Current Clinical Pharmacology, 2020, *15*, 152-163

CLINICAL TRIAL STUDY

First-in-Human Study of the Safety, Tolerability, Pharmacokinetics and -Preliminary Dynamics of Neuroprotectant 2-Iminobiotin in Healthy Subjects

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ARTICLE HISTORY

Received: June 05, 2019
Revised: July 18, 2019
Accepted: July 19, 2019

DOI:
10.2174/1574884714666191017111109

Abstract: ***Background*:** 2-iminobiotin (2-IB) is an investigational neuroprotective agent in development for the reduction of brain cell injury after cerebral hypoxia-ischemia.

***Objective*:** The present first-in-human study evaluated the safety, tolerability, pharmacokinetics (PK) and -dynamics (PD) of 2-IB in healthy male subjects, intravenously infused with or without Captisol® as a solubilizing agent.

***Methods*:** This randomized, double-blind, placebo-controlled, dose-escalation study was executed in 2 groups of 9 healthy male subjects. A single dose of 2-IB 0.6 mg/kg or placebo was infused over periods between 15 min and 4 h, and repeated doses escalating from 0.6 mg/kg to 12 mg/kg, or placebo were infused every 4 h for 6 administrations in total.

***Results*:** Single and multiple doses of 2-IB up to 6 doses of 6 mg/kg with and without Captisol® were safe and well-tolerated in healthy male subjects. 2-IB proved to be a high-clearance drug with a volume of distribution slightly exceeding total body water volume, and with linear PK that appeared not to be affected by the presence of Captisol®.

***Conclusion*:** Sulfobutyletherbeta-cyclodextrin (SBECD) in Captisol® had a low-clearance profile with a small volume of distribution, with time-independent PK. Preliminary PD characterization of repeated iv dosing of 2-IB in an acute peripheral hypoxic ischemia model in healthy subjects did not reveal any notable effects of 2-IB, noting that this model was not selected to guide efficacy in the currently pursued indication of cerebral hypoxia-ischemia.

Keywords: 2-iminobiotin, healthy subjects, nitric oxide synthase, pharmacokinetics, neuroprotection, birth asphyxia, cardiac arrest.

1. INTRODUCTION

2-Iminobiotin (2-IB) is a novel injectable pharmaceutical product with anti-oxidative properties that is currently in clinical development to reduce brain cell injury after cerebral hypoxia-ischemia. Potential indications include birth asphyxia, cardiac arrest, as well as (congenital) heart defects or coronary bypass surgery.

Birth asphyxia comprises impaired gas exchange between the mother and the fetus, leading to Hypoxia-Ischemia (HI). HI triggers a cascade of events, including free radical production, Nitric Oxide Synthases (NOS) activation, mitochondrial failure, inflammation and apoptosis, with neonatal Hypoxic-Ischemic Encephalopathy (HIE) as a potential clinical manifestation in the short term [1]. Long-term sequelae of birth asphyxia include seizures, neuropsychiatric disorders, and effects on motor function (cerebral palsy), cognition, vision, sensation, and attention [2].

Birth asphyxia accounts for 23% of neonatal morbidity and mortality worldwide [3], and despite serious efforts, neonatal mortality below 5 years of age has not improved the last decade [4]. Therapeutic hypothermia has now been established as a safe method to reduce mortality and morbidity for HIE with an effect size of about 15% in Western countries [5]. Since still 45% of neonates affected by HIE have an unfavorable neurodevelopmental outcome at the age of 2 years old, additional neuroprotective strategies in combination with hypothermia are needed [1, 2, 6]. In low-income countries where the highest burden of HIE falls, and therapeutic hypothermia has not been established as a safe and

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2212-3938/20 $65.00+.00

effective treatment strategy, a low-cost and effective solution is urgently needed [7, 8].

Cardiac arrest describes the loss of cardiac function, leading to the absence of systemic circulation. Globally, it is estimated that on average, less than 10% of all patients with out-of-hospital cardiac arrest will survive [9]. However, early basic life support and early defibrillation using automated external devices will improve survival rates in the upcoming years [10]. In survivors of cardiac arrest, about 30-50% experience cognitive problems [11, 12] and this incidence will continue to grow.

2-IB exerts its neuroprotective effect by inhibition of neuronal and inducible isoform of NOS. It is intended to modulate the pathophysiological pathways triggered by oxygen shortage in the brain in the early phase after hypoxia-ischemia [13, 14]. Based on *in vitro* cell culture data and an *in vivo* model in piglets, the neuroprotective 2-IB concentration in cerebrospinal fluid has been estimated to approximately 30 ng/mL [15, 16]. 2-IB does not affect endothelial NOS, and is thereby expected not to affect blood pressure and cerebral perfusion.

As a starting point for the clinical development of 2-IB in reducing brain injury after hypoxia-ischemia, the present first-in-human study with 2-IB was designed and executed. This escalating dose study evaluated the safety, tolerability Pharmacokinetics (PK) and pharmacodynamics (PD; oxygenation and hemodynamics) of intravenously (iv) administered 2-IB in healthy subjects. For the higher 2-IB dose levels, the polyanionic beta-cyclodextrin derivative Captisol® was used to increase formulation strength and thereby reducing infusion volume, safety and PK of Captisol® were evaluated as well.

2. MATERIALS AND METHODS

2.1. Ethics, Good Clinical Practice, and Privacy

The clinical study protocol, protocol amendments and the Informed Consent Forms (ICFs) were reviewed and approved by an Independent Ethics Committee (Stichting Beoordeling Ethiek Bio-Medisch Onderzoek, Assen, The Netherlands). The study was conducted in accordance with the principles of the Declaration of Helsinki including amendments in force up to and including the time the study was conducted. The study was conducted in compliance with the International Conference on Harmonisation (ICH) E6 Guideline for Good Clinical Practice (GCP) (Committee for Proprietary Medicinal Products (CPMP) guideline CPMP/ICH/135/95), and compliant with the European Union Clinical Trial Directive (EU CTD): Directive 2001/20/EC. All participants were informed verbally and in writing of the objectives, procedures and risks of study participation including possible side effects and potential interactions. In addition, the insurance cover provided during the study was explained. Participants signed the ICF during the pre-study screening visit, within 3 weeks prior to the first dosing day and before any study-related procedures were started. All personal details were treated as confidential by the Medical Investigator and staff at PRA Health Sciences, and handling of personal data was in compliance with the Dutch Data Protection Act. Personal details were stored in databases that have been registered with the Dutch Data Protection Authority.

