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ORIGINAL RESEARCH ARTICLE

SAFE-ROCK: A Phase I Trial of an Oral Application of the ROCK Inhibitor Fasudil to Assess Bioavailability, Safety, and Tolerability in Healthy Participants

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Abstract

Background The intravenous (IV) formulation of Rho-kinase (ROCK) inhibitor fasudil has been approved for the treatment of subarachnoid haemorrhage since 1995. Additionally, fasudil has shown promising preclinical results for various chronic diseases, including neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's disease, and dementia, in which long-term intravenous (IV) administration might not be suitable.

Objective The objective of this study was to assess the absolute bioavailability of oral, in comparison to IV, application of the approved formulation of fasudil (ERIL®) and to evaluate the safety and tolerability of the oral application of fasudil.

Methods This was a phase I, single-center, open-label, randomized, two period cross-over clinical trial in healthy women and men. By applying a cross-over design, each subject served as their own control. Two treatments were investigated, separated by a wash out phase of at least 3 days. Oral fasudil was administered once on day 1 to assess pharmacokinetics and three times on day 2, at an interval of 8 ± 1 h, to assess safety and gastrointestinal tolerability. For pharmacometrics of IV fasudil, it was administered once on day 1. Plasma profiles of fasudil and its active metabolite hydroxyfasudil after oral or IV administration were measured by liquid chromatography electrospray tandem mass spectrometry. Tolerability was assessed as proportion of subjects without significant drug intolerance, and safety was assessed by the proportion of subjects without clinical or laboratory treatment-associated serious adverse events. Gastrointestinal safety was assessed by applying the gastrointestinal symptom rating scale (GSRS).

Results Fourteen subjects aged 30–70 years were included in this trial. After oral administration, fasudil concentrations in blood were mostly very low [1.4 g/L; coefficient of variation (CV) 41.0%]. After IV application, the peak concentration was 100.6 µg/L (CV 74.2%); however, a high variance in peak concentrations were assessed for both treatments. The maximal concentrations of hydroxyfasudil in blood were similar after oral and IV treatment [111.6 µg/L (CV 24.1%) and 108.4 µg/L (CV 19.7%), respectively]. Exposure of hydroxyfasudil (assessed as AUC_{0-tz}) differed between both treatments, with 449 µg × h/L after IV treatment and 309 µg × h/L after oral treatment. Therefore, the absolute bioavailability of hydroxyfasudil after the oral treatment was approximately 69% of the IV treatment. No serious adverse events (SAEs) occurred during this trial, and good tolerability of oral fasudil (90 mg/day) was documented.

Conclusions Oral fasudil was generally well tolerated in the studied population, and no safety concerns were identified. However, systemic bioavailability of oral hydroxyfasudil corresponded to 69%, and dose adjustments need to considered. The results presented here lay grounds for future trials of fasudil in chronic diseases, which require an oral long-term application. This trial was registered with EudraCT (no. 2019-001805-26).

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Key Points

In this first publicly available bioavailability study comparing oral and intravenous (IV) administered Rho-kinase inhibitor fasudil in its licenced formulation, we demonstrate a bioavailability of 69% for the active metabolite hydroxyfasudil after oral compared with IV treatment and good gastrointestinal tolerability.

Based on these findings, clinical trials on oral fasudil in its licenced formulation for neurodegenerative diseases, such as amyotrophic lateral sclerosis, dementia, and Parkinson's disease, as well as vascular diseases and metabolic diseases can be planned, yet adaptions of the dosage need to be considered.

1 Introduction

Small molecule inhibitors targeting the Rho-associated coiled-coil kinase (ROCK) pathway have gained significant attention in recent years due to their potential therapeutic implications in a wide range of diseases. ROCK presents itself with a plethora of different downstream effectors regulating cell shape, cell motility, cellular survival and cell cycle pathways [1]. Activation of ROCK leads to phosphorylation of myosin light chain and consecutive actomyosin contraction [2]. Additionally, by activating the LIM domain kinases, ROCK activation leads to increased number of stable actin filaments and reduced actin turnover, therefore hindering cell regeneration and growth [3]. Other targets phosphorylated by ROCK include ezrin, radixin, moesin, adducin, profilin, PTEN, MAP2, CRMP2, GFAP, vimentin, and many more [3].

In parallel with the vast physiological effects of ROCK, ROCK inhibition offers a wealth of pharmaceutical applications for various acute and chronic diseases. ROCK inhibition promotes vasodilatation, reduces vascular smooth muscle contraction, and preserves endothelial integrity, leading to alleviated cerebral vasospasm following subarachnoid hemorrhage [4], improved clinical outcome in ischemic stroke [5], ameliorated pulmonary hypertension [6], and increased ischemic threshold of angina patients during exercise [7]. In addition to implications in cardiovascular diseases, in some cancer entities, such as breast and bladder cancer, elevated expression of ROCK was observed, associated with late stages and metastasis, while ROCK inhibition reduced migration and tumor growth in animal models [8]. Furthermore, preclinical work also indicates beneficial effects in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) [3, 9–11]. In the central nervous system, ROCK was shown to regulate axonal regeneration and neuronal survival [12]. ROCK inhibition resulted in beneficial effects on inflammation, survival, motor functions, and histological parameters in animal models of ALS and PD [13–24].

