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ORIGINAL ARTICLE

First-in-human trial to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of STR-324, a dual enkephalinase inhibitor for pain management

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Aim: Dual enkephalinase inhibitors (DENKIs) are involved in the regulation of nociception via opioid receptors. The novel compound STR-324 belongs to the DENKI pharmacological class. This first-in-human study evaluated the safety, tolerability, pharmacokinetics and pharmacodynamics of STR-324 in healthy male participants.

Methods: This was a randomised, double-blind, placebo-controlled ascending dosing study in two parts: in part 1, 30 participants received 0.004-11.475 mg h^{-1} of STR-324 or placebo (ratio 4:1) by 4 h intravenous infusion in a two-group, partial cross-over design with four treatment periods separated by 1 month wash-out, and in part 2, 48 participants divided into three groups received either the active drug (1.25-11.25 mg h^{-1}) or placebo (ratio 3:1) by 48 h intravenous infusion. Safety and tolerability parameters, pharmacokinetics and pharmacodynamic effects on neurocognitive and neurophysiological tasks and on a nociceptive test battery were evaluated.

Results: No clinically relevant changes in safety parameters were observed. All treatment-emergent adverse events were mild and transient. The pharmacokinetics of STR-324 could not be determined due to most concentrations being below quantifiable limits. STR-324 metabolite concentrations were measurable, showing dose proportionality of C_{max} and AUC_{inf} with an estimated $t_{1/2}$ of 0.2-0.5 h. Significant changes in pharmacodynamic parameters were observed, but these were not consistent or dose-dependent.

Conclusion: STR-324 displayed favourable safety and tolerability profiles at all doses up to 11.475 mg h^{-1}. Although pharmacokinetic characterisation of STR-324 was limited, dose proportionality could be assumed based on major metabolite data

The authors confirm that the PI for this paper was Prof. Geert Jan Groeneveld and that he had direct clinical responsibility for patients.

assayed as proxy. No clear effects on nociceptive thresholds or other pharmacodynamic measures were observed.
Trial registry: EudraCT (2014-002402-21) and toetsingonline.nl (63085).

KEYWORDS
DENKI, dual enkephalinase inhibitor, first-in-human, opioids, pain management, phase 1

1 | INTRODUCTION

Currently, the standard of care for patients who suffer from pain includes a wide range of analgesic compounds, depending on the perceived severity of the pain. In 1987, the World Health Organization (WHO) published a three-step analgesic ladder as the guideline for developing treatment plans for pain, originally intended for use in cancer pain management.[1] At the third step, for the highest severity of pain, **opioids** are indicated. Although the efficacy of opioids on severe acute pain has been established, emerging data showed that use of opioids for chronic noncancer pain is controversial due to the lack of evidence of the long-term efficacy in that patient population. In addition, opioids are associated with many adverse effects, including respiratory depression induced by high doses administered in an uncontrolled setting, eventually leading to hypoxaemia and death.[2] Moreover, opioids are reported to have a relatively high potential for abuse compared to other analgesic drug classes. Opioid use disorder is a major source of morbidity and mortality worldwide.[3] This class of drugs has increasingly been prescribed in recent decades, and a concurrent rise in the number of fatalities attributable to opioid misuse and overdose has been reported.[4] Therefore, there is an unmet need for highly effective analgesics with fewer associated risks, especially for the treatment of chronic pain. New drugs with less potential for abuse are preferred over classic opioid therapies, if available.

A class of opioidergic drugs that has recently gained attention is that of dual enkephalinase inhibitors (**DENKIs**). In healthy humans, the neural process of nociception is regulated by endomorphines including **enkephalins**, which play a major role in the modulation of pain. However, this natural modulation is of short duration. Enkephalins are synthesised intracellularly and stored in large synaptic vesicles, only to be released locally in response to nociceptive information. Enkephalins are present at the different levels of signal transmission from the peripheral sensory neurons through the spinal dorsal horn up to the brain. Outside the cells, enkephalins briefly interact with opioid receptors, reducing nociceptive transmission, before their action is disturbed by the metallopeptidases **aminopeptidase-N** (APN) and **neprilysin** (NEP). These two peptidases generate inactive metabolites and thus contribute to the modulation of nociceptive signalling. No enkephalinase inhibitors are yet available for the treatment of pain. Inhibiting both APN and NEP, and thereby increasing the bioavailability of enkephalins is a new therapeutic paradigm in the management of pain. DENKIs have the capacity of inhibiting both peptidases APN and NEP. Enkephalinase inhibitors have the advantage of being active only when enkephalins are produced in response to a pain sensation.[5]

What is already known about this subject

- There is an unmet need for highly effective analgesics with an improved benefit-risk ratio compared to commonly prescribed opioids.
- Enkephalins play a role in the modulation of pain.
- Increasing enkephalin bioavailability by inhibiting both aminopeptidase-N and neprilysin, is a new therapeutic paradigm in the management of pain.