2.2. Study Design and Plan

This was a phase 1, randomized, double-blind, placebo-controlled, dose-escalation study with 2 groups of 9 healthy male subjects, each receiving a single iv infusion (group 1, period 1) or repeated iv infusions (group 1, periods 2 and 3 and group 2) of 2-IB or placebo in a cross-over design over 3 study periods. Treatments were randomized such that each subject received 2 out of 3 foreseen dose levels of 2-IB and once *placebo*. There was a washout period of at least 7 days between each dosing period. In this first-in-human study, subjects participating at the lowest dose level (group 1, period 1), were dosed according to a sentinel dosing design to ensure optimal safety. Of these 3 sentinel subjects, 2 subjects were dosed with 2-IB over a duration of 4 h, and 1 subject with placebo. The remaining subjects of group 1 were dosed based on satisfactory safety and tolerability results of the first 24 h following dosing of the sentinel subjects. After completion of each period of group 1 and periods 1 and 2 of group 2, interim safety evaluations were performed. After completion of group 1, interim PK evaluation was performed, to reconfirm the dosing schedule and PK sampling for the subsequent periods. Subjects were discharged at 24 h post-dose (group 1, period 1) or 48 h post-dose (group 1, periods 2 and 3, and all periods for group 2), and returned for a follow-up visit 7 to 14 days after the last blood sample had been taken.

2.3. Study Population

A total of 18 subjects were to be enrolled into 2 groups of 9 subjects. All subjects were healthy male volunteers, in the age range 18-55 years, inclusive, with a BMI in the range 18-28 kg/m^2, inclusive, and bodyweight not exceeding 90 kg.

2.4. Investigational Product

2-IB drug substance was provided by SAFC Pharma (Gillingham, UK). Captisol® (sulfobutylether-beta-cyclodextrin; SBECD) was obtained from Cydex Pharmaceuticals, Inc. (Lenexa, Kansas, USA). Drug product was prepared by the PRA pharmacy and consisted of 2-IB 1.0 mg/mL in pH 4.0 citrate buffer (group 1), and 2-IB 4.0 mg/mL in pH 4.0 citrate buffer and 5% Captisol® (group 2).

2.5. Treatments

Subject group 1 was initiated at a 2-IB dose level of 0.6 mg/kg (n=6) or placebo (n=3) in 3 subgroups of 3 subjects each, receiving the active drug (n=2) or placebo (n=1) by a single infusion duration of 4 h, 1 h, or 15 min, respectively. In the 2nd period, the same dose level of 0.6 mg/kg or placebo was infused in 15 min every 4 h on Day 1 (6 administrations in total) in all subjects. In the 3rd period, the dose was escalated to 2 mg/kg or placebo infused in 15 min every 4 h on Day 1 (6 administrations in total) in all subjects.

Subject group 2 was initiated at a 2-IB dose level of 2 mg/kg (n=6) or placebo (n=3), both delivered in 5% Captisol®,

infused in 15 min every 4 h on Day 1 (6 administrations in total). In periods 2 and 3, 2-IB dose levels were increased to 6 mg/kg and 12 mg/kg, respectively, or placebo, both in 5% Captisol®, and infused in 15 min (period 2) or 30 min (period 3) every 4 h on Day 1 (6 administrations in total). The co-administration with 5% Captisol® resulted in SBECD dose levels of 25, 75 and 150 mg/kg per infusion, for the 2-IB or placebo dose levels of 2, 6, and 12 mg/kg, respectively.

2.6. Assessments

Safety assessments from the time the informed consent form was signed until completion of the follow-up visit comprised recording of adverse events, clinical laboratory (including clinical chemistry, hematology, coagulation, and urinalysis), vital signs (including supine systolic and diastolic blood pressure, pulse rate, respiratory rate, and oral body temperature), 12-lead ECGs (each period before the first dose and at 6 h, 10 h, 24 h (Groups 1 and 2), and 30 h 34 h, and 48 h (Group 2 only) after the start of the first infusion, inspection of the infusion site in the forearm, and Treatment-Emergent Adverse Events (TEAE). The intensity of TEAE was graded using the following criteria:

- Mild: No interference with the subjects' daily activities and does not require mandatory corrective/symptomatic treatment.
- Moderate: Moderate interference with the subjects' daily activities and/or requires minimal medical intervention or corrective treatment.
- Severe: Major and unacceptable interference with the subjects' daily activities and requires mandatory corrective/symptomatic treatment, possibly hospitalization.

For the assessment of PK of 2-IB (groups 1 and 2) and Captisol® (group 2 only) in plasma, blood samples were collected pre-dose, and at selected time points during and after iv infusion, up to 12 h after start of the infusion when given as a single dose. For the repeated iv infusions, blood samples were collected pre-dose prior to each infusion, at selected time points for up to 4 h after the first infusion, and at selected time points for up to 12 h after the last infusion. Collected blood samples were immediately cooled in ice water, and were processed within 30 minutes by centrifugation for 10 minutes at 4ºC and 1500g. Plasma was transferred into 2 polypropylene tubes of 3.5 mL, and immediately capped, frozen and stored at -70ºC until analysis.

For the assessment of urinary excretion kinetics of 2-IB, urine was collected over 0-4 h and 4-12 h fractions after the start of the single iv infusion, and over 0-4 h and 4-12 h fractions after the start of the last iv infusion. Voided urine was stored in a refrigerator immediately. The total volume of the urine collected during each interval was determined, using the weight of the collected urine and assuming a specific gravity of 1 g/mL. A 10 mL sample of each homogenised fraction was transferred into a polypropylene container, which was stored in the same way as the plasma samples.

Exploratory PD assessments comprised oxygenation and hemodynamics using near-infrared spectroscopy by an automated device (PortaMon, Artinis Medical Systems B.V., Zetten, Netherlands). This assessment was applied in the subjects receiving 2-IB doses as from 2.0 mg/kg or placebo, *i.e*. pre-dose of the first infusion and at 15 min and 2.5 h after the start of the third infusion in Period 3 of Group 1, and at the same three time points (pre-dose before 1st infusion and at 15 min and 2.5 h after the start of the 3rd infusion) in all periods of Group 2. The near-infrared spectroscopy device was placed on the volar side of the lower arm. Arterial occlusion was established by cuff inflation for 2 minutes. Oxygenated Hb (O2Hb), deoxygenated Hb (HHb), total hemoglobin (tHb), and tissue saturation index were registered before occlusion (4 minutes), during occlusion, and up to 8 minutes after the start of reperfusion.