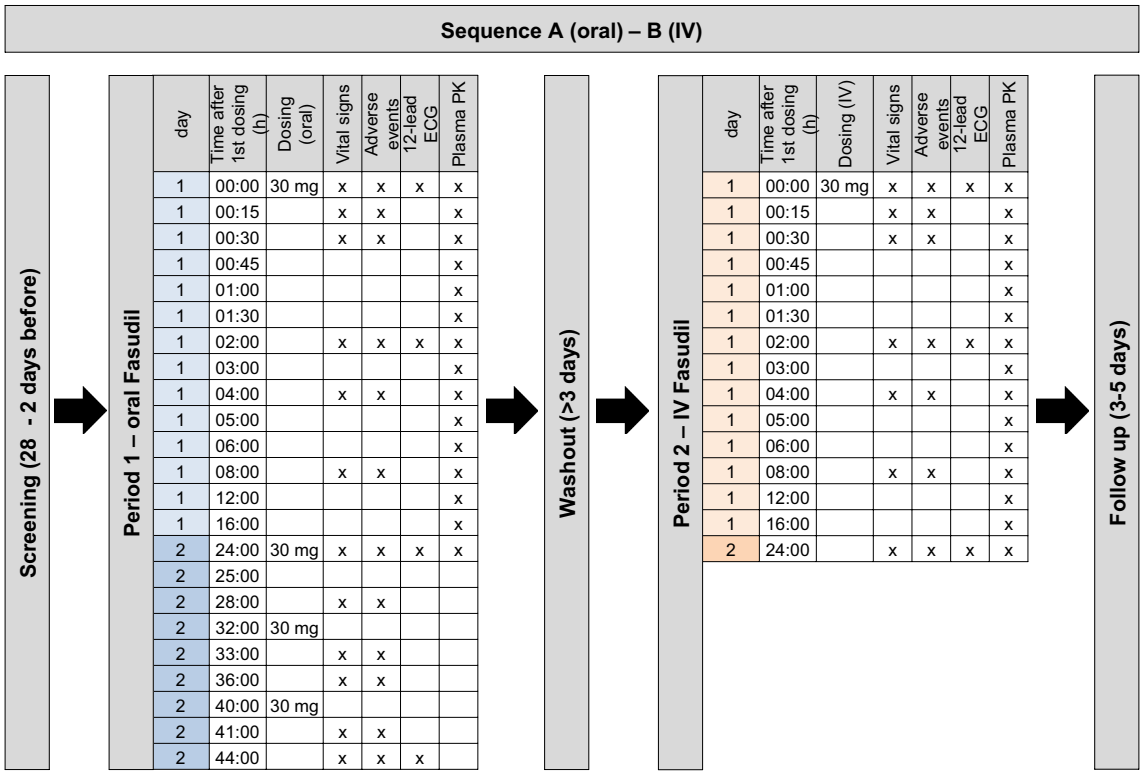
The small molecule fasudil is the only ROCK inhibitor approved for systemic application and has been used in Japan and China since 1995 for the treatment of vasospasms following subarachnoid haemorrhage. It is marketed under the name ERIL® and approved for intravenous (IV) treatment up to three times daily for two weeks. After (IV) administration, fasudil is rapidly converted in the liver to its active metabolite hydroxyfasudil [25], corresponding to a serum half-life of 20 min for fasudil and 100–200 min for hydroxyfasudil [26, 27], followed by renal excretion. Both, fasudil and hydroxyfasudil, are potent inhibitors of ROCK, with hydroxyfasudil being slightly more potent. Significant amounts of hydroxyfasudil are taken up in the brain, making it an interesting candidate for the treatment of diseases of the central nervous system [28]. In an exploratory phase I trial in healthy subjects, oral bioavailability of an investigational formulation of fasudil was studied. No absolute bioavailability was estimated in this trial, since only oral treatment without reference IV treatment was applied [29]. In general, both licensed IV formulation and nonlicensed oral formulation were well tolerated [4, 26, 29]. However, for the long-term treatment of chronic diseases, an oral application is preferred.

Here we present results from the first phase I trial investigating bioavailability, safety, and tolerability of the oral application of the approved and commercially available formulation of fasudil in a cross-over design. The primary objective of this study is to evaluate the absolute bioavailability of an oral administration of a 5% glucose solution containing 30 mg fasudil compared with an IV administration of a 5% glucose solution containing 30 mg fasudil. Additionally, safety and tolerability of an oral administration against an IV administration were evaluated.

2 Methods

2.1 Investigational Drugs

The investigational medicinal product (IMP) was 30 mg fasudil hydrochloride (ERIL®) and was imported from the manufacturer, Asahi Kasei Pharma Corporation, Tokyo, Japan, by the sponsor and provided to the study site. The ATC-code is C04AX32. For IV application, the IMP was diluted in 100 ml 5% glucose solution. It was applied via



day	Time after 1st dosing (h)	Dosing (oral)	Vital signs	Adverse events	12-lead ECG	Plasma PK
1	00:00	30 mg	x	x	x	x
1	00:15		x	x		x
1	00:30		x	x		x
1	00:45					x
1	01:00					x
1	01:30					x
1	02:00		x	x	x	x
1	03:00					x
1	04:00		x	x		x
1	05:00					x
1	06:00					x
1	08:00		x	x		x
1	12:00					x
1	16:00					x
2	24:00	30 mg	x	x	x	x
2	25:00					
2	28:00		x	x		
2	32:00	30 mg				
2	33:00		x	x		
2	36:00		x	x		
2	40:00	30 mg				
2	41:00		x	x		
2	44:00		x	x	x	

day	Time after 1st dosing (h)	Dosing (IV)	Vital signs	Adverse events	12-lead ECG	Plasma PK
1	00:00	30 mg	x	x	x	x
1	00:15		x	x		x
1	00:30		x	x		x
1	00:45					x
1	01:00					x
1	01:30					x
1	02:00		x	x	x	x
1	03:00					x
1	04:00		x	x		x
1	05:00					x
1	06:00					x
1	08:00		x	x		x
1	12:00					x
1	16:00					x
2	24:00		x	x	x	x

Fig. 1 Trial schedule (sequence A–B). Exemplary time and events of the SAFE-ROCK trial for a participant randomly allocated to sequence treatment A (oral) followed by treatment B (IV). *ECG* electrocardiogram, *PK* pharmacokinetic measurements

intravenous catheter at a constant rate of infusion using a CE-certified volumetric infusion pump over a period of 30 min in a reclined position (30°–45°). To allow a complete delivery IMP, the infusion system was flushed with 50 mL 5% glucose solution. For oral application, the IMP was diluted in 20 mL 5% glucose solution and applied with 250 mL of tap water.