What this study adds

- STR-324, a dual enkephalinase inhibitor targeting aminopeptidase-N and neprilysin, displayed favourable safety and tolerability profiles at doses up to 11.475 mg h^{-1} in healthy males.
- Pharmacokinetic characterisation of STR-324 is challenging due to its rapid metabolism.
- The pharmacodynamic effects of STR-324 are yet to be observed in the target population.

STR-324 is the pyroglutamised form of **opiorphin** and both penta-peptides are naturally present in humans as endogenous compounds with an opiorphin:STR-324 ratio of 70:30.[6] Like its parent molecule, STR-324 is a DENKI that targets APN and NEP.[7,8] A schematic representation of the putative mode of action of STR-324 is presented in Figure 1. In preclinical studies (unpublished) a favourable safety and analgesic activity profile of STR-324 supported the administration in humans for further research.[9,10] Preclinically, measurements of STR-324 appeared challenging but its main metabolite allowed adequate characterisation. Isolated as a compound, STR-324 has not been previously administered to humans. STR-324 is currently being developed as a therapeutic candidate for pain management. In contrast to other compounds in its drug class, STR-324 is naturally present in the human body and therefore, theoretically, has a reduced risk for toxicities of any type. The observation of an improved benefit-risk ratio compared to commonly prescribed opioids, such as morphine and fentanyl, would justify a place for STR-324 in the armamentarium for the management of pain.

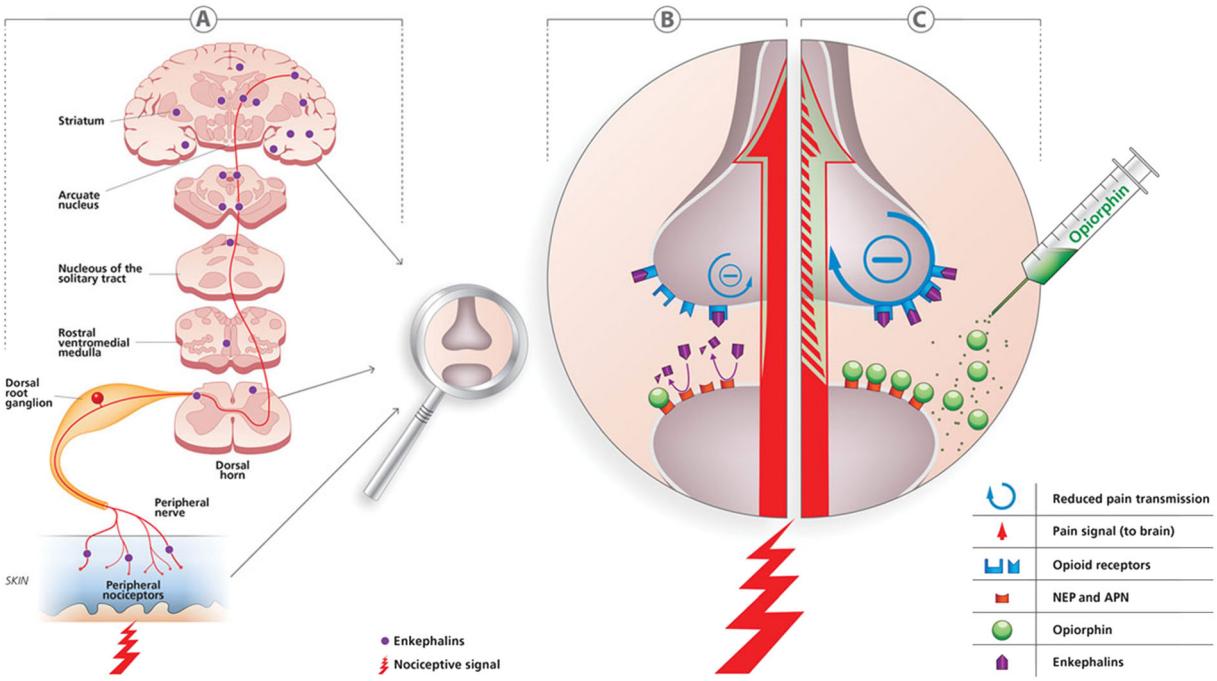

FIGURE 1 Mechanism of action of opiorphin/STR-324.[9] APN, aminopeptidase-N; NEP, neprilysin

The aim of this study was to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of STR-324 intravenous infusions in healthy males.

2 | METHODS

This first-in-human, randomised, double-blind, placebo-controlled, ascending dosing study was conducted at the Centre for Human Drug Research (Leiden, the Netherlands), in accordance with the principles of the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice (ICH GCP) and ethical principles as referenced in EU Directive 2001/20/EC. The protocol was approved by the Medical Review and Ethics Committee of the BEBO foundation (Assen, the Netherlands).

This trial was prospectively registered in EudraCT (number 2014-002402-21) and at toetsingonline.nl (CHDR-1725, ABR-number 63085).