2.7. Bioanalysis

Bioanalysis of plasma and urine samples for 2-IB levels (groups 1 and 2) was performed by validated liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method (NOTOX B.V.,'s-Hertogenbosch, Netherlands). Sample volumes of 50 µL were processed by solid-phase extraction (Oasis™ HLB 96-well extraction plate, Waters Corporation, Milford, MA, USA). 2-IB was eluted using 250 µL of methanol and dried under nitrogen, and after reconstitution in 100 µL of eluent (90/10/0.1 (v/v/v) water/acetonitrile/formic acid), an 8 µL sample was injected on the LC-MS/MS system. Chromatographic conditions comprised an Acquity Ultra-High Performance Liquid Chromatography (UHPLC) system with 515 High-Performance Liquid Chromatography (HPLC) pump 2, and UPLC column and guard columns (Waters Corporation, Milford, MA, USA). The mobile phase consisted of a water/acetonitrile/formic acid gradient, with a flow of 0.7 mL/min. Detection was by API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Sciex Toronto, Canada) with electrospray ionisation in positive ion mode. 2-Iminobiotin-1'-^{15}N,4,5-$^{13}C_2$ was used as an internal standard. The Lower Limit of Quantification (LLOQ) of 2-IB in human EDTA plasma was 5.00 ng/mL, with a dynamic range of 5.00-5000 ng/mL. For the bioanalytical method of 2-IB in urine, the LLOQ was 50.0 ng/mL, with a dynamic range of 50.0-25000 ng/mL.

Bioanalysis of plasma samples for SBECD (group 2) was performed using a validated assay by liquid chromatography coupled with mass spectrometry (Analytical Bio-Chemistry Laboratories, Inc. (ABC) (Columbia, Missouri, USA). A 50 µL aliquot of each human plasma sample was extracted using protein precipitation, and injected on a HPLC system consisting of a Hypersil silica column (250 x 4.6 mm, 5 micron particle size; Thermo Fisher Scientific, Waltham, MA, USA) at 40°C with a flow-rate of 0.4 mL/min. The mobile phase consisted of a gradient of A: 100 mM ammonium acetate in water, and B: 50/50 (v/v) acetonitrile/methanol. The effluent was monitored on an API-3000 mass spectrometric system with turbo-ion spray ionization in negative ion mode and multiple reaction monitoring detections (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). Sulfobutylether-γ-cyclodextrin was used as an internal standard. The method had an LLOQ of 5.00 µg/mL, and operated over a dynamic range of 5.00 to 200 µg/mL.

2.8. Data Analysis

Plasma PK parameters were estimated from the concentration-time profiles using non-compartmental analysis methods for all subjects in the PK population. In estimating the PK parameters, any below limit of quantification values at the beginning of the profile (pre-dose) were set to zero; Below limit of quantification values embedded between 2 quantifiable values were set to missing, values after the last quantifiable point, if any, were considered missing. The time interval of the terminal elimination phase was determined by visual inspection of the concentration-time profiles on the semi-logarithmic scale. If considered feasible, linear regression on the e-log-transformed concentration values versus the actual times within this interval were used to estimate elimination half-life ($t_{1/2el}$). At least 3 datapoints were required to obtain reliable estimates for $t_{1/2el}$ and associated parameters. In calculations of AUC parameters, the log-linear trapezoidal rule (WinNonlin method 1) was used. Plasma and urine PK parameters were evaluated by descriptive statistics (including the geometric mean) by treatment. PK parameters were calculated based on planned doses of 2-IB and SBECD and were not adjusted for deviations from doses actually administered if any.

In the exploratory PD assessments, every subject acted as his own reference. During the arterial occlusion, the oxygen consumption in the muscle was measured as $mVO_2=\Delta O_2Hb/\Delta t$. After the release of the arterial occlusion, the reoxygenation velocity was calculated as $\Delta reoxy = \Delta O_2Hb/\Delta t$ in the first 3 seconds, and its half time ($t_{0.50}$) was calculated as: $t_{0.50}= (t\ (O_2Hb_{max}) - t(O_2Hb_{min}))/2)$, O_2Hb_{max} being the highest value of O_2Hb after its overshoot after cuff release, and O_2Hb_{min} being the original value prior to occlusion. The tissue saturation index was measured during baseline measurement and was defined as $TSI = O_2Hb/(O_2Hb+HHb)*100\%$. Data sets were verified for quality and interpretability by visual inspection prior to analysis, and were rejected if meeting any of the following criteria: (1) instable pressure during occlusion, defined as coefficient of determination (R^2) below 0.98 for the second-order fit of the O_2Hb trace during occlusion, (2) unrealistic tHb change defined as absolute change of tHb exceeding 2/3 of the absolute change of HHb, or (3) disturbed reoxygenation defined as R^2 below 0.90 for the first-order fit of the O_2Hb trace during the first 45 seconds of the reoxygenation. The analysis was done by the analysis of variance, maintaining a p-value of <0.05 for statistical significance. Due to the exploratory nature of the study, no a priori correction was made for multiple comparisons subsequently performed on the data sets.

3. RESULTS AND DISCUSSIONS

3.1. Safety

Treatment with single and multiple doses of 2-IB with and without Captisol® by iv infusion of up to 6 doses of 6 mg/kg was safe and well-tolerated by all healthy male subjects. There were no clinically relevant differences in the clinical laboratory, vital signs, ECG, or physical examinations between placebo and all active treatments (up to and including the dose level of 12 mg/kg). A total of 86 TEAEs were reported for 17 subjects (77% of the 22 subjects who received any study medication). Most of the 86 TEAEs were mild in intensity, 7 TEAEs were moderate in intensity, and no severe events were reported. Four of the moderate TEAEs occurred in subjects treated with 6 x 12 mg/kg 2-IB + Captisol® (nausea, dizziness, and malaise in a single subject, and nausea in a second subject), and one moderate event each occurred in subjects treated with placebo + Captisol® (constipation), 6 x 2 mg/kg 2-IB + Captisol® (upper respiratory tract infection), and 6 x 6 mg/kg 2-IB + Captisol® (infusion site pain). With the note that numbers of subjects for each treatment were low, a relation between 2-IB dose and incidence of TEAEs was not evident. In addition, the incidence of TEAEs was similar for treatment with placebo or placebo + Captisol®. The most frequently reported TEAEs, *i.e.* those that occurred in ≥ 10% of subjects, were: infusion site pain (15 events, 9 subjects, 41%), headache (15 events, 8 subjects, 36%), back pain (5 events, 4 subjects, 18%), nausea (6 events, 3 subjects, 14%), and dizziness (5 events, 3 subjects, 14%).