2.2 Study Design and Participants

This was a phase I, single-center, open-label, randomized, two period cross-over clinical trial in 14 healthy women and men aged 30–70 years (EudraCT-no. 2019-001805-26). Two treatments were investigated, separated by a wash out phase of at least 3 days. For treatment A (test), oral fasudil was dosed once on day 1 to establish the pharmacokinetic (PK) profile followed by three times at an interval of 8 ± 1 hours on day 2 of the respective period to assess safety and gastrointestinal tolerability. For treatment B (reference), IV fasudil was dosed on day 1 of the respective period (Fig. 1). Subjects were randomly assigned to one of the treatment sequences (A–B or B–A) according to a randomization list generated using the Statistical Analysis System (SAS, version 9.4, Cary, NC). A cross-over design is the preferred design for a clinical trial evaluating the absolute bioavailability, because it removes the inter-subject variability from the comparison between formulations/treatments. Each subject serves as their own control, meaning that the comparison between the formulations/treatments is based on intra-subject comparison. This explorative clinical trial was expected to generate PK data to establish the bioavailability of an oral administration for the further development program of an oral fasudil formulation. Relevant deviations in kidney and liver function were excluded so as not to interfere with the metabolism of IMP. A detailed description of inclusion and exclusion criteria can be found as supplementary material (Table S1). Fourteen healthy women and men were enrolled in this clinical trial. The subjects were recruited from the clinical trial center subject pool and by public advertisement. The approval of both, the ethics committee (no. PVN7203) and the German Federal Institute for Drugs and Medical Devices (BfArM) were obtained prior to the start of the clinical trial, and the study was performed in accordance with good clinical practice and the Declaration of Helsinki. The clinical trial was designed in line with the "Guideline on the Investigation of Bioequivalence" (CPMP/EWP/QWP/1401/98 Rev. 1 of 20 January 2010). The study was conducted from 25 February 2020 through

4 January 2021, at the CTC North study ward at the University Medical Center Hamburg-Eppendorf, Hamburg, Germany. All participants signed an informed consent form as evidence of consent. For reporting of this trial, the CONSORT guidelines were applied (Supplementary Table S2) [30].

2.3 Procedures

Screening procedures had to be completed between 28 days and 2 days prior to receiving the first dose of clinical trial medication. The following events were performed: acknowledging extended hygiene measures, filling out questionnaire for self-assessment of current state of health, measuring body temperature, SARS-CoV-2 polymerase chain reaction (PCR) test, inclusion/exclusion criteria check, obtaining medical history and demographics, physical examination, vital signs, height, and weight (BMI), ECG, clinical chemistry, hematology, serology, urine pregnancy test, urine drug screen, and an alcohol breath test. A complete list of screening procedures performed can be found in the supplementary material (Table S3). All laboratory assays were performed according to the laboratory's normal procedures. Reference ranges were supplied by the laboratory and used to assess the laboratory data for clinical significance and out-of-range pathological changes. Out-of-range laboratory values were assessed for clinical significance by a physician. Abnormal laboratory values that were unexpected or not explained by the subject's clinical condition may have been, at the discretion of the physician, principal investigator, or sponsor, repeated until confirmed, explained, or resolved as soon as possible. The following laboratory assessments were performed: blood samples (7.5 mL) for serum biochemistry were collected into a serum separator gel tube at the time points described in Table S3. The following parameters were assessed: aspartate transaminase (AST); creatinine clearance (GFR, using CKDEPI); alanine transaminase (ALT); alkaline phosphatase (AP); gamma glutamyl transferase (GGT); total bilirubin; creatinine; and lactate dehydrogenase (LDH). Blood samples (2.6 mL) for serum hematology were collected into tube a tube containing potassium ethylene diamine tetra-acetic-acid (EDTA) anticoagulant at the time points described. The following parameters were assessed: hemoglobin; hematocrit; red blood cells (RBC); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); total and differential white blood cell (WBC) count (lymphocytes, neutrophils, eosinophils, basophils, monocytes); and platelet count. In all females of childbearing potential regular pregnancy test (urine or serum) was performed as indicated in Table S3. During the screening period only, serum analyses for the presence of human immunodeficiency virus (HIV) antibody (HIV I and HIV II), HCV antibody, and HBsAg were performed. Drug and alcohol tests were performed as indicated or at the physician's discretion. Blood samples of 9.8 mL (2 × 4.9 mL EDTA) for PK analysis were collected at the following time points (in hours after dosing): day 1: predose (within 30 min before dose administration), 0:15, 0:30, 00:45, 1:00, 1:30, 2:00, 3:00, 4:00, 5:00, 6:00, 8:00, 12:00, 16:00 h; day 2: 24:00 h. Within 90 min after sampling, samples were centrifuged at 1.100*g* (10 min, 4 °C) and stored in polypropylene tubes at −80 °C. During oral application, participants were fed 2, 6, and 12 h after dosing on day 1 and 2, 5.5, 10, and 12 h after dosing on day 2.

2.4 Outcomes

As primary endpoints of absolute bioavailability we investigated the Area under the concentration time curve from zero up to the last measured concentration (AUC_{0-tz}), Area under the concentration time curve from zero extrapolated to infinity ($AUC_{0-\infty}$) and highest measured concentration determined in the measuring interval (C_{max}) of fasudil and its active metabolite hydroxyfasudil. Blood levels and further PK characteristics of fasudil and hydroxyfasudil after the oral or the IV administration were analyzed as secondary objectives. Tolerability was assessed as proportion of subjects without significant drug intolerance during the treatment (oral or IV) period, and safety was assessed by the proportion of subjects without treatment-associated serious adverse event (SAE). Subjects were questioned in a general way to ascertain if AEs have occurred (e.g., "Have you had any health problems since the last time you came to the clinic/since you were last questioned?"). This open, standardized questioning was done discretely to prevent subjects from influencing each other. Spontaneous reports of AEs were also recorded as well as AEs that were observed by the investigator, the physician, or a staff member. All AEs were reviewed, confirmed, and classified by a qualified, designated physician. In additionally, the gastrointestinal symptom rating scale (GSRS) was applied, a validated disease-specific questionnaire used to evaluate common symptoms of gastrointestinal disorders [31]. It contains 15 items related to signs and symptoms experienced by the subject, each rated on a seven-point Likert scale from no discomfort (score = 1) to very severe discomfort (score = 7). In this way, the total score is comprised between 15 and 105.

