childbearing potential were required to use a reliable method of contraception for at least 60 days prior to study entry, during the study and for 2 weeks post-dose.

Subjects were excluded from participating in the study if they had clinically significant abnormalities on ECG, physical examination, or laboratory analyses; a significant history of disease that might interfere with the aim of the study; active viral or bacterial infection; positive result for human immunodeficiency virus (HIV), hepatitis B or C; surgery in the past 60 days; medications within the past 2 weeks including over-the-counter medication, herbal or traditional Chinese remedies (but allowing oral contraceptives for women); vaccination within the past 4 weeks; high-level use of alcohol, caffeine or tobacco.

Methods

This phase I study was of single- and multiple-dose, open-label design and was performed at a single center (Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Phase I Unit, Shanghai, China). The trial was designed by the sponsors (Zambon S.p.A. Italia) and the academic authors. It was registered at www.clinicaltrials. gov, No. NCT03881163.

All subjects enrolled in the study received the same treatment schedule with IV NAC. On day 1 at 08.00 ± 1 h, subjects received a single dose of NAC 600 mg IV under fasting conditions. After a washout of 3 days, subjects received repeat doses of NAC 600 mg IV on days 4 and 5 at 08.00 ± 1 h and 20.00 ± 1 h and on day 6 at 08.00 ± 1 h (total of five doses). Standardized meals were provided except for breakfast on day 1 and day 6 due to the requirement for fasting prior to administration of the IV NAC, i.e., on the days of PK blood sampling.

Study medication was provided in vials containing NAC 300 mg, sodium hydroxide 74 mg, and disodium edetate 3 mg with water for injection to a total volume in each vial of 3 mL. Study medication was administered by IV infusions over at least 5 minutes as NAC 600 mg (two vials of 3 mL each) diluted in 10 mL NaCl 0.9% saline (total infusion volume 16 mL).

For pharmacokinetic evaluation, venous blood samples (up to 10 mL) were collected from an indwelling catheter in a forearm vein. The first mL of blood was discarded at each collection point, with the remaining blood sample collected into blood collection tubes. Blood was sampled prior to the first dose on Day 1, at 5 min (end of infusion), and at 8, 12, 15, 20, 25, 30, 60 min and 2, 4, 6, 8, 10, 12, 24 and 32 h post-dose. On days 4 and day 5, blood was sampled pre-dose and on day 6 pre-dose and at 5 min (end of infusion), 8, 12, 15, 20, 25, 30, 60 min and 2, 4, 6, 8, 10, 12, 24 and 32 h post-dose. Urine was sampled post-dose on day 1 and day 6 at 0-4, 4-8, 8-12, 12-24 and 24-32 h, with the bladder required to be emptied before the end of each collection period.

Concentrations of total NAC in plasma and urine were determined at a certified bioanalytical laboratory according to Good Laboratory Practice using a fully validated modern liquid chromatograph mass spectrometry/mass spectrometry method, with a lower limit of quantification of 10 ng/mL.

After a single dose of IV NAC, plasma PK parameters measured and/or calculated included: maximum NAC plasma concentration (C_{max}); time to achieve C_{max} (T_{max}); terminal elimination rate constant (K_{el}); half-life ($T_{1/2}$); area under concentration-time curve to last observed concentration-time [t] ($AUC_{(0-t)}$); AUC extrapolated to infinity ($AUC_{(0-inf)}$); AUC to 12 hours post-dose ($AUC_{(0-12)}$); percentage of $AUC_{(0-inf)}$ obtained by extrapolation ($\%AUC_{extra}$); total body clearance (CL_t); and volume of distribution (V_d).

After multiple doses of IV NAC, plasma PK parameters measured and/or calculated at steady

state (ss) included C_{ss_max}; T_{ss_max}; trough NAC plasma concentration at t=12 hours post-dose (C_{ss_min}); $AUC_{ss(0-t)}$; $AUC_{ss(012)}$; average NAC plasma concentration (C_{ss-avg}); degree of fluctuation over one dosing interval (DF%); and accumulation ratio (R).