The 12 mg/kg dose regimen was well tolerated by 4 out of 6 subjects. However, 2 subjects discontinued study treatment due to TEAEs at that dose level, due to nausea of moderate-intensity and dizziness, abdominal pain and headache of mild intensity in one subject, and due to nausea and dizziness of moderate intensity, as well as pallor (mild intensity) and malaise (moderate intensity) in the second subject. With that observation, it was concluded that the highest tolerable 2-IB dose in this trial was 6 administrations 6 mg/kg per day, *i.e.* 36 mg/kg/day.

Iv administered Captisol® proved to be tolerated well; the most common reported TEAEs on dosing of 5% Captisol® without 2-IB were a headache (2 out of 3 subjects) and injection site pain (3 out of 3 subjects). One adverse event of moderate-intensity was reported on treatment with placebo + Captisol®, *viz.* a case of constipation. All adverse events reported on the administration of Captisol® recovered.

3.2. Pharmacokinetics of 2-IB

In the subjects in group 1, receiving a single 2-IB dose level of 0.6 mg/kg infused over 4 h, 1 h, or 15 min, respectively, 2-IB plasma profiles peaked consistently with the infusion periods (Fig. **1**), resulting in median peak times of 4.0 h, 1.0 h, and 0.26 h (Table **1**). Mean systemic clearance values of 2-IB appeared to be high, ranging from 0.39 L/h.kg to 0.48 L/h.kg, and approximating liver plasma flow. The volume of distribution somewhat exceeded total body water volume. Systemic clearance and thereby AUC appeared to be independent of infusion rate, with the note that at the fastest infusion rate of 15 min, clearance was numerically smaller by 20% compared to the other regimens (Table **1**). The interpretation of this minor difference is limited by the small number of observations in the group sizes of two subjects each.

Pharmacokinetic profiles observed after repeated iv infusions of 2-IB over 15 min or 30 min in healthy subjects proved to be consistent with those observed in the single-dose phase of the study. With 6 subsequent doses administered each 4 h, accumulation of the study was minimal, and trough levels between doses were low (Fig. **2**). The exposure

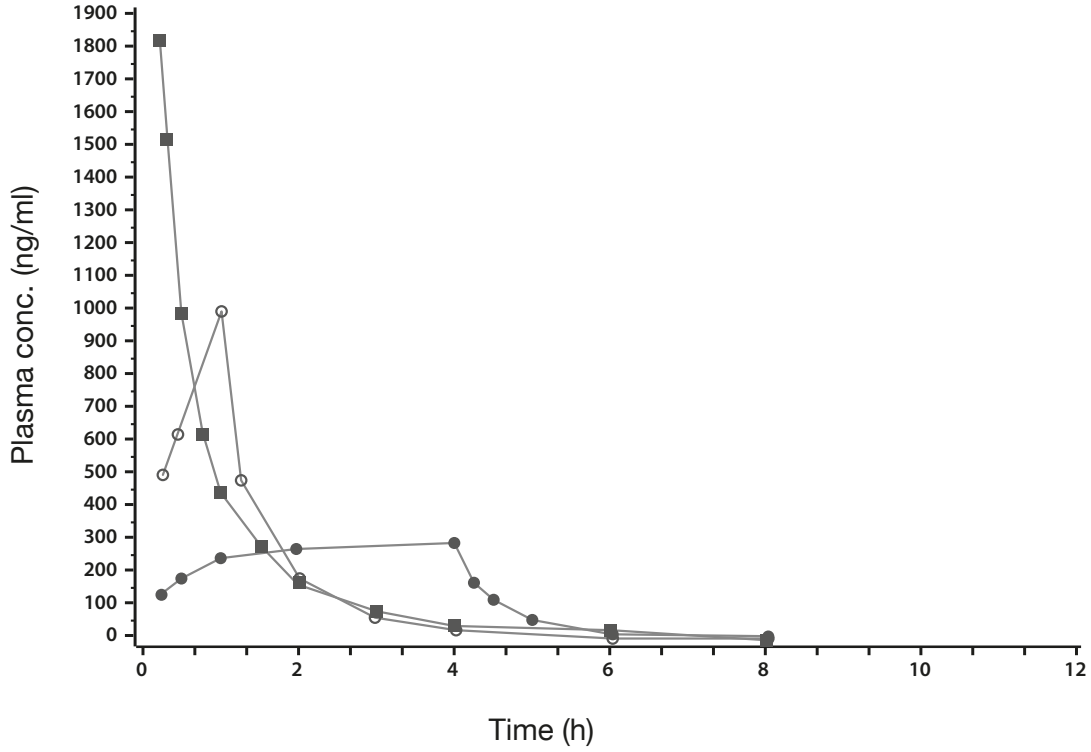

Fig. (1). 2-IB plasma concentration-time profiles after iv infusion of 0.6 mg/kg 2-IB over 4 h (solid circles), 1 h (open cicles) and 15 min (filled squares) in 3 groups of 2 healthy subjects each.

Table 1. Geometric mean (%CV) pharmacokinetic parameters of 2-IB in plasma, after iv infusion of 0.6 mg/kg 2-IB over 4 h, 1 h and 15 min in 3 groups of 2 healthy subjects each.

Parameter	Infusion Regimen		
	0.6 mg/kg in 4 h	**0.6 mg/kg in 1 h**	**0.6 mg/kg in 15 min**
t_{max} (h) (median [range])	4.00 (4.00-4.00)	1.00 (1.00-1.00)	0.26 (0.25-0.27)
C_{max} (ng/mL)	296 (4)	984 (15)	1808 (36)
$AUC_{(0-4)}$ (ng*h/mL)	1005 (0)	1175 (28)	1408 (15)
AUC_{inf} (ng*h/mL)	1258 (2)	1256 (31)	1528 (11)
$t_{1/2el}$ (h) (median [range])	1.16 (1.07-1.25)	1.20 (1.06-1.34)	1.48 (1.24-1.72)
CL (L/h/kg)	0.477 (2)	0.478 (31)	0.393 (11)
V_z (L/kg)	0.796 (13)	0.820 (15)	0.827 (34)
$A_{e(0-4h)}$ (% of dose)	71 (5)	79 (21)	90 (2)
$A_{e(0-12h)}$ (% of dose)	91 (3)	84 (19)	96 (1)