2.1 | Study participants

Healthy male volunteers aged 18-45 years, with a body mass index of 18-30 kg/m^2, could be enrolled after successfully completing a medical screening. No female participants were included in this trial due to the reported hormonal influence on pain thresholds in women. Written informed consent was obtained from all participants before any study-specific procedures were performed. Key exclusion criteria were any clinically significant medical conditions, in particular any conditions that could affect sensitivity to cold and pain, any active or chronic disease or condition that could interfere with the conduct of the study, previous history of seizures or epilepsy, pain tolerance >80% on nociceptive test battery as determined at screening, history of any substance use disorder, smoking >5 cigarettes (or equivalent products) per day, alcohol consumption ≥21 units/week and use of any medication, especially analgesics, within 14 days before dosing. Participants were required to practice effective contraception throughout the study.

2.2 | Study design

This ascending dosing study consisted of two parts. Part 1 had a partial crossover, four out of five design with 30 participants divided into two equal groups following an interleaving dosing schedule with 4-week wash-out periods. Participants received escalating STR-324 doses and randomly placed placebo in four visits. Of the 15 participants per group, 12 received a placebo once and three received active drug during all visits. Part 2 had a parallel study design and included 48 participants divided into three equal groups. In this part, participants randomly received placebo or one of the three STR-324 dose levels over a 48 h infusion, randomised in a 3:1 ratio (active versus placebo). Dose levels in part 2 were selected based on tolerability in part 1 and expected analgesic activity but did not exceed the protocol-defined maximum dose of 11.475 mg h^{-1} for 24 h (based on preclinical no observed adverse effect level [NOAEL]). Administration of each new dose level was performed using a sentinel approach. An overview of the treatment

groups and dose levels is presented in Tables 1 and 2. All doses were prepared by the pharmacy of Leiden University Medical Centre (Leiden, the Netherlands).

2.3 | Safety and tolerability assessments

Safety and tolerability were assessed based on treatment-emergent (serious) adverse events (TE[S]AEs), clinical laboratory tests, vital signs, electrocardiogram (ECG) and continuous Holter monitoring and continuous oxygen saturation. TE(S)AEs were recorded from the time the participant signed consent until the follow-up visit and were classified according to MedDRA v20.1. Measurements of safety parameters were repeatedly performed throughout the study period.

Because increased levels of angiotensin II (Ang-II) have been reported in rats after a bolus injection of opiorphin,[11] Ang-II plasma levels were measured pre-dose and directly after infusion stop as additional safety measure; whole blood was collected in K2EDTA BD Vacutainer tubes and analysed by Ardena Bioanalytical Laboratory, the Netherlands, using a qualified ELISA method (EIA-ANGII, RayBiotech, Norcross, USA). The lower limit of quantification (LLOQ) was 5 pg/mL.

2.4 | Pharmacokinetic assessments

To characterise the pharmacokinetics (PK) of STR-324 and its major metabolite, called STR-324M, blood samples were taken at various time points and urine was collected according to specified intervals (Appendix 1). Based on preclinical data, measurement of plasma concentrations of STR-324 was expected to be challenging. However, metabolisation occurred systemically in blood and STR-324M allowed for adequate characterisation. Therefore, it was decided to use the plasma concentrations of the inactive metabolite STR-324M as a proxy of the exposure of the parent product in humans. Blood was collected in K2EDTA BD Vacutainer tubes, immediately mixed with ethanol in a prefilled polypropylene tube and stored at ≤−70 °C. For each urine PK collection interval, each fraction of collected urine was homogenised and a sample was taken to be handled similar to blood samples.

All PK samples were analysed by Ardena Bioanalytical Laboratory, the Netherlands, using a specific good laboratory practice (GLP) validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method compliant with the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) guidelines. Briefly, products were extracted by acidifying the received clinical samples, followed by

TABLE 1 Treatment groups and baseline characteristics (mean and SD)

	Treatment STR-324 (or matching placebo)				
	Part 1 (4 h infusion)		Part 2 (48 h infusion)		
Treatment group	1	2	3	4	5
Participants, *N*	15	21	16	16	16
Dose level (mg h^{-1})	0.004-5.748	0.021-11.475	1.25	3.75	11.25
Age, y					
Mean (SD)	27.4 (6.4)	26 (6.9)	23.6 (3.1)	22.9 (2.1)	22.9 (3.0)
Range	20-40	19-45	19-32	20-27	18-29
Gender, %					
Male	100	100	100	100	100
Ethnicity, *N* (%)					
Caucasian	12 (80)	18 (85.7)	9 (75)	11 (91.7)	12 (100)
Mixed	1 (6.7)		1 (8.3)	1 (8.3)	
Black/African	1 (6.7)	3 (14.3)	1 (8.3)		
Other	1 (6.7)		1 (8.3)		
Height, cm					
Mean (SD)	182.2 (7.2)	184.3 (6.6)	184.2 (5.2)	182.1 (6.3)	183.7 (6.5)
Range	169.4-193.1	172.9-199.4	176.1-197.4	169.7-194.6	171.8-202.4
Weight, kg					
Mean (SD)	76.8 (11.1)	77.9 (10.3)	76.1 (9.1)	71.7 (9.8)	73.8 (8.8)
Range	56.1-93.8	59.5-96.4	58.7-95.8	58.4-93.0	60.7-91.3
BMI, kg/m^2					
Mean (SD)	23.1 (2.3)	22.9 (2.2)	22.5 (2.6)	21.6 (2.4)	21.8 (1.7)
Range	19.5-26.5	19.4-26.5	18.1-28.0	17.6-26.3	19.5-25.7

Abbreviations: BMI, body mass index; *N*, sample size; SD, standard deviation; y, years.