Urine PK parameters assessed after single dosing included: volume of urine during each collection interval (V_{ur}); amount excreted in the urine at each collection interval ($Ae_{(t1-t2)}$); cumulative amount excreted up to tz, where tz is 4, 8, 12, 24, 32 hours ($Ae_{(0-tz)}$); total amount excreted in urine up to 32 hours ($Ae_{(0-t)}$); the fraction of drug excreted in urine calculated as fe=Ae/Dose up to tz hours ($Fe_{(0-tz)}$); the total fraction of NAC dose excreted in urine up to 32 hours ($Fe_{(0-t)}$); renal clearance (CL_R). After multiple dosing, total amounts of NAC excreted in urine from the last multiple-dose to 32 hours ($Ae_{ss(0-t)}$) was assessed.

Safety and tolerability were assessed by treatment-related adverse events (TEAEs, defined as an adverse event that occurred or worsened after the first dose of IV NAC), physical examination, weight, ECGs, vital signs, and clinical laboratory analyses (hematology, blood chemistry and urinalysis). Adverse event monitoring started immediately after informed consent was obtained up to the final follow-up visit on day 8, or in the case of early discontinuation from the study at an early termination visit.

Statistical Analysis

No formal sample size calculation was performed. A sample size of 24 subjects was considered sufficient for the descriptive purposes of the study.

PK and safety results were analyzed using descriptive statistics. Unless otherwise stated, continuous data were summarized in terms of the mean, standard deviation (SD), median, minimum, maximum, and number of observations. Categorical data were summarized in terms of the number of subjects providing data at the relevant time point (n), frequency counts and percentages.

Analysis of PK parameters was performed on the dataset that included all enrolled subjects who fulfilled protocol requirements with respect to IV NAC intake and had evaluable PK data for the planned analyses, with no major protocol deviation that might affect PK results. Safety and tolerability analyses were performed on the dataset, including all enrolled subjects who received at least one dose of IV NAC.

PK parameters were calculated by non-compartmental analysis methods based on actual sampling times relative to dosing, with no imputation of missing data and inclusion in the analysis of any subjects with missing concentration data provided that at least C_{max} and $AUC_{(0-t)}$ could be reliably calculated. The sampling schedule was considered adequate if $\%AUC_{extra}$ was <20% for more than 80% of individual PK profiles.

The quality of log-linear regression (reflecting the reliability of extrapolated PK parameters) had to be demonstrated by a determination coefficient $R^2 \geq 0.8$. When considered unreliable, individual extrapolated parameters were to be reported as not calculated (NC).

Below quantification limit (BQL) values were imputed in the PK concentration dataset based on BQLs prior to the first incidence of a measurable concentration assigned to zero, with BQLs after the last incidence of a measurable concentration or BQLs between measurable concentrations set to missing.

Results

Study Subjects

All 24 subjects received the planned study medication and completed the study without major protocol violations. Demographics and subject characteristics are shown in Table I. The age of subjects ranged from 19 to 40 years, with 75% of subjects being male. More than 90% of subjects had never consumed alcohol, tobacco, or caffeine.

Single-Dose Plasma NAC Pharmacokinetics

Mean plasma concentrations of NAC over time on Day 1 are shown in tab 1A, with plasma PK parameter results shown in Table II. Following a single dose of 600 mg IV NAC, plasma concentrations peaked rapidly, as expected, with most subjects attaining C_{max} by the end of the 5-minute infusion. The remaining subjects reached C_{max} between 8 and 15 minutes post-dose. At the end of the infusion, plasma NAC levels started to fall in a multiphasic manner. The geometric mean C_{max} was 83.30 μg/mL (CV% 30.7%) and was reached in a median T_{max} of 0.083 h (range 0.08-0.25 h).

All 24 subjects had quantifiable NAC plasma levels up to 12 hours post-dose, resulting in very similar geometric mean $AUC_{(0-12h)}$ and $AUC_{(0-t)}$, namely 81.87 h*μg/mL (CV 14.0%) and 87.16 h*μg/

Table I. Subject demographic and baseline characteristics.