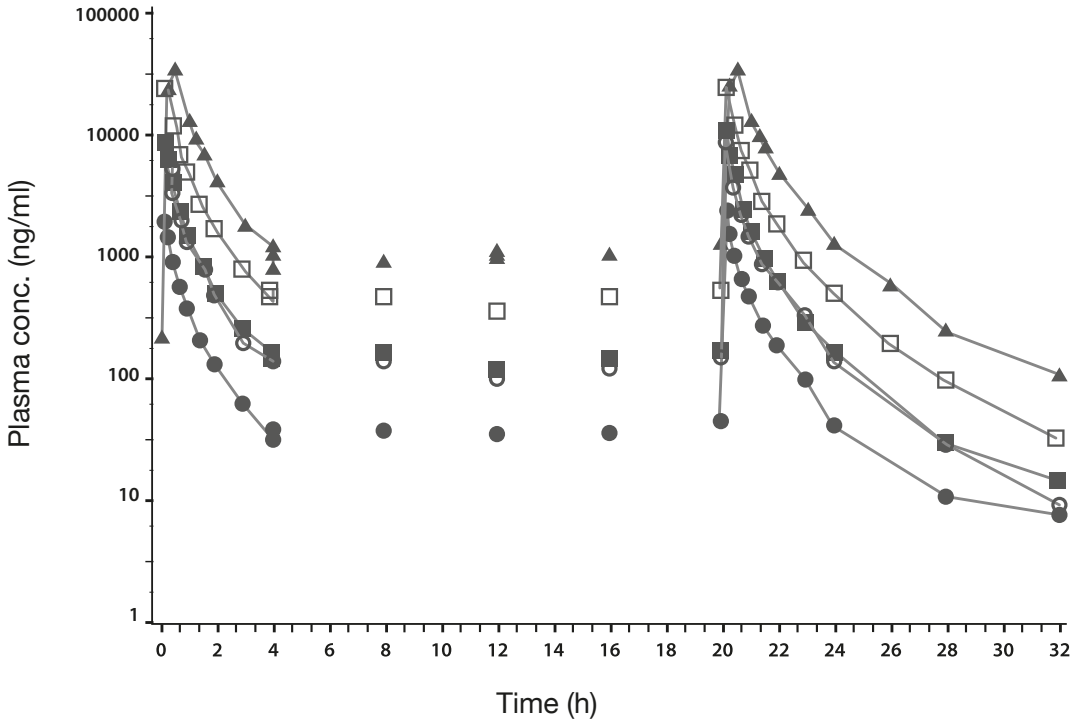

Fig. (2). 2-IB plasma concentration - time profiles after repeated iv infusions of 0.6 mg/kg (solid circles) and 2 mg/kg (open circles) of 2-IB without Captisol® in 15 min, of 2mg/kg (solid squares), and 6 mg/kg 2-IB (open squares) with Captisol® in 15 min, and of 12 mg/kg 2-IB with Captisol® (closed triangles) over 30 min, in groups of 6 healthy subjects each.

Table 2. Geometric mean (%CV) pharmacokinetic parameters of 2-IB in plasma, after repeated iv infusions of 0.6 mg/kg, 2 mg/kg, and 6 mg/kg 2-IB over 15 min, and 12 mg/kg 2-IB over 30 min, in groups of 6 healthy subjects each.

Para-meter	2-IB Dosing Regimen				
	6 x 0.6 mg/kg	6 x 2 mg/kg	6 x 2 mg/kg	6 x 6 mg/kg	6 x 12 mg/kg
	in 15 min				in 30 min
	without Captisol®		in 5% Captisol®		
First infusion					
t_{max} (h) (median [range])	0.26 (0.25-0.28)	0.25 (0.25-0.25)	0.25 (0.25-0.27)	0.27 (0.25-0.32)	0.52 (0.52-0.53)
C_{max} (ng/mL)	1794 (17)	6992 (27)	8360 (26)	21805 (28)	34304 (24)
$AUC_{(0-4)}$ (ng*h/mL)	1170 (18)	4337 (23)	5065 (20)	14358 (25)	33372 (12)
CL (L/h/kg)	0.513 (18)	0.461 (18)	0.395 (17)	0.418 (22)	0.360 (11)
Last infusion					
t_{max} (h) (median [range])	0.25 (0.25-0.27)	0.25 (0.25-0.25)	0.25 (0.25-0.27)	0.28 (0.27-0.30)	0.50 (0.50-0.52)
C_{max} (ng/mL)	2225 (13)	8212 (19)	10418 (18)	22541 (19)	34175 (18)
$AUC_{(0-4)}$ (ng*h/mL)	1441 (17)	5098 (19)	5965 (15)	15145 (21)	36009 (7)

(Table 2) contd….

Para-meter	2-IB Dosing Regimen				
	6 x 0.6 mg/kg	6 x 2 mg/kg	6 x 2 mg/kg	6 x 6 mg/kg	6 x 12 mg/kg
	in 15 min				in 30 min
	without Captisol®		in 5% Captisol®		
Last infusion					
AUC_{inf} (ng*h/mL)	1546 (20)	5479 (20)	6402 (16)	16448 (22)	39956 (8)
$t_{1/2el}$ (h) (median [range])	1.55 (0.80-2.62)	1.96 (1.28-2.16)	1.52 (1.20-3.32)	2.12 (1.74-3.67)	2.37 (1.93-3.93)
CL (L/h*kg)	0.416 (17)	0.392 (18)	0.335 (17)	0.396 (20)	0.333 (8)
V_{ss} (L/kg)	0.462 (7)	0.428 (10)	0.340 (23)	0.452 (15)	0.417 (23)
$A_{e(0-4h)}$ (% of dose)	100 (6)	76 (22)	63 (31)	27 (15)	26 (42)