TABLE 2 Summary of treatment-emergent adverse events possibly or probably related to treatment in both study parts (by MedDRA preferred term)

Part 1 (4 h infusion)																		
Dose level (mg h^{-1})	**0.004**		**0.021**		**0.106**		**0.319**		**0.956**		**2.869**		**5.748**		**11.475**		**Placebo**	
Participants, *N*	12		12		12		12		12		12		12		12		24	
Treatment relationship	PROB	POS	PROB	POS	PROB	POS	PROB	POS	PROB	POS	PROB	POS	PROB	POS	PROB	POS	PROB	POS
TEAE, *N*																		
Fatigue				1		1				1		1				1		
Dizziness		1				1		1		1								3
Headache		1		1		2				1		1		2				2
Somnolence				3								1		1		3		4
Nausea										1				1				
Noncardiac chest pain				1														
Muscle spasms																1		

Part 2 (48 h infusion)										
Dose level (mg h^{-1})	**1.25**		**3.75**		**11.25**		**Placebo**			
Participants, *N*	12		12		12		12			
Treatment relationship	PROB	POS	PROB	POS	PROB	POS	PROB	POS		
TEAE, *N*										
Fatigue				1						
Dizziness				2		1				1
Headache				3		1				1
Somnolence										2
Nausea		1								
Diarrhoea		1								
Palpitations										1
Chest pain		1								
Feeling abnormal		1								
Infusion site pruritus		1								
Neck pain		1								

Abbreviations: IV, intravenous; MAD, multiple ascending doses; MedDRA, Medical Dictionary for Regulatory Activities; *N*, sample size; POS, possibly; PROB, probably; SAD, single ascending doses; SC, subcutaneous; TEAE, treatment-emergent adverse event.

evaporation and reconstitution. Chromatographic separation was performed on a Waters BEH300 C18 column (Waters Chromatography BV, Milford Massachusetts, U.S.A.) using gradient elution and quantification using an API 6500 + LC-MS/MS system with a turbo ion spray probe (AB Sciex, Framingham Massachusetts, U.S.A. and Shimadzu, Kyoto, Japan). Data acquisition was performed using Analyst software (version 1.6.3) from AB Sciex. Following peak area integration, regression was also performed using Analyst. Concentrations were calculated using nine-point curves with weighted linear regression. The LLOQ for the PK assay was 0.1 ng/mL for blood, 2 ng/mL for urine.

2.5 | Pharmacodynamic assessments

To determine the effect of STR-324 on pain thresholds, measurements using a validated battery of nociceptive tests were performed at set times throughout the day. The nociceptive test battery was previously validated and used to show the analgesic profile of a wide variety of compounds.[12–15] At screening, a training session was performed to reduce possible learning effects, and to exclude participants conform the eligibility criteria. Assessments were performed with the participant sitting comfortably in a chair, leg raised, in a quiet room that was fitted with ambient lighting. Each participant was assigned to a separate room to minimise any distraction. During part 1 of the study, the test battery was performed twice predose as baseline assessment and six times over 5 h postdose on each study drug administration day. In part 2 the test battery was performed twice predose and at 1, 8, 24, 48 and 56 h postdose. Nociceptive tests used in this study are listed in Supporting Information Table S2.

In part 2 a series of neurocognitive and neurophysiological measurements was also performed, using a validated test battery measuring a wide range of central nervous system (CNS) functions as described previously.[16] Additionally, pupillometry was performed using a digital camera with flash, and pupil/iris ratio was calculated as a measure of pupil size (Qpupil, Leiden University Medical Center, Leiden, the Netherlands). A complete overview of the measurements performed is provided in the Supporting Information.

In part 2, the 49-item Addiction Research Center Inventory (ARCI)[17] and bowel function index (BFI)[18] were taken to explore the opioidergic drug effects of STR-324.

2.6 | Exploratory biomarkers

Given STR-324 inhibits the action of APN and NEP, a reduction in the plasma levels of these two molecules may be measurable. In literature, Big Endothelin 1 (Big ET-1) has been reported to be an indirect biomarker for the effects of two neprilysin inhibitors[19] and was included as proxy biomarker for NEP in this study.

APN and Big ET-1 concentrations in peripheral blood were included as exploratory endpoints in part 2 only. The blood samples to measure APN were collected in sodium heparin tubes, and for Big ET-1 K2EDTA BD Vacutainer tubes were used. Both biomarkers were analysed by the Ardena Bioanalytical Laboratory using qualified ELISA methods. The LLOQs were 0.400 nmol/well for APN and 0.100 fmol/well for Big ET-1.