Demographic and baseline characteristics	N = 24, n (%) or mean ± SD
Sex:	
Male	18 (75.0)
Female	6 (25.0)
Age (years)	30.6 ± 5.18
Chinese ethnicity	24 (100)
Weight (kg)	66.85 ± 7.58
Height (cm)	169.0 ± 7.92
BMI (kg/m^2)	23.36 ± 1.65
Alcohol consumption:	
Current	2 (8.3)
Former	0
Never	22 (91.7)
Tobacco consumption:	
Current	2 (8.3)
Former	0
Never	22 (91.7)
Caffeine consumption†:	
Current	0
Former	0
Never	23 (95.8)

†Data on caffeine consumption available for n = 23.

mL (CV 17.4%), respectively. At 24 and 32 hours, plasma NAC levels were still quantifiable in 13 and three subjects, respectively. Low to moderate variability in NAC plasma concentrations was observed with CV% for geometric mean C_{max} of 31% and $AUC_{(0-t)}$ of 17%.

The sampling schedule was adequate to capture the majority of the NAC plasma concentration-time curve, with a geometric mean percentage of $AUC_{(0-inf)}$ ($\%AUC_{extra}$) obtained by extrapolation of 6.97% (CV 26.8%). Extrapolation to infinity could be performed for all 24 subjects, with geometric mean $AUC_{(0inf)}$ slightly higher than $AUC_{(0-t)}$ at 93.94 h*μg/mL (CV 18.0%). Estimates of $T_{1/2}$ were deemed reliable in all subjects, with a geometric mean for $T_{1/2}$ of 7.13 h (CV 56.8%).

Multiple-dose Plasma NAC Pharmacokinetics

The NAC plasma concentration over time curve following the last repeat dose on day 6 is shown in Figure 1b, with plasma PK parameter results shown in Table III. Following twice daily administration of 600 mg IV NAC for 3 days, almost all subjects attained C_{max} at the end of the 5-minute infusion, with NAC levels falling in a multiphasic manner thereafter. NAC plasma levels were quantifiable in all but one subject until 32 hours post-dose. The elimination curve could be clearly determined in all 24 subjects.

C_{max} was approximately 20% higher at steady state than after a single dose, with similar T_{max} after single and multiple dosing. Total exposure was slightly higher following multiple dosing compared with a single dose, with $AUC_{(0-12)}$ and $AUC_{(0-t)}$ 13% and 34% higher at steady state, respectively.

Accumulation ratio R was approximately 1.13, indicating only slight accumulation with multiple dosing.

Low to moderate variability in NAC plasma concentrations was observed, similar to that seen following single-dose administration (multiple dose CV for geometric mean C_{max} of 29% and for $AUC_{(0-t)}$ of 15%).

Pre-dose NAC levels on Day 4 were BQL in all subjects, with geometric mean trough concentrations on Day 5 of 1.39 μg/mL (CV 22.1%).

Urine Parameters

Following both single- and multiple-dose IV administration of 600 mg NAC, the cumulative amount of NAC excreted in urine appeared to plateau by approximately 12 hours post-dose (data not shown).

The amount of NAC excreted over the first 32 hours post single administration appeared to be greater than that following multiple dose administration [single dose $Ae_{(0-32)}$ geometric

mean 93490 μg (CV 48.5%) *vs.* 68980 μg (CV 68.1%) following multiple dosing].

Approximately 15% of the total NAC dose was excreted over the first 32 hours following single-dose administration [geometric mean $Fe_{(0-32)}$ 0.1558 (CV 48.5%)], in keeping with extensive metabolism and transformation of NAC. Renal clearance of NAC following single dose IV administration was 995.2 mL/h (CV 50.2%).

Safety and Tolerability

IV NAC was well tolerated after both single and multiple dosing. A total of 7 (29.2%) subjects experienced at least one TEAE during the study [3 (12.5%) subjects in the single-dose phase and 5 (20.8%) in the multiple-dose phase, with one subject experiencing a TEAE in both phases]. All TEAEs were mild in severity.

All TEAEs reported were in the system organ class (SOC) 'Investigations' [2 (8.3%) in the single dose phase and 4 (16.7%) in the multiple-dose phase] and 'Gastrointestinal disorders' [1 (4.2%) each in the single-dose phase and multiple-dose phase] (Table IV).