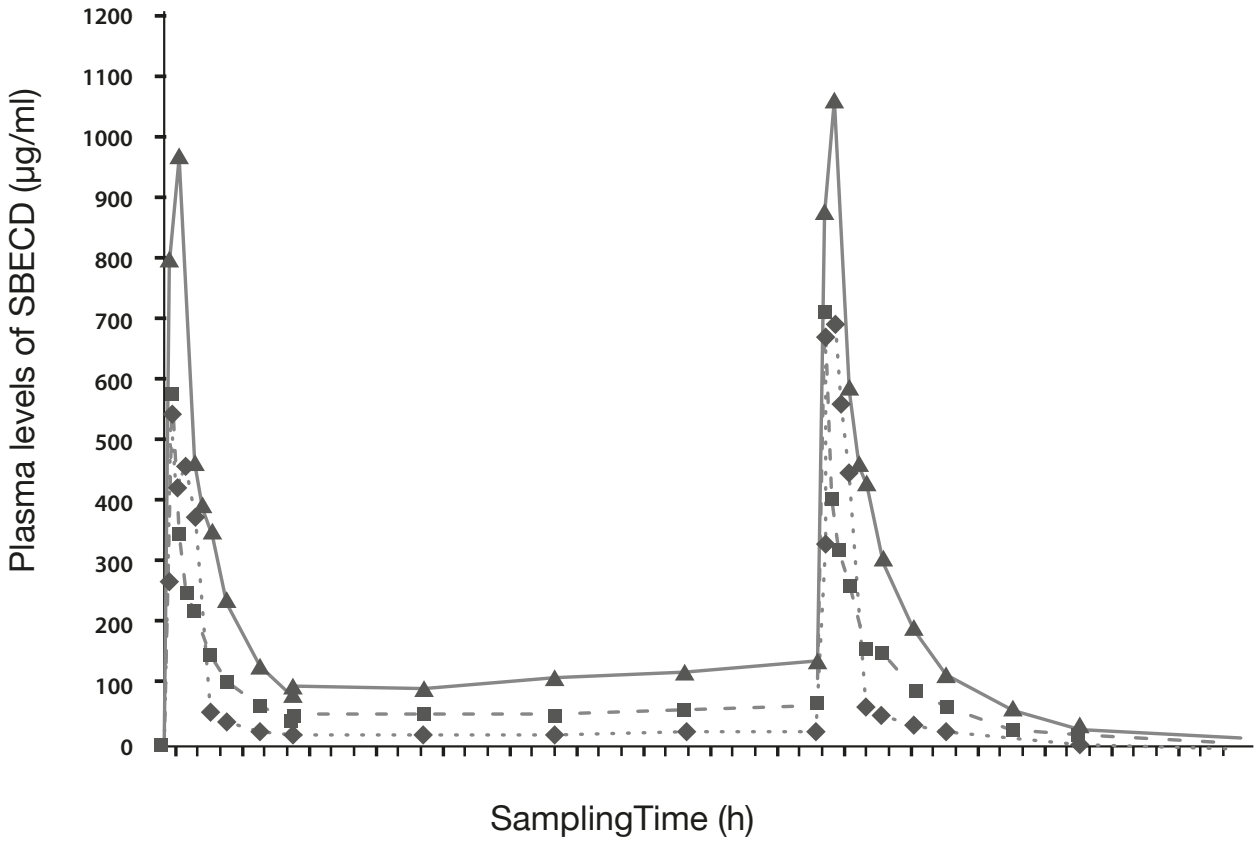

Fig. (3). Mean SBECD plasma concentration - time profiles following iv infusion of 5% Captisol® *placebo* solution at 25 mg/kg (period 1; dotted line), 75 mg/kg (period 2; dashed line) for 15 min, and 150 mg/kg (period 3; solid line) for 30 min each 4 h in groups of 3 healthy subjects for each period.

to 2-IB appeared to be generally dose-proportional throughout the range of doses and regimens (Fig. **2**; Table **2**).

Captisol® did not have an appreciable effect on the PK parameters of 2-IB, although an approximately 17% higher AUC was observed in the 2 mg/kg 2-IB with Captisol® group compared to the 2 mg/kg without Captisol® group (Table **2**). Between-subject variability of repeated-dose PK data proved to be limited, with coefficients of variation typically below 20%, and not exceeding 28% in any instance (Table **2**).

Renal clearance is an important route of elimination of 2-IB. Mean cumulative amounts of renally excreted, unchanged 2-IB varied between 84% and 96% of the administered dose over the first 12 h after single iv doses, for the three infusion regimens (Table **1**). After repeated iv infusion, the dose administered iv over the last dosing interval was recovered completely over the 4-h urinary fraction in case of the lowest dose tested, *i.e*. 0.6 mg/kg each 4 h (Table **2**). With increasing dose, the fraction of the dose recovered from the 4-h urine fraction consistently decreased, with a mean 26% fraction of the dose recovered over 4 h after dosing 12 mg/kg each 4 h (Table **2**). Since apparent elimination half-lives remained similar over the dose range, data suggest a relative increase of non-renal elimination pathways for 2-IB with an increase of dose, concurrently with a relative decline of the renal elimination route.

3.3. Pharmacokinetics of SBECD

After iv infusion of 5% Captisol® placebo solution, the SBECD plasma concentration-time profiles were consistent with the iv infusion regimen, peaking at or shortly after the end of infusion, and showing a rapid decline (Fig. **3**). Residual amounts at the end of the 4-h dosing interval contributed to a modest extent of accumulation after repeated dosing.

On iv infusion of Captisol®, SBECD pharmacokinetics were consistent with a low-clearance compound with a small volume of distribution over the dose range tested (Table **3**).

Table 3. Arithmetic mean (%CV) pharmacokinetic parameters of SBECD in plasma, after repeated iv infusions of 25 mg/kg and 75 mg/kg in 5% Captisol® placebo solution in 15 min, and 150 mg/kg in 5% Captisol® *placebo* solution over 30 min, in groups of 3 healthy subjects each.

Parameter	5% Captisol® dosing regimen		
	6 x 25 mg/kg	6 x 75 mg/kg	6 x 150 mg/kg
	in 15 min		in 30 min
First infusion			
t_{max} (h) (median [range])	0.50 (0.33-0.50)	0.25 (0.25-0.25)	0.50 (0.25-0.50)
C_{max} (µg/mL)	693 (30)	590 (23)	996 (16)
$AUC_{(0-4)}$ (µg*h/mL)	548 (3)	625 (9)	1355 (15)
AUC_{inf} (µg*h/mL)	578 (2)	705 (5)	1500 (19)
$t_{1/2el}$ (h) (median [range])	1.18 (1.11-1.73)	1.38 (1.12-1.70)	1.11 (1.03-1.42)
CL (L/h/kg)	0.0433 (2)	0.107 (5)	0.102 (17)
V_Z (L/kg)	0.0837 (26)	0.217 (24)	0.171 (3)
Last infusion			
t_{max} (h) (median [range])	0.33 (0.33-0.50)	0.25 (0.25-0.25)	0.50 (0.50-0.50)
C_{max} (ng/mL)	863 (12)	725 (12)	1082 (22)
$AUC_{(0-4)}$ (µg*h/mL	726 (8)	799 (9)	1658 (30)
$t_{1/2el}$ (h) (median [range])	1.83 (1.27-2.22)	1.47 (1.31-1.57)	1.34 (1.16-1.36)
CL (L/h/kg)	0.0346 (8)	0.0945 (10)	0.0953 (25)
V_Z (L/kg)	0.0885 (29)	0.198 (17)	0.175 (23)