2.7 | Statistical analysis

The primary endpoint (safety of STR-324) was descriptively reported, summarizing TEAEs by system organ class and preferred term. For the PK parameters, a noncompartmental analysis was performed. PK parameters assessed for each individual profile are shown in Tables 3 and 4. Additionally, urinary excretion (%) was calculated. The methods of analysis of pharmacokinetic and pharmacodynamic endpoints are provided in the supporting information (**Appendix 3**). Noncompartmental analysis and programming of PK tables and figures was conducted with R 3.6.1 for Windows (R Foundation for Statistical Computing/R Development Core Team, Vienna, Austria, 2019). All safety and statistical programming was conducted with SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

Since statistical testing was exploratory, statistical hypothesis testing and power calculations were not performed.

2.8 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[20–22]

3 | RESULTS

Between 20 February 2018 and 07 November 2018, 36 participants were enrolled in part 1 of the study and 48 participants in part 2. Due to the premature discontinuation of six participants in part 1, replacement participants were enrolled to complete data for all study visits (Figure 2). Demographics and other baseline characteristics are presented in Table 1.

3.1 | Safety and tolerability

An overview of TEAEs that were assessed as at least possibly related to treatment can be found in Table 2. Two serious adverse events (SAEs) occurred postdose but were not considered related to STR-324 administration: acute appendicitis in part 1 and testicular torsion in part 2. No participants withdrew from the study due to adverse effects. Headache, fatigue and somnolence were frequently reported in all STR-324 dose groups, but no dose-dependent increase in adverse events and no evident differences compared to placebo were

TABLE 3 Summary of STR-324 pharmacokinetics

	Dose level (mg h^{-1})	*N*	Mean	SD	CV	GeoMean	GeoCV	Median	Min.	Max.
Part 1 (4 h infusion)	**2.869**									
	C_{max}, ng/mL	12	11.94	41.27	345.74	1.32	377198.74	0	0	143
	t_{max}, h	3	3.4	1.21	35.66	3.23	43.29	4.08	2	4.12
	AUC_{last}, ng*h/mL	12	12.75	44.09	345.95	0.89	3006866.09	0	0	152.76
	5.748									
	C_{max}, ng/mL	12	0.07	0.08	109.74	0.14	25.62	0.05	0	0.19
	t_{max}, h	6	3.69	0.83	22.43	3.58	29.14	4	2	4.08
	AUC_{last}, ng*h/mL	12	0.09	0.12	131.88	0.14	110.79	0.01	0	0.28
	11.475									
	C_{max}, ng/mL	12	0.2	0.04	18.9	0.2	19.5	0.2	0.15	0.26
	t_{max}, h	12	3.08	1.23	39.87	2.81	48.93	4	1.5	4.08
	AUC_{last}, ng*h/mL	12	0.59	0.22	36.67	0.55	43.39	0.62	0.28	0.93
Part 2 (48 h infusion)	**3.75**									
	C_{max}, ng/mL	12	0.06	0.07	125.78	0.14	16.57	0	0	0.16
	t_{max}, h	5	21.01	18.14	86.36	11.73	300.34	24	1	48.02
	AUC_{last}, ng*h/mL	12	1.37	2.2	160.13	2.6	94.35	0	0	6.13
	11.25									
	C_{max}, ng/mL	12	0.21	0.08	38.38	0.23	21.45	0.22	0	0.32
	t_{max}, h	11	35.33	18.99	53.76	26.8	118.4	48.02	4.22	48.2
	AUC_{last}, ng*h/mL	12	8.62	3.51	40.77	9.17	23.34	8.53	0	14.58

$AUC_{0\text{-}last}$, area under the concentration-time curve from time 0 to last quantifiable concentration; C_{max}, maximum concentration; CV, coefficient of variation; GeoCV, geometric mean coefficient of variation; GeoMean, geometric mean; h, hour; *N*, sample size; SD, standard deviation; t_{max}, time to reach C_{max}.

observed. In part 2 there were five mild adverse events recorded related to the infusion site versus zero in the placebo group. However, no dose-dependent increase in events was observed. Additionally, the events did not reoccur after infusion cannula replacement. Most adverse events were mild in severity. Seven moderate adverse events were reported, in part 1 only. All moderate and severe adverse events were classified as being unlikely or not related to the study drug and were reported in both STR-324 and placebo groups. No clinically relevant changes in blood chemistry, haematology, urinalysis, Ang-II plasma levels, vital signs or ECG tests were identified.