TEAEs in two (8.3%) subjects were considered at least possibly related to IV NAC (one TEAE of

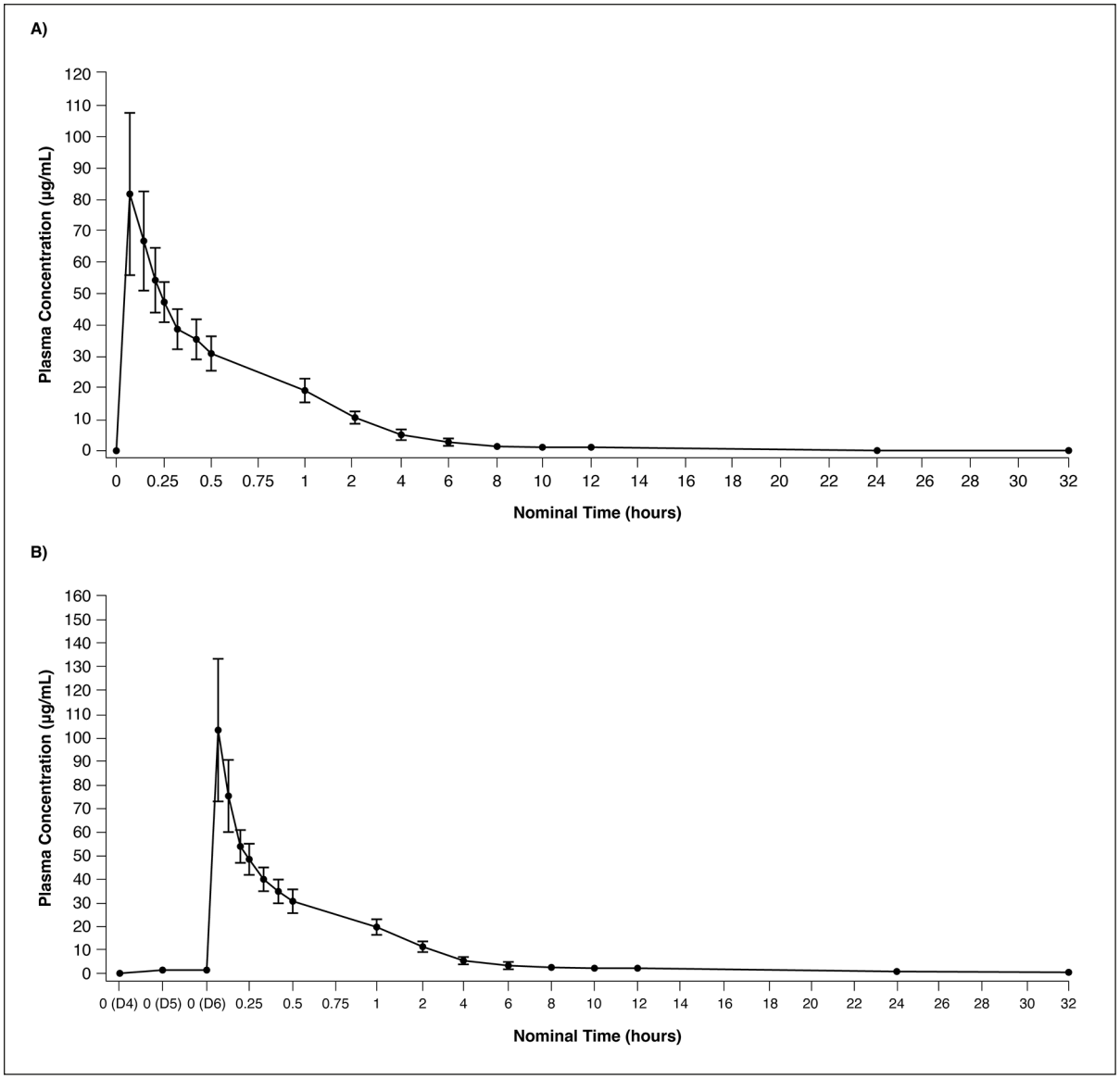

Figure 1. Mean (± SD) NAC plasma concentration *vs.* time. The outlier plasma concentration of 8,550 μg/mL for one subject at 5 minutes post-dose on day 6 was excluded from the statistical analysis.

Table II. Plasma PK parameters following a single dose of IV NAC 600 mg (n = 24).