2.5 Quantification of Fasudil and Hydroxyfasudil

For pharmacokinetic analyses, plasma samples were collected as described. Simultaneous quantification of fasudil and hydroxyfasudil was carried out in plasma matrix by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS), following a previously published protocol. [32]

For this, a QTRAP® 5500 LC-MS/MS System (Sciex, Framingham, MA) with a HPLC pump 1260 (Agilent Technologies, Santa Clara, CA) and a CTC Autosampler PAL HTCxt (Axel Semrau GmbH, Sprockhövel, Germany) was used. This method is based on protein precipitation as sample preparation followed by reverse phase chromatography coupled to tandem mass spectrometry (triple quadrupole). Chromatographic separation of matrix components and analytes was carried out with a C18-reversed-phase column using a solvent gradient. Electrospray ionization in positive mode (ESI / +) generates and detects selected ion fragments in the mass spectrometer. The quantification was carried out via a matrix replenishment with a standard solution. The calibration standards and quality control samples, containing low, medium, and high concentrations of fasudil or hydroxyfasudil, were prepared by spiking blank human K2-EDTA plasma using two different stock solutions. For each analytic run, remeasured quality control samples needed to be within ± 20% for the low quality control and ± 15% for medium and high quality control. Method validation comprised of the determination of the linear range, the limit of detection (LOD), and the precision. The linear range has been validated from 1–500 µg/L for both fasudil and hydroxyfasudil with a lower limit of detection (LLOD) of 1 µg/L. The intra-assay coefficient of variation at 50 µg/L ($N = 10$) was 3.6% for fasudil and 3.7% for hydroxyfasudil. The recovery rates from plasma were 118% for fasudil and 124% for hydroxyfasudil. Sample stability (human plasma) under different storage conditions [repeated freeze/thaw cycles, short- (24 h) and long-term (30 day) storage] for fasudil and hydroxyfasudil has been reportedly very good (> 94%). [32] The measurement was done in a single batch.

2.6 Statistical Analyses

All statistical calculations were carried out using SAS language and procedures (SAS 9.4 version SAS-Institute, Cary NC) and R Version 4.1.0 (R Core Team, Vienna, Austria). WinNonlin version 8.1.0.3530 (Phoenix 64) was used for the derivation of the PK parameters. The individual subject values for concentrations and as well as secondary and subsidiary PK parameters are tabulated with descriptive statistics. Two-sample *t*-tests or paired Wilcoxon–Mann–Whitney tests were performed for comparison of metric variables between groups, whereas Fisher's exact tests were performed for the analysis of categorical data. Absolute bioavailability of fasudil and hydroxyfasudil was estimated by the geometric mean ratios resulting from estimates of analysis of variance (ANOVA) model for each primary endpoint on the log-scale. Ratios of the geometric means (test/reference) as well as their two-sided 90% confidence intervals (CIs) were presented. For each endpoint, the difference between the means [log(test)-log(reference)] were derived from the ANOVA as least squares means (LS-MEANS), whereas the residual error and the quantiles from the *t*-distribution were used to calculate the two-sided 90% CIs. These values were back transformed to the original scale. Additionally, the two-sided 90% CIs were calculated based on the residual error from the ANOVA. This corresponds to the two one-sided *t*-test procedures, each at a significance level of 5%. Since the clinical trial is of exploratory nature, no formal hypothesis test and associated acceptance range are specified. In a post hoc analysis, terminal half-life ($t_{½}$) and time to peak (t_{max}) were analyzed on the group level by unpaired *t*-tests.

3 Results

3.1 Participants

Altogether, 50 healthy subjects were screened for this trial; 36 of the screened subjects were not randomized, out of which 34 subjects failed in screening (i.e., not meeting inclusion criteria and/or meeting any exclusion criteria, screening failure due to COVID-19 pandemic). One subject was not randomized, but selected for the replacement purpose and one subject withdrew for personal reasons. A total of 14 subjects were randomly allocated to one of two treatment sequences [test treatment A (oral) followed by reference treatment B (IV) or vice versa]. Out of these 14 randomized and dosed subjects, 13 subjects (93%) completed the trial as per the protocol. One subject discontinued the trial prematurely as this subject had a positive drug screening result. An overview of subject enrolment and completion/disposition by treatment is shown in Fig. 2. Demographics of the participants are depicted in Table 1.

3.2 Pharmacokinetics

The PK profiles of fasudil and hydroxyfasudil were evaluated to assess absolute bioavailability of the oral application (test) compared with the IV application (reference) of fasudil. Due to one early withdrawal, plasma PK concentration data were available for 13 subjects after test application and for 14 subjects after reference application. In period 1, presumably due to an analytic error, one participant had a baseline hydroxyfasudil concentration of > 5% of C_{max} during reference treatment, and therefore, no PK profile for hydroxyfasudil was calculated for this subject. For two subjects, deviations from planned sampling times were reported as protocol deviations. These deviations were deemed of minor severity and therefore not

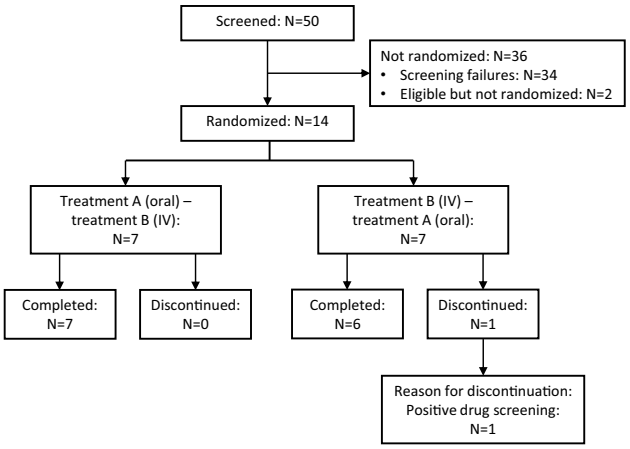


Fig. 2 Subject enrolment and treatment regime. Treatment A (test) refers to oral treatment, and treatment B (reference) refers to intravenous treatment with fasudil

Table 1 Demographics of study participants

Parameter	Value
n	14
Age, years, mean (SD)	49.6 (10.5)
Sex, female, *n* (%)	8 (57%)
Race, Caucasian/white, *n* (%)	14 (100%)
Ethnicity, not Hispanic/Latino, *n* (%)	14 (100%)
Height, cm, mean (SD)	173.9 (9.4)
Weight, kg, mean (SD)	76.75 (13.08)
BMI, kg/m^2, mean (SD)	25.22 (2.23)

SD standard deviation, *BMI* body mass index

excluded from analysis. Measurements below the LLOD were set to zero.