3.2 | Pharmacokinetics

Following intravenous administration of STR-324, plasma concentration-time profiles were characterised. STR-324 plasma concentrations were below the limit of quantification (BLQ) for most dose levels during the 4 and 48 h infusion periods. Due to the paucity of results, it was not considered appropriate to undertake a full toxicokinetic interpretation of these data except for the highest dose level in part 1. A summary of C_{max}, t_{max} and AUC_{last} for STR-324 is provided in Table 3. In contrast, plasma concentrations for the metabolite STR-324M were measurable for all dose levels with the exception for dose level 1 in part 1 where concentrations were BLQ. PK profiles of STR-324M for all dose levels are presented in Figure 3. Plasma concentrations of the metabolite STR-324M increased immediately after the infusion started and decreased rapidly after the end with a bi-exponential decline. A summary of the PK parameters is shown in Table 4. Terminal half-life and half-life related parameters could not be determined for the first four dose levels of part 1, as only the first part of the bi-exponential decay was above the LLOQ. Infusion rate normalised C_{max} and dose normalised AUC_{last} for STR-324M were equal across dose levels (indicative for a dose proportional PK). All observations for STR-324 in urine were BLQ except at the highest dose in either study part. Excretion of the metabolite into urine ranged from 12.5% to 17.7% in part 1 (dose levels 0.004 to 11.475 mg h^{-1}, respectively), which is in line with the dose-linear exposure and stable elimination constant. Similarly, excretion of the metabolite into urine ranged from 16.2% to 16.5% in the 1.25 to 11.25 mg h^{-1} dose range in part 2.

3.3 | Pharmacodynamics

No consistent, dose-dependent pharmacodynamic effects related to STR-324 could be observed in this study. Significant differences from placebo in multiple parameters were observed at varying dose levels for the cold pressor, electrical burst, pressure pain and conditioned

TABLE 4 Summary of STR-324 M pharmacokinetics

	Dose level (mg h^{-1})	N	Mean	SD	CV	GeoMean	GeoCV	Median	Min	Max
Part 1 (4 h infusion)	**0.956**									
	C_{max}, ng/mL	11	7.01	1.58	22.57	6.85	22.29	6.93	5.25	9.54
	t_{max}, h	11	2.32	1.15	49.44	2.08	52.93	2	1	4
	AUC_{last}, ng*h/mL	11	25.64	6.24	24.33	24.97	24.35	24.74	18.15	35.61
	AUC_{inf}, ng*h/mL	11	25.81	6.28	24.33	25.14	24.31	24.87	18.25	35.85
	$t_{1/2}$, h	11	0.49	0.13	26.76	0.48	27.42	0.5	0.31	0.77
	CL, L/h	11	99.92	23.23	23.25	97.4	24.31	98.46	68.29	134.12
	2.869									
	C_{max}, ng/mL	12	30.35	29.31	96.57	25.12	55.43	22.9	16.8	123
	t_{max}, h	12	3.1	1.18	38	2.84	49.74	4	1	4.12
	AUC_{last}, ng*h/mL	12	89.42	32.45	36.29	85.65	29.09	85.17	54.46	187.25
	AUC_{inf}, ng*h/mL	12	89.6	32.48	36.25	85.83	29.08	85.31	54.55	187.49
	$T_{1/2}$, h	12	0.5	0.1	20.54	0.49	19.63	0.47	0.39	0.68
	CL, L/h	12	88.43	21.67	24.51	85.58	29.08	86.1	39.17	134.65
	5.748									
	C_{max}, ng/mL	12	45.01	9.69	21.54	44.02	22.6	46.6	31.6	58.3
	t_{max}, h	12	2.88	1.4	48.72	2.49	66.34	4	1	4.02
	AUC_{last}, ng*h/mL	12	163.34	33.82	20.7	160.11	21.23	166.25	116.3	221.89
	AUC_{inf}, ng*h/mL	12	163.55	33.88	20.72	160.31	21.24	166.53	116.37	222.16
	$t_{1/2}$, h	12	0.56	0.13	22.93	0.55	24.66	0.62	0.37	0.7
	CL, L/h	12	93.51	19.75	21.12	91.63	21.24	88.23	66.12	126.23
	11.475									
	C_{max}, ng/mL	12	87.47	15.21	17.39	86.33	16.78	82.75	70	117
	t_{max}, h	12	3.04	1.21	39.93	2.76	52.5	4	1	4
	AUC_{last}, ng*h/mL	12	323.39	56.71	17.54	318.95	17.44	306.29	233.19	423.13
	AUC_{inf}, ng*h/mL	12	323.68	56.77	17.54	319.23	17.44	306.43	233.68	423.68
	$T_{1/2}$	12	0.64	0.08	12.76	0.64	12.32	0.63	0.56	0.81
	CL, L/h	12	93.23	16.07	17.23	91.95	17.58	95.89	69.34	125.73
Part 2 (48 h infusion)	**1.25**									
	C_{max}, ng/mL	12	10.23	1.78	17.41	10.1	15.84	9.66	8.27	14.9
	t_{max}, h	12	18.35	15.57	84.9	11.16	169.33	16	2	48.08
	AUC_{last}, ng*h/mL	12	435.76	89.31	20.5	428.62	18.46	414.79	357.04	665.63
	AUC_{inf}, ng*h/mL	12	435.93	89.34	20.49	428.79	18.46	415	357.17	665.83
	$t_{1/2}$, h	12	0.49	0.09	18.2	0.48	19.91	0.49	0.3	0.6
	CL, L/h	12	90.85	14.94	16.44	89.57	18.46	92.62	57.68	107.53
	3.75									
	C_{max}, ng/mL	12	35.56	7.61	21.39	34.79	22.37	34.95	23.1	45.8
	t_{max}, h	12	16.67	16.05	96.28	8.56	242.03	8.01	1	48.02
	AUC_{last}, ng*h/mL	12	1443.61	352.5	24.42	1400.16	27.26	1383.86	739.52	2031.29
	AUC_{inf}, ng*h/mL	12	1443.85	352.56	24.42	1400.4	27.26	1384	739.63	2031.64
	$t_{1/2}$, h	12	0.68	0.18	26.19	0.66	28.63	0.72	0.44	0.93
	CL, L/h	12	85.24	25.96	30.45	82.27	27.26	83.45	56.71	155.77
	11.25									
	C_{max}, ng/mL	12	86.96	13.68	15.73	86.02	15.27	81.55	71.4	115
	t_{max}, h	12	8.77	9.65	109.98	4.93	177.96	8	1	32
	AUC_{last}, ng*h/mL	12	3609.86	661.29	18.32	3556.46	18.04	3535.46	2861.62	4828.51