	C_{max} (µg/mL)	T_{max} (h)	K_{el} (1/h)	$T_{1/2}$ (h)	$AUC_{(0-t)}$ (h*µg/mL)	$AUC_{(0-inf)}$ (h*µg/mL)	$AUC_{(0-12\ h)}$ (h*µg/mL)	$\%AUC_{extra}$ (%)	CL_t (L/h)
Mean±SD	86.53±22.08	–	0.11±0.057	8.11±4.05	88.43±15.57	95.39±17.31	82.63±11.44	7.19±1.80	6.48± 1.15
CV (%)†	25.5	–	51.4	50.0	17.6	18.1	13.8	25.0	17.7
Median (range)	82.90 (34.80 - 129.0)	0.083 (0.08 - 0.25)	0.081 (0.043 - 0.21)	8.53 (3.23 - 16.26)	86.23 (60.03 - 120.2)	91.55 (63.80 - 129.5)	81.29 (60.02 - 109.0)	7.04 (3.81 - 10.16)	6.56 (4.63 - 9.40)
Geometric mean	83.30	–	0.097	7.13	87.16	93.94	81.87	6.97	6.39
Geometric CV (%)‡	30.7	–	56.8	56.8	17.4	18.0	14.0	26.8	18.0

†CV (%) = SD/Mean×100. ‡GEO CV (%) = sqrt (exp (v)-1)×100, v is variance of ln (concentration).

Table III. Plasma PK parameters following a single dose of IV NAC 600 mg (n = 24).

	C_{max} (µg/mL)	T_{max} (h)	C_{ss_min} (µg/mL)	$AUC_{ss(0-t)}$ (h*µg/mL)	$AUC_{ss(0-12h)}$ (h*µg/mL)	C_{ss_avg} (µg/mL)	DF% (%)	R
Mean±SD	104.7±29.61	–	1.94±0.42	117.9±17.7	93.50±12.98	7.79±1.08	1332±370.3	1.13±0.067
CV (%)†	28.3	–	21.9	15.0	13.9	13.9	27.8	5.9
Median (range)	104.5 (61.50 - 181.0)	0.083 (0.07 - 0.13)	1.85 (1.31 -3.17)	115.1 (81.64 - 153.20)	93.04 (66.32 - 120.9)	7.75 (5.53 - 10.08)	1406 (613.7 - 2113)	1.13 (0.97 - 1.27)
Geometric mean	100.8	–	1.90	116.6	92.63	7.72	1279	1.13
Geometric CV (%)‡	29.1	–	20.9	15.1	14.1	14.1	30.7	6.0

†CV (%) = SD/Mean×100. ‡GEO CV (%) = sqrt (exp (v)-1)×100, v is variance of ln (concentration). The outlier plasma concentration of 8,550 µg/mL for one subject at 5 minutes post-dose on day 6 was excluded from the statistical analysis of PK results following consultation at the Data Review Meeting.

nausea in the single-dose phase and one TEAE of retching in the multiple-dose phase).

There were no severe TEAEs, serious adverse events (SAEs), deaths, or TEAEs leading to discontinuation or interruption of study medication.

No trends were observed over time in clinical laboratory evaluations, vital signs, physical examination, weight, or ECG findings. One subject had an abnormal post-baseline reading for bilirubin and urate, reported as TEAEs.

Discussion

The current study provides pharmacokinetic and safety data following single and multiple dosing of IV NAC in Chinese subjects in a phase 1 study carried out according to Good Clinical Practice and using modern bioanalytical methods.

Following single and multiple dose IV administration of 600 mg NAC, plasma concentrations of NAC peaked rapidly at the end of the injection, exhibited low to moderate variability, and were eliminated with a $T_{1/2}$ of approximately 8 hours. Plasma NAC levels returned to BQL at 32 h post-dose for most subjects.

Peak and total exposure of NAC were slightly higher following multiple-dose IV administration compared with single-dose IV administration. However, multiple dosing of NAC demonstrated only a slight accumulation of the drug.

Following both single and multiple-dose IV administration of 600 mg NAC, the cumulative amount of NAC excreted in urine appeared to plateau by approximately 12 hours post-dose. Only a minor fraction of the administered dose was excreted over 32 hours, consistent with the well-recognized extensive metabolism and transformation of NAC with final excretion as sulphate or sulphur[14]. Renal clearance of NAC following IV administration was 995.2 mL/h.