3.2.1 Fasudil

The primary PK parameters of fasudil after administration of oral (test) and IV (reference) treatment differed considerably (Fig. 3). For 4/14 subjects (28.6%), no fasudil concentration above LLOD was obtained after oral application. It was predefined in the study protocol that if the number of missing values is more than three in total, or more than two consequent values, the applicability of any interpolations should be discussed and decided within the trial team. Due to insufficient number of measurements, no $AUC_{0-\infty}$ and $t_{½}$ were computed for oral fasudil. AUC_{0-tz} was 0.29 µg × h/L [geometric mean (geoMean); coefficient of variation (CV) 96.2%] for oral treatment and 55.6 µg × h/L (CV 55.9%) for IV treatment. C_{max} was 1.4 g/L (CV 41.0%) for oral treatment and 100.6 µg/L (74.2%) for IV treatment. Results from the linear-mixed effect model comparing both treatments are given in Table 2. However, the validity of the model might be limited, as only nine subjects had data available for both periods that could be used in the model, and the geometric CV was high for C_{max} and AUC_{0-tz}. t_{max} was comparable between oral and IV treatment [median (range): 0.25 h (0.00 – 0.50 h) vs. 0.375 h (0.25 – 0.53 h), respectively, $P = 0.30$]. $t_{½}$ of fasudil after infusion was 0.55 h (56.4%).

3.2.2 Hydroxyfasudil

In contrast, concentrations of active metabolite hydroxyfasudil after oral (test) and IV (reference) treatment were obtained for all subjects (Fig. 4). Oral and IV treatment resulted in comparable C_{max} [oral: geoMean (CV): 111.6 µg/L (24.1%) vs. IV: 108.4 µg/L (19.7%)]. However, exposure between treatments differed, with AUC_{0-tz} of 295.2 µg × h/L (30.9%) versus 438.8 µg × h/L (22.5%) and $AUC_{0-\infty}$ of 310.3 µg × h/L (31.9%) versus 459.8 µg × h/L (22.3%) for of oral versus IV treatment, respectively. Thus, IV treatment led to a higher amount of measurable hydroxyfasudil in the blood. Estimated by a linear mixed-effect model of the full analysis set, hydroxyfasudil concentration after oral treatment is 65% compared with IV treatment (Table 3). The linear models were repeated with subjects as fixed effects. The obtained results were similar to the random effects models. t_{max} was comparable between oral and IV treatment [median (range): 0.50 h (0.25–3.00 h) versus 0.75 h (0.50–0.92 h), respectively; $P = 0.27$)]. Furthermore, $t_{½}$ did not differ significantly [geoMean (CV) 5.54 h (33.6%) versus 5.70 h (13.4%), respectively; $P = 0.50$].

3.3 Safety

For the oral application of 30 mg fasudil, no safety concerns were identified, and it was found to be generally well tolerated. No serious adverse events (SAEs) occurred in the course of this trial. One subject (7.1%) experienced two pretreatment adverse events. These events were deemed not related to the IMP or to the trial procedure. Two subjects experienced a total of two treatment emergent adverse events (TEAEs). One TEAE was reported in a subject receiving oral fasudil, whereas the other one was reported