TABLE 4 (Continued)

Dose level (mg h^{-1})	N	Mean	SD	CV	GeoMean	GeoCV	Median	Min	Max
AUC_{inf}, ng*h/mL	12	3610.45	661.47	18.32	3557.03	18.05	3536.08	2862.14	4829.5
$t_{1/2}$, h	12	0.76	0.08	10.13	0.76	9.54	0.75	0.67	0.96
CL, L/h	12	98.58	17.12	17.37	97.17	18.05	98.09	71.57	120.76

$AUC_{0\text{-last}}$, area under the concentration-time curve from time 0 to last quantifiable concentration; $AUC_{0\text{-inf}}$, AUC from time 0 to infinity; CL, clearance; C_{max}, maximum concentration; CV, coefficient of variation; GeoCV, geometric mean coefficient of variation; GeoMean, geometric mean; h, hour; *N*, sample size; SD, standard deviation; t_{max}, time to reach C_{max}; $t_{1/2}$, terminal half-life.

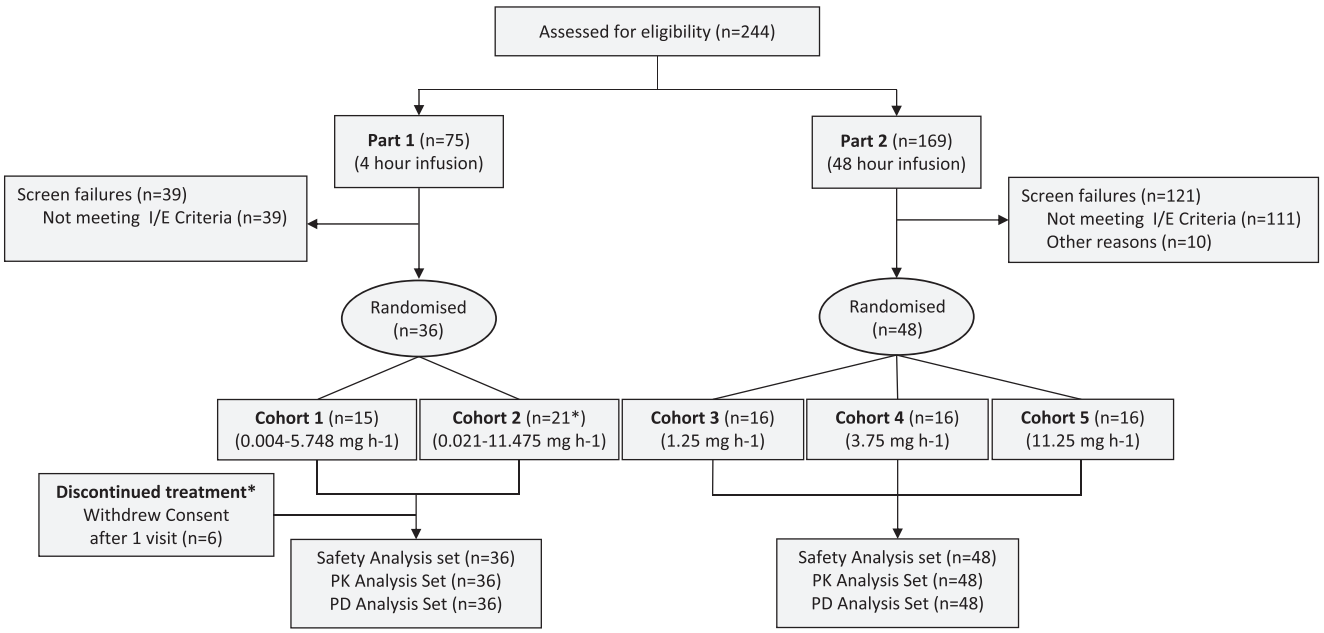

FIGURE 2 Study flow diagram. *Four initially randomised participants withdrew consent after one visit due to personal reasons. Replacement participants completed the remaining visits, of whom one withdrew consent after one treatment period and a second replacement participant was used. In addition, this replacement participant withdrew consent after one treatment period and a third replacement participant was used