Comparability across previous studies of PK parameters following single or repeat doses of IV NAC in healthy subjects is low, making a comparison of the current study with prior findings difficult. Each of the three previous studies included small numbers of subjects (each study with $\leq$ 10 subjects[12,13,15]. A further study is not considered relevant as it used constant rate infusions to achieve target constant NAC plasma concentrations during cycling exercise in healthy male subjects[16]. The studies by Borgstrom[12] and Olsson[13] used older bioanalytical methods that would miss a large part of the analyte due to the precipitation of blood proteins before reducing NAC's intermolecular disulphide bonds. However, the mean clearance (CLt) reported by Olsson[13] is very similar to that found in the current study (7.26 L/h *vs.* 6.48 L/h). The study by Jones et al[15] of the bioavailability of NAC after a single IV dose of 600 mg injected over 3 minutes in subjects with chronic liver disease and in 6 healthy controls used an improved bioanalytical method to analyze the total NAC plasma concentration capable of avoiding understimations. Mean AUC in the healthy controls of 93.9 (±9.6) was very similar to that observed in the current study, although CL_R, $T_{1/2}$ from the healthy controls are inconsistent with the current study. This could be due to the small number of subjects (n=6) included in the previous study.

The current single and multiple-dose study of IV NAC provides PK and safety data from a larger number of healthy subjects than previously reported in the literature for this dose and route of administration. It is the first report of the

Table IV. Summary of treatment emergent adverse events.

System organ class preferred term	NAC 600 mg (n = 24), n (%)		
	Single dose	Multiple dose	Total
Number of subjects with at least one TEAE	3 (12.5)	5 (20.8)	8 (33.3)
Investigations	2 (8.3)	4 (16.7)	6 (25)
Urine output increased	0 (0)	3 (12.5)	3 (12.5)
Blood bilirubin increased	0 (0)	1 (4.2)	1 (4.2)
Blood uric acid increased	1 (4.2)	0 (0)	1 (4.2)
Weight decreased	1 (4.2)	0 (0)	1 (4.2)
Gastrointestinal disorders	1 (4.2)	1 (4.2)	2 (8.3)
Nausea	1 (4.2)	0 (0)	1 (4.2)
Retching	0 (0)	1 (4.2)	1 (4.2)

TEAEs were classified according to MedDRA version 22.0.

PK of NAC following single and multiple IV dosing in Chinese subjects. It provides important information to guide the therapeutic use of this agent as a mucolytic when given by the IV route in this population.

Conclusions

Altogether, our data on PK and safety provide evidence of the use of single or multiple dosing of IV NAC as a mucolytic agent in Chinese subjects. These data highlight the possible management of Chinese patients with respiratory diseases associated with abnormal mucus production, in which the IV route is preferred.

Conflict of Interest

X. Zhang and L. Wang are employees of Hainan Zambon Pharmaceutical Co., Ltd; A. F. D. Di Stefano is an employee of CROSS Research SA; V. Zanin and P. Magrone are employees of Zambon S.p.A. Italia; J. Sun and Y. Yuan declare no conflict of interest. The sponsor had no role in conducting the analysis but was involved in the interpretation of data, preparation review and final approval of the manuscript.

Acknowledgments

The authors thank Aashni Shah, BSc, Lara Vecchi, Ph.D. and Valentina Attanasio, Ph.D. (Polistudium srl, Milan, Italy) for medical writing and editorial assistance. This was funded by Zambon S.p.A. Italia. The contract research organization, CROSS Research SA, was responsible for monitoring the study. The authors and the sponsor thank the subjects for agreeing in participating to the study.

Funding

Zambon S.p.A. Italia funded the research and provided the study medication.

Authors' Contributions

All the authors are responsible for the conception and design of the study; J. Sun and Y. Yuan are responsible for the enrollment of patients, collection and analysis of data; all the authors contributed to data analysis and interpretation, writing, revision of the manuscript and final approval of the manuscript.