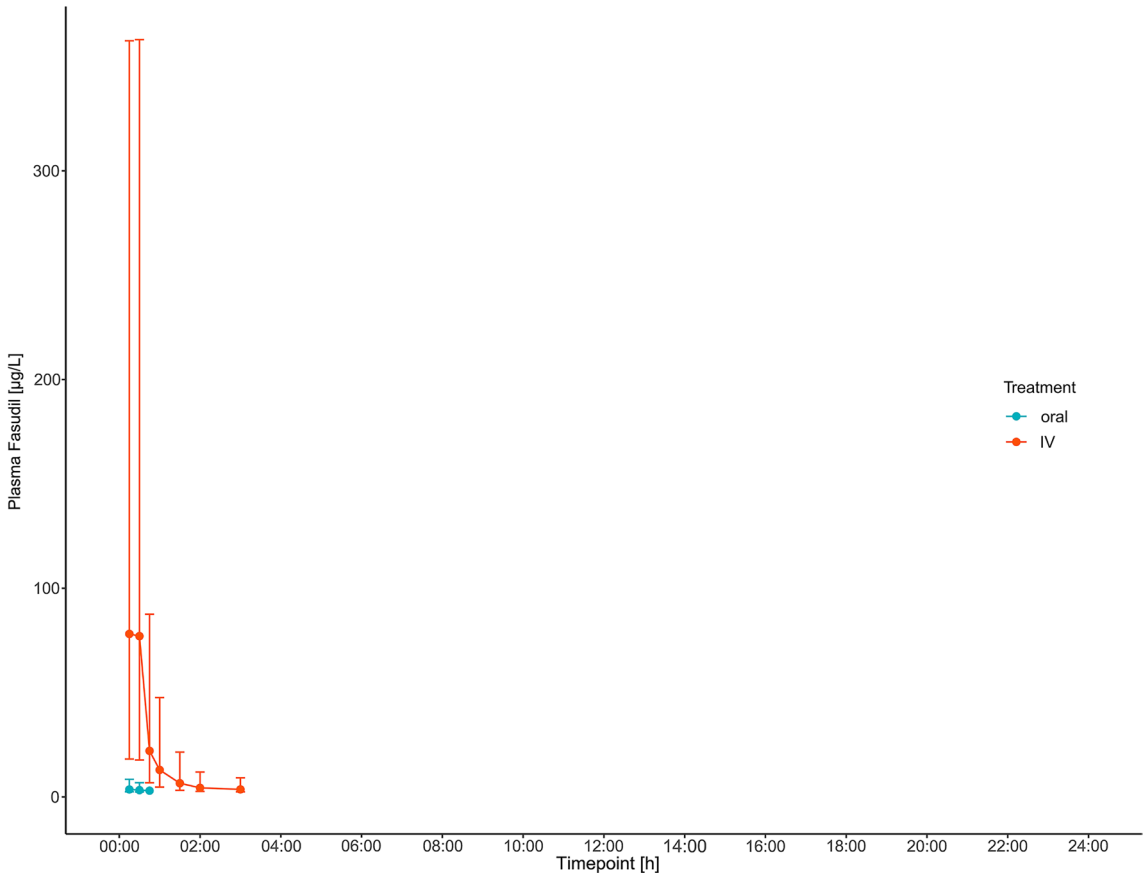


Fig. 3 Plasma concentrations of fasudil over time. Exposure of oral and IV treatment was generally low due to rapid hepatic metabolization; however, bioavailability differed significantly. Points indicate geometric mean; error bars represent 95% confidence interval, calculated for each time point

Table 2 Analysis of variance of primary pharmacokinetic variables of fasudil

Parameter	*n*	LS mean		LS mean ratio (oral/IV treatment)	
		treatment A (oral)	treatment B (IV)	Point estimate (%, 90% CI)	Intra-subject CV (%)
AUC_{0-tz} (µg × h/L)	14	0.29	55.58	0.52 (0.31–0.87)	70.46
C_{max} (µg/L)	14	1.41	100.65	1.41 (0.92–2.17)	56.92

AUC_{0-tz} area under the concentration time curve from zero up to the last measured concentration, C_{max} highest measured concentration determined in the measuring interval, *CI* confidence interval, *CV* coefficient of variation, *LS*, least square, *n* number of evaluable subjects included in the model. Results are based on a linear mixed-effects model with sequence, period, and treatment as fixed effects and subject nested within sequence as a random effect

in a subject receiving IV treatment. Thus, the amount of TEAE was equal for each treatment group. Overall, only one TEAE "headache" in one subject (7.1%) from IV treatment was assessed as related to the IMP. The remaining TEAE "fatigue" in one subject was assessed as not related to the IMP. All reported TEAEs from both treatment groups were of "mild" severity and had an outcome of "recovered/resolved" by the end of the trial. Additionally, there were no relevant changes in safety laboratory, vital signs, ECG, or in physical findings when comparing pre- and post-trial results. There were no other clinically relevant observations related to safety in this trial. Gastrointestinal tolerability after oral administration was assessed by the gastrointestinal symptom rating scale (GSRS). Overall, oral fasudil was well tolerated. No significant increase in subscales abdominal pain (abdominal pain, hunger pains, and nausea), reflux

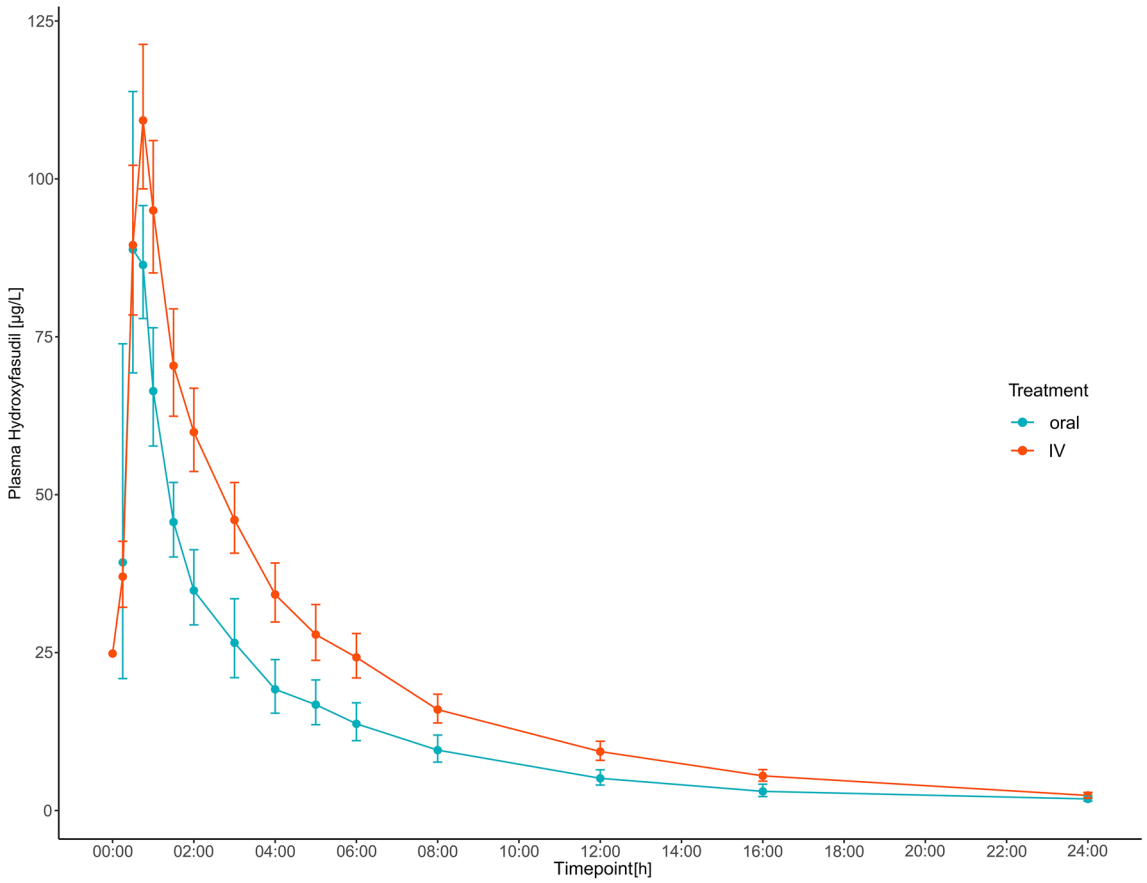


Fig. 4 Plasma concentrations of hydroxyfasudil over time. Exposure of oral and IV treatment differed significantly, highest measurable concentration was comparable. Points indicate geometric mean; error bars represent 95% confidence interval, calculated for each time point