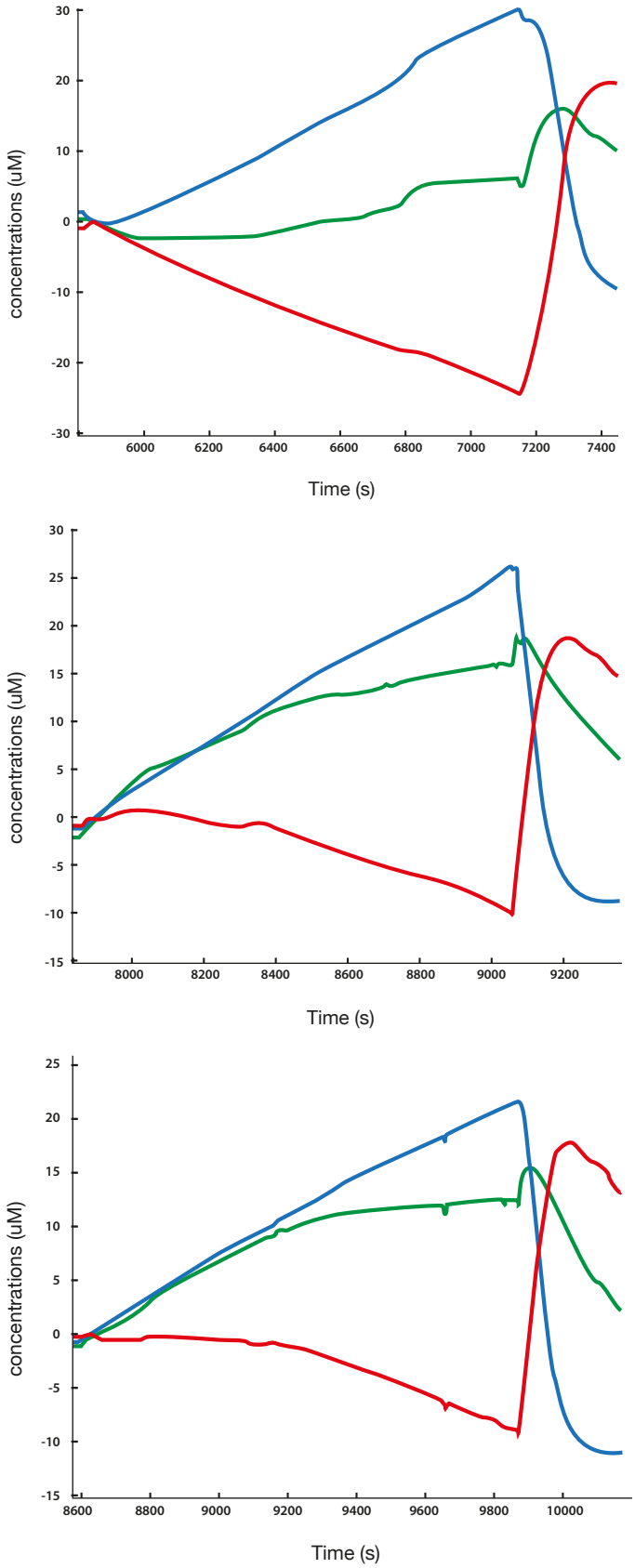

Fig. (4). Oxygenated Hb (O2Hb; red), deoxygenated Hb (HHb; blue), and total hemoglobin (tHb; green) traces by near infrared spectroscopy in an individual subject, collected prior to the 1st dose (top panel), during infusion of the 3rd dose (middle panel) and 2.5 hours after the 3rd dose (bottom panel) of 2-IB 12 mg/kg. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 4. Arithmetic mean (%CV) oxygenation parameters by near infrared spectroscopy prior to the first iv infusion, and during (15 min) and after (2.5 h) after the 3d infusion of 2 mg/kg and 6 mg/kg 2-IB in 5% Captisol® in 15 min, and 12 mg/kg 2-IB in 30 min, in 9 healthy subjects; [n] presents the number of reported observations passing quality criteria; *p<0.05 versus placebo.

Parameter	Time Point	2-IB Dosing Regimen					
		2 mg/kg in 15 min		6 mg/kg in 15 min		12 mg/kg in 30 min	
		Active	Placebo	Active	Placebo	Active	Placebo
Oxygen consumption (μM/s)	Before 1st dose	0.158 (26) [5]	0.155 (76) [3]	0.160 (41) [4]	0.126 (19) [2]	0.138 (21) [4]	0.153 (31) [3]
	During 3d dose	0.149 (34) [5]	0.124 (65) [3]	0.167 (6) [4]	0.144 (9) [3]	0.105* (11) [5]	0.150 (27) [3]
	After 3d dose	0.127 (29) [5]	0.136 (77%) [3]	0.125 (43) [5]	0.148 (22%) [3]	0.102 (46) [6]	0.158 (17%) [3]
Reoxygenation velocity (μM/s)	Before 1st dose	2.47 (11) [4]	4.02 (52) [2]	3.11 (37) [4]	2.13 (33) [2]	3.00 (65) [4]	2.41 (2) [2]
	During 3d dose	3.02 (24) [5]	2.66 (30) [3]	3.02 (9) [4]	2.67 (54) [3]	2.52 (30) [5]	2.72 (31) [3]
	After 3d dose	2.47 (46) [5]	2.60 (57%) [3]	2.30 (52) [5]	2.65 (47%) [3]	2.98 (77) [6]	2.37 (60%) [3]
Half-recovery time (s)	Before 1st dose	6.43* (7) [4]	5.30 (5) [2]	6.20 (27) [4]	6.75 (7) [2]	7.03 (37) [4]	8.05 (24) [2]
	During 3d dose	5.92 (15) [5]	4.87 (28) [3]	5.53 (20) [4]	6.77 (13) [3]	5.02 (32) [5]	4.07 (19) [3]
	After 3d dose	7.50 (64) [5]	5.37 (35%) [3]	5.78 (29%) [5]	5.70 (13%) [3]	4.38 (14) [6]	5.93 (32%) [3]
Tissue saturation index (%)	Before 1st dose	63.0 (12) [5]	65.8 (4) [3]	65.7 (9) [6]	62.6 (5) [3]	64.4 (8) [6]	64.3 (5) [3]
	During 3d dose	64.0 (6) [6]	66.1 (5) [3]	68.2 (5) [6]	65.2 (5) [3]	67.3 (4) [6]	66.5 (7) [3]
	After 3d dose	61.9 (11) [6]	64.1 (4%) [3]	66.5 (6) [6]	66.5 (3%) [3]	65.2 (4) [6]	62.1 (12%) [3]

The elimination half-life of SBECD was short, *i.e.* ranging from 1.03 to 1.73 h at the first dose, and independent of the dose. Clearance, distribution volume and elimination half-life were time-independent, with similar values observed after the last dose compared to the first dose (Table **3**). Between-subject variability of PK data proved to be small to moderate, with coefficients of variation not exceeding 30% in any instance (Table **3**). At the higher doses of 75 mg/kg and 150 mg/kg, clearance and distribution volume were dose-independent. At the low dose of 25 mg/kg though, exposures were relatively high, reflected by relatively low calculated values for SBECD clearance and distribution volume (Table **3**). The t_{max} values at that dose, viz. ranging from 0.33 to 0.50 h and exceeding the infusion period of 15 min, are suggestive for a dosing artefact that remained unexplained. In terms of 2-IB profiles, this apparent protracted exposure profile proved absent in the subjects receiving the same dose of 25 mg/kg Captisol® in presence of 2-IB (Table **2**).