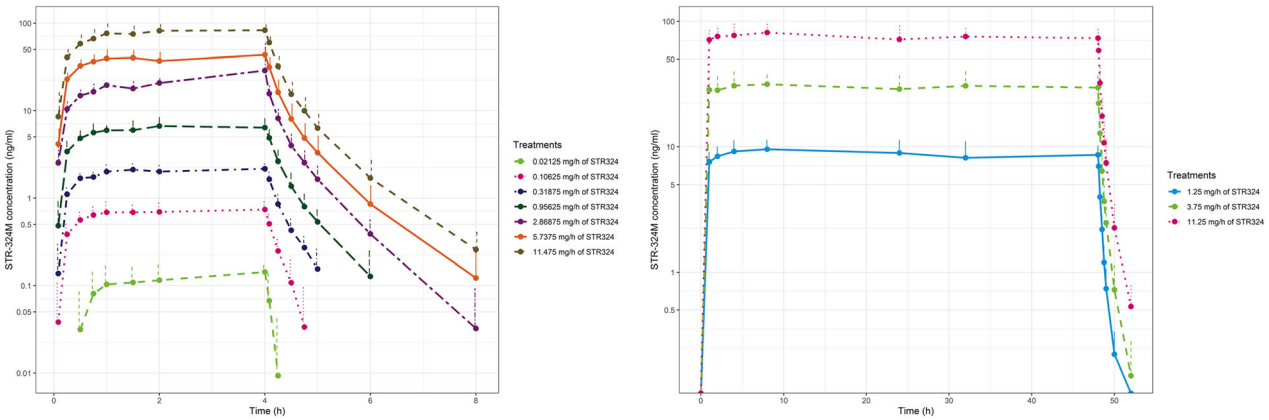

FIGURE 3 Means with standard deviations of concentration-time profiles of STR-324M in plasma, per treatment (part 1 left, part 2 right). The last point in the graph reflects the last measurable concentration after discontinuation of the infusion

pain modulation response in both study parts. Results of the nociceptive tests are shown in the Supporting Information.

Of the other pharmacodynamic results measured, the following effects are noteworthy: a significant decrease in pupil/iris ratio (miosis) was observed during the 48 h infusions at all three doses in part 2 for both the right and left eyes (overall treatment effect $P < .05$) when compared to placebo. The largest decrease was measured at the lowest dose in both the right (−0.04272 [95% confidence interval [CI] −0.06099, −0.02444, $P < .05$]) and left eyes (−0.04278 [95% CI −0.06544, −0.02012, $P < .05$]). Example changes from baseline graphs are shown in Figure 4. Compared to placebo, a significant increase in APN activity of 57.7% (95% CI 1.5-113.8, $P < .05$) was observed at the highest dose of STR-324 in part 2. The increase was observed during the first 24 hours of dosing only.

4 | DISCUSSION

In this study, we combined a typical first-in-human study design in healthy volunteers with extensive nociceptive and CNS testing, with the aim to not only determine the safety, tolerability and pharmacokinetic profile of STR-324 but to also obtain an impression of its analgesic and opioid-like (adverse) effects potential.

The presented safety data indicates that STR-324 has a favourable safety and tolerability profile in healthy males at all doses up to 11.475 mg h^{-1} for 48 h of infusion. Although some adverse effects were recorded as being possibly related to the study drug, the absence of dose-dependency and of evident differences with participants who received placebo reject any relatedness to STR-324. Although several participants prematurely discontinued participation, none withdrew consent as a result of events related to the study drug.

As expected from preclinical data, measuring plasma concentrations of STR-324 was challenging and limited its pharmacokinetic characterisation. STR-324M, its main metabolite, therefore served as a proxy of the plasma concentrations and exposure of STR-324. No significant, dose-dependent analgesic or other pharmacodynamic effects related to STR-324 could be observed in this study except for the paradoxical increase in APN at the higher dose in part 2.

Based on the sparse concentrations measured in this study, we can confirm that STR-324 is a short-lived compound that is quickly metabolised and distributes outside the blood compartment almost immediately after administration. This is in line with preclinical findings, where the parent compound provided analgesia only during a short period of time when given by bolus opiorphin[23] and proved highly unstable after administration by intravenous infusion. This instability hampered the pharmacokinetic characterization of STR-324 in animals, in contrast to its metabolite. In this study, PK profiles of STR-324 and its main, inactive metabolite were characterised in plasma and urine. We anticipated the challenge of measuring plasma concentrations of STR-324 by using the metabolite as a proxy to get an impression of the PK profile of the parent compound and to support dose escalations. The metabolite could be consistently measured throughout the infusion periods and dose proportionality was observed. Similarity between profiles was confirmed by visual inspection of available individual plots of the parent. Therefore, the notion that the PK of STR-324 could be monitored appropriately and also showed dose proportionality (albeit only briefly available in plasma) was assumed to be justified.

Several DENKIs are currently being developed for the treatment of pain. This is the first published report of a study that aimed to demonstrate the analgesic effects of a DENKI with use of evoked pain models in humans. In response to a painful stimulus, enkephalins are released into the synaptic cleft and interact with opioid receptors for