Table 3 Analysis of variance of primary pharmacokinetic variables of hydroxyfasudil

Parameter	*n*	LS mean		LS mean ratio (oral/IV treatment)	
		treatment A (oral)	treatment B (IV)	Point estimate (%, 90% CI)	Intra-subject CV (%)
AUC_{0-tz} (µg × h/L)	14	288.65	447.39	64.52 (60.32–69.01)	9.04
$AUC_{0-\infty}$ (µg × h/L)	14	303.07	467.96	64.76 (60.11–69.78)	10.04
C_{max} (µg/L)	14	111.19	107.98	102.97 (89.53–118.43)	19.65

AUC_{0-tz} area under the concentration time curve from zero up to the last measured concentration, $AUC_{0-\infty}$ area under the concentration time curve from zero extrapolated to infinity, C_{max} highest measured concentration determined in the measuring interval, *CI* confidence interval, *CV* coefficient of variation, *LS* least square, *n* number of evaluable subjects included in the model. Results are based on a linear mixed-effects model with sequence, period, and treatment as fixed effects and subject nested within sequence as a random effect

syndrome (heartburn and acid regurgitation), diarrhea syndrome (diarrhoea, loose stools, and urgent need for defecation), and constipation syndrome (constipation, hard stools, and feeling of incomplete evacuation) was observed. However, a slight, statistically significant ($P = 0.048$) increase could be observed for the indigestion syndrome (borborygmus, abdominal distension, eructation, and increased flatus). The average symptom severity in this domain did not reach the level of "minor discomfort". One subject reported an increase in "passing gas or flatus" from "minor discomfort" before treatment to "mild discomfort" after the treatment (Fig. 5).

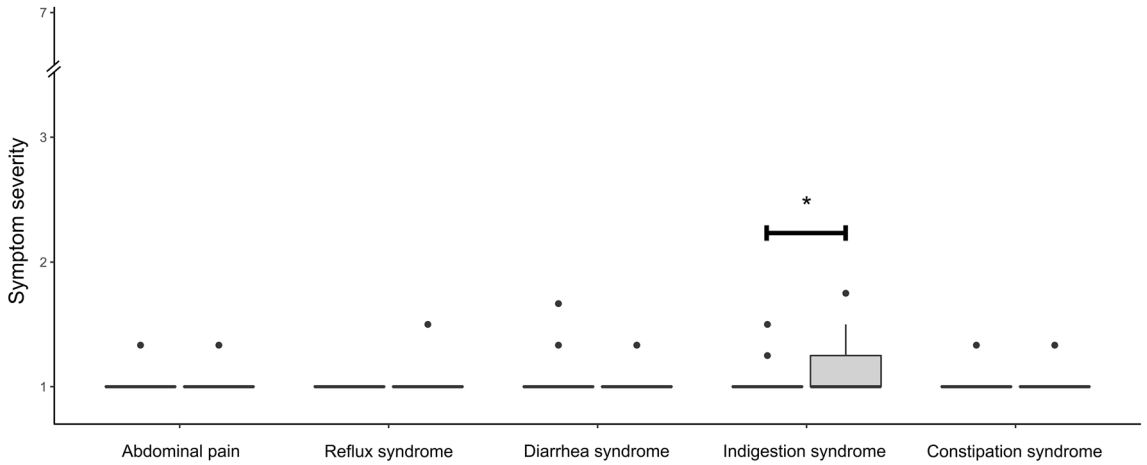


Fig. 5 Gastrointestinal Symptom Rating Scale subscales of oral fasudil. Scale is corresponding: 1 = no discomfort to 7 = very severe discomfort. It was assessed only in the period of treatment A (test, oral treatment). The scores of the subscales are calculated as the average of the individual item scores. Left column refers pretreatment response and right column post-treatment. If adequate, the interquartile range (IQR) is depicted as the box (25th percentile), the range of the data as whiskers (75th percentile), and points represent outliers. Paired Wilcoxon–Mann–Whitney test was performed to assess significant changes in responses. *P = 0.048 pre-treatment versus post-treatment

4 Discussion

This phase I, two-period crossover trial an absolute bioavailability of 69% was documented of the oral application of the approved and commercially available formulation of fasudil (ERIL®) in comparison to the reference IV application of fasudil. Additionally, oral application of fasudil raised no safety concerns and gastrointestinal tolerability of the oral administration of 90 mg/day fasudil could be established. To our knowledge this is the first published bioavailability study of the licensed formulation of fasudil comparing oral and IV administration. As described previously, fasudil is metabolized rapidly after IV administration, resulting in a serum half-life of 0.3–0.4 h [26, 32]. The precise etiology of the observed high inter-subject variability of C_{max} after IV administration remains elusive. Internal quality control did not identify any analytic biases associated with the observed variability. However, fasudil undergoes swift hepatic metabolism with discernible inter-subject variability, suggesting that the observed variations may be attributed, in part, to differences in metabolization kinetics among subjects. Oral fasudil experiences an even more pronounced first pass effect, resulting in low concentrations and high variances of fasudil. Hinderling et al. [29] studied the bioavailability of investigational solutions, powder and immediate-release tablets of fasudil hydrochloride and found comparable systemic concentrations of hydroxyfasudil, the active metabolite of fasudil, independent of the release site in the intestine. Pharmacokinetics of fasudil were not addressed in this study. However, the peak concentration of hydroxyfasudil tended to be smaller after colonic release compared with oral or ileal administration. Additionally, aqueous solution and immediate-release tablets displayed comparable systemic availability in this study [29]. In the present study, the maximal concentrations and time to peak concentration of hydroxyfasudil in the blood were comparable between the oral and IV treatments. For hydroxyfasudil, an exposure (AUC) of 400–555 µg × h/L has been documented after IV applications (30 mg fasudil over 30 min) [26, 32] and 300 µg × h/L for investigational oral solution administration of 40 mg of fasudil hydrochloride [29]. Our findings using the licensed formulation of fasudil are in line with this, with AUC_{0-tz} for hydroxyfasudil of 449 µg × h/L after IV treatment and 309 µg × h/L after oral treatment. Based on our findings, the absolute bioavailability of hydroxyfasudil after the oral treatment was approximately 69% of the IV treatment. For studies employing oral fasudil, this must be considered, and the dosage must be adapted.