3.4. Pharmacodynamics

The PD data set available for analysis comprised the results of subjects in group 2 only, since measurements of group 1 in period 3 could not be analysed due to an unplanned deviation from experimental procedures by error. Fig. **4** displays the typical O_2Hb, HHb and tHb pre- and post-dose values in a 2-IB (12 mg/kg) treated subject.

Overall, and irrespective of treatment and assessment time point, the mean oxygen consumption for all subjects was 0.14 ± 0.05 μM/s. The mean reoxygenation velocity was 2.8 ± 1.1 μM/s. The mean half-recovery time was 5.7 ± 1.5 s, and the mean tissue saturation was 65.1 ± 4.5 %. For the data sets per treatment and per assessment time point, between-subject variation proved to be substantial in several cases, and no consistent differences were observed between assessment periods, and when comparing active treatment versus placebo (Table **4**). The observed statistically significant differences between active treatment and placebo for oxygen consumption post-dose at the top dose of 12 mg/kg, and for half recovery time pre-dose at the lowest dose tested (Table **4**) are to be interpreted with caution in view of the exploratory nature of the study and absence of statistical correction for multiplicity.

Summarizing, under the conditions of the present study, no statistically significant differences in oxygenation values during acute hypoxic ischemia could be shown in healthy subjects experiencing a brief period of peripheral ischemia followed by reperfusion. Using this peripheral methodology, no effects of 2-IB could be demonstrated at repeated iv doses of 2, 6 and 12 mg/kg of 2-IB. With the experimental error in group 1, limiting results analysis to group 2 only, this PD characterisation of 2-IB is to be considered preliminary only.

CONCLUSION

Single and multiple iv doses of 2-IB with and without Captisol® up to 6 doses of 6 mg/kg proved to be safe and well-tolerated in healthy male subjects, covering the target AUC of 6000 ng.h/mL expected to be efficacious in birth asphyxia [16]. Tolerability of the top dose of 12 mg/kg appeared to be limited, with 2 out of 6 subjects withdrawn due to non-serious adverse events, *i.e*. mainly nausea and dizziness. 2-IB proved to be a high-clearance drug with a volume of distribution somewhat exceeding total body water volume; the compound was close to completely excreted renally after single doses, but renal elimination within 4 h after dosing decreased with increasing dose after repeated dosing. 2-IB PK was linear over the dose range tested, with low inter-individual variability, and little accumulation on repeated dosing. Captisol® did not have an appreciable effect on the PK parameters of 2-IB. On iv dosing of Captisol®, SBECD pharmacokinetics implied a low-clearance profile with a small volume of distribution and moderate interindividual variability. Its clearance, distribution volume and elimination half-life proved time-independent when comparing values after the last dose with the first dose.

The pharmacodynamic characterization of repeated iv dosing of 2-IB in Captisol® in an acute peripheral hypoxic ischemia model in healthy subjects did not reveal any notable effects of 2-IB under the conditions of the model. It is noted that this model was not selected to provide guidance for efficacy in the currently pursued indication of cerebral hypoxia-ischemia.

LIST OF ABBREVIATIONS

2-IB	=	2-iminobiotin
$A_{e(0-t)}$	=	Amount excreted into urine over collection interval 0-t hours
AUC_{0-t}	=	Area under the plasma concentration versus time curve up to sampling point t hours
AUC_{inf}	=	Area Under the plasma Concentration versus time curve extrapolated to infinity
Cl	=	Systemic clearance
C_{max}	=	Peak plasma concentration
CPMP	=	Committee for Proprietary Medicinal Products
ECG	=	Electrocardiogram
EU CTD	=	European Union Clinical Trial Directive
GCP	=	Good Clinical Practice
HHb	=	Deoxygenated Hemoglobin
HI	=	Hypoxia-Ischemia
HIE	=	Hypoxic-Ischemic Encephalopathy
HPLC	=	High-Performance Liquid Chromatography
ICF	=	Informed Consent Form
ICH	=	International Conference on Harmonisation
LC	=	Liquid Chromatography
MS	=	Mass Spectrometry
MS/MS	=	Tandem (dual) Mass Spectrometry
mVO_2	=	Oxygen consumption in the muscle
NOS	=	Nitric Oxide Synthase
O_2Hb	=	Oxygenated Hemoglobin
PD	=	Pharmacodynamics
PK	=	Pharmacokinetics
R^2	=	Coefficient of determination
SBECD	=	Sulfobutylether-beta-cyclodextrin
$t_{1/2el}$	=	Elimination half-life
TEAE	=	Treatment-Emergent Adverse Event
tHb	=	Total hemoglobin
t_{max}	=	Time to peak plasma concentration
TSI	=	Tissue Saturation Index
UPLC	=	Ultra-High-Performance Liquid Chromatography
V_{ss}	=	Apparent volume of distribution in steady state

V_z = Apparent volume of distribution during terminal elimination phase

Δreox = Reoxygenation velocity

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The clinical study protocol was approved by an Independent Ethics Committee (Stichting Beoordeling Ethiek Bio-Medisch Onderzoek, Assen, The Netherlands). It was conducted in compliance with the International Conference on Harmonisation (ICH) E6 Guideline for Good Clinical Practice (GCP) (Committee for Proprietary Medicinal Products (CPMP) guideline CPMP/ICH/135/95), and compliant with the European Union Clinical Trial Directive (EU CTD): Directive 2001/20/EC.

HUMAN AND ANIMAL RIGHTS

All research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/).

CONSENT FOR PUBLICATION

All participants were informed verbally and in writing of the objectives, procedures and risks of study participation including possible side effects and potential interactions.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

EH, JJL and JH are employees of PRA Health Sciences. JJL and JH performed this study as part of contract research sponsored by Neurophyxia B.V. CPS, PL and LL are consultants for and stakeholders in Neurophyxia B.V.

ACKNOWLEDGEMENTS

EH interpreted the study results and drafted the 1st version of the manuscript. JJL co-designed and performed the clinical study, and revised the manuscript. JH co-designed the study, analyzed the study data and revised the manuscript. CPS and PL contributed to the design of the study and revised the manuscript. LL interpreted the study results and revised the manuscript.

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