Ethics Approval and Informed Consent

The protocol of the study has been approved by the Ethic Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. Prior to inclusion in the study, each subject provided an informed consent form upon having received extensive oral and written education about the study. The study was conducted in accordance with Good Clinical Practice (GCP), ICH topic E6 (R2 and CFDA GCP) guidelines, the rules of the Declaration of Helsinki, all applicable local law requirements and regulations concerning the privacy and security of personal information, including the General Data Protection Regulation (GDPR) (EU) 2016/679.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1) Stey C, Steurer J, Bachmann S, Medici TC, Tramèr MR. The effect of oral N-acetylcysteine in chronic bronchitis: a quantitative systematic review. Eur Respir J 2000; 16: 253-262.
2) Santus P, Danzo F, Zuffi A, Pini S, Saad M, Visconti A, Radovanovic D. Oxidative stress and viral Infections: rationale, experiences, and perspectives on N-acetylcysteine. Eur Rev Med Pharmacol Sci 2022; 26: 8582-8590.
3) Oliva A, Pallecchi L, Rossolini GM, Travaglino F, Zanatta P. Rationale and evidence for the adjunctive use of N-acetylcysteine in multidrug-resistant infections. Eur Rev Med Pharmacol Sci 2023; 27: 4316-4325.
4) Mohanty RR, Padhy BM, Das S, Meher BR. Therapeutic potential of N-acetyl cysteine (NAC) in preventing cytokine storm in COVID-19: review of current evidence. Eur Rev Med Pharmacol Sci 2021; 25: 2802-2807.
5) Di Marco F, Foti G, Corsico AG. Where are we with the use of N-acetylcysteine as a preventive and adjuvant treatment for COVID-19? Eur Rev Med Pharmacol Sci 2022; 26: 715-721.
6) Tang W, Zhu D, Wu F, Xu JF, Yang JP, Deng ZP, Chen XB, Papi A, Qu JM. Intravenous N-acetylcysteine in respiratory disease with abnormal mucus secretion. Eur Rev Med Pharmacol Sci 2023; 27: 5119-5127.
7) Burgunder JM, Varriale A, Lauterburg BH. Effect of N-acetylcysteine on plasma cysteine and glutathione following paracetamol administration. Eur J Clin Pharmacol 1989; 36: 127-131.
8) De Caro L, Ghizzi A, Costa R, Longo A, Ventresca GP, Lodola E. Pharmacokinetics and bioavailability of oral acetylcysteine in healthy volunteers. Arzneimittelforschung 1989; 39: 382-386.
9) Borgstrom L, Kagedal B. Dose dependent pharmacokinetics of N-acetylcysteine after oral dosing to man. Biopharm Drug Dispos 1990; 11: 131-136.
10) Greene SC, Noonan PK, Sanabria C, Peacock WF. Effervescent N-acetylcysteine tablets versus oral solution n-acetylcysteine in fasting healthy

adults: An open-label, randomized, single-dose, crossover, relative bioavailability study. Curr Ther Res Clin Exp 2016; 83: 1-7.

11) Papi A, Di Stefano AFD, Radicioni M. Pharmacokinetics and safety of single and multiple doses of oral N-acetylcysteine in healthy Chinese and Caucasian volunteers: An open-label, Phase I clinical study. Adv Ther 2021; 38: 468-478.
12) Borgstrom L, Kagedal B, Paulsen O. Pharmacokinetics of N-acetylcysteine in man. Eur J Clin Pharmacol 1986; 31: 217-222.
13) Olsson B, Johansson M, Gabrielsson J, Bolme P. Pharmacokinetics and bioavailability of reduced and oxidized N-acetylcysteine. Eur J Clin Pharmacol 1988; 34: 77-82.
14) Sheffner AL, Medler EM, Bailey KR, Gallo DG, Mueller AJ, Sarett HP. Metabolic studies with acetylcysteine. Biochem Pharmacol 1966; 15: 1523-1535.
15) Jones AL, Jarvier D. Simpson D. Pharmacokinetics of N-acetylcysteine are altered in patients with chronic liver diseases. Aliment Pharmacol Ther 1997; 11: 787-791.
16) Brown M, Bjorksten A, Medved I, McKenna M. Pharmacokinetics of intravenous N-acetylcysteine in men at rest and during exercise. Eur J Clin Pharmacol 2004; 60: 717-723.