In recent years and in addition to the approved indication of fasudil, fasudil has been tested for different cardiovascular diseases, such as stable angina, pulmonary hypertension, and stroke. Especially in the context of neurodegenerative diseases, the inhibition of ROCK by fasudil emerges as a promising and innovative approach, targeting various relevant disease mechanisms. Evidence indicates its ability to enhance axonal regeneration, reduce neuronal cell death, regulate microglial activity, and positively impact both survival and motor function in preclinical models of ALS and PD [3]. As of 4 October of 2023, 13 clinical trials are registered with the trial register of the National Library of Medicine, Bethesda, USA (clinicaltrials.gov), six of which investigate

oral formulations of fasudil (Supplementary Table S4). Results from the present trial provide relevant insight into the pharmacokinetic properties of fasudil, identify the need for adaptions of the dosage, and can serve as guideline for future clinical trials evaluating oral fasudil in novel patient populations. In vein with ample preclinical evidence for beneficial effects of fasudil in neurodegenerative diseases, including ALS and PD [3], we conducted a phase II trial of IV fasudil in patients with ALS (NCT03792490). Based on findings of the present study, we planned and currently conduct a phase II trial of oral fasudil in its licensed formulation in patients with Parkinson's disease (NCT05931575), applying the adapted dosage [33].

In general, our data suggest that fasudil can be obtained in its approved formulation from the original manufacturer opening new treatment possibilities by oral application. Oral administration allows ambulatory treatment and reduces the economic burden on healthcare systems. It results in comparable systemic availability with IV treatment while being generally safer for the patients. In addition, oral treatment will make long-term treatment with fasudil accessible for clinical trials. To date, oral fasudil (tablets) have been studied for a treatment period of 8 weeks in patients with stable angina and 12 weeks in patients with pulmonary hypertension [7, 34]. Both studies reported satisfactory long-term safety and tolerability. However, to translate promising preclinical findings of fasudil to chronic diseases, additional trials evaluating an even longer application of the drug are needed.

Overall, orally administered fasudil was generally well tolerated in the studied population. No severe adverse events occurred, and no systemic side effects were observed. The evaluation of adverse events, clinical laboratory parameters, physical findings, vital signs, and gastrointestinal safety raised no concerns regarding subject safety and drug tolerability. This is in line with previous studies investigating oral and IV fasudil, where no indications of safety concerns were uncovered [4–7, 26, 27, 29, 32, 35–38]. For both applications, adverse events were mild and self-resolving, and fasudil was well tolerated with mild changes in laboratory tests of kidney and liver function, headache or hypotonia. The largest safety data set published so far is derived from the postmarketing surveillance of IV fasudil for the treatment of vasospasms after subarachnoid hemorrhage [4]. In this patient population, hemorrhages occurred during fasudil treatment in rare cases (1.7%); however, they were not significantly exceeding events during placebo treatment (1.4%).

The following caveat applies for this study: this trial was conducted in healthy subjects. How bioavailability or safety is altered in patients with conditions affecting the gastrointestinal tract remains to be determined. Altered gastrointestinal passage time could change bioavailability of fasudil and, thus, the concentration of the active metabolite hydroxyfasudil. This could, for example, apply for patients with Parkinson's disease, known to have prolonged intestinal passage time, or patients with chronic diarrhea [39, 40]. Furthermore, the study had a restricted participant pool, and the evaluation of oral safety was conducted over a relatively brief duration. Nevertheless, these conditions provided sufficient justification for designing subsequent trials to explore the oral administration of fasudil.

In summary, oral application of fasudil in its approved formulation was generally well tolerated and showed good oral bioavailability of its active metabolite hydroxyfasudil. This study provides valuable insights for planning clinical trials involving the oral application of fasudil.

5 Conclusions

The suitability of oral application of fasudil is supported by its established safety and the attainment of adequate bioavailability. Oral fasudil did not raise any safety concerns; however, generally low, and highly variable concentrations of fasudil were documented. For the active metabolite of fasudil, hydroxyfasudil, an absolute bioavailability of 69% was found after oral application. Therefore, it is crucial to recognize the imperative for dose adjustments in subsequent applications. This trial provided evidence for an ongoing clinical trial applying oral fasudil in patients with PD. It also sets the groundwork for future trials exploring the use of oral fasudil in different chronic conditions.

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Conflicts of Interest Paul Lingor is an inventor on a patent for the use of Fasudil for the treatment of amyotrophic lateral sclerosis (EP 2825175 B1, US 9.980,972 B2). Gabriela Zurek heads the LC-MS/MS department of the Medical Laboratory Bremen, where the determination of total fasudil and hydroxyfasudil was performed. All other authors declare that there are no conflicts of interest.

Ethics Approval The approval of both, the ethics committee (no. PVN7203) and the German Federal Institute for Drugs and Medical Devices (BfArM) were obtained prior to the start of the clinical trial and the study was performed in accordance with good clinical practice and the Declaration of Helsinki.

Consent to Participate All participants signed an informed consent form as evidence of consent.

Consent for Publication Not applicable.

Data Availability Statement Data are available from the corresponding author upon reasonable request.

Code Availability Statement Not applicable.

Author Contributions Paul Lingor developed the trial concept. Paul Lingor, Claus Hemker, and Jörg Peine wrote the trial protocol. Jörg Peine coordinated the trial execution. Josef Höfler detailed the statistical aspects of the study in the study protocol. Josef Höfler and Andreas Wolff performed the statistical analysis. Gabriela Zurek was responsible for the pharmacokinetic analyses. Andreas Wolff and Paul Lingor interpreted the study results. Andreas Wolff wrote the manuscript. All authors critically reviewed the manuscript. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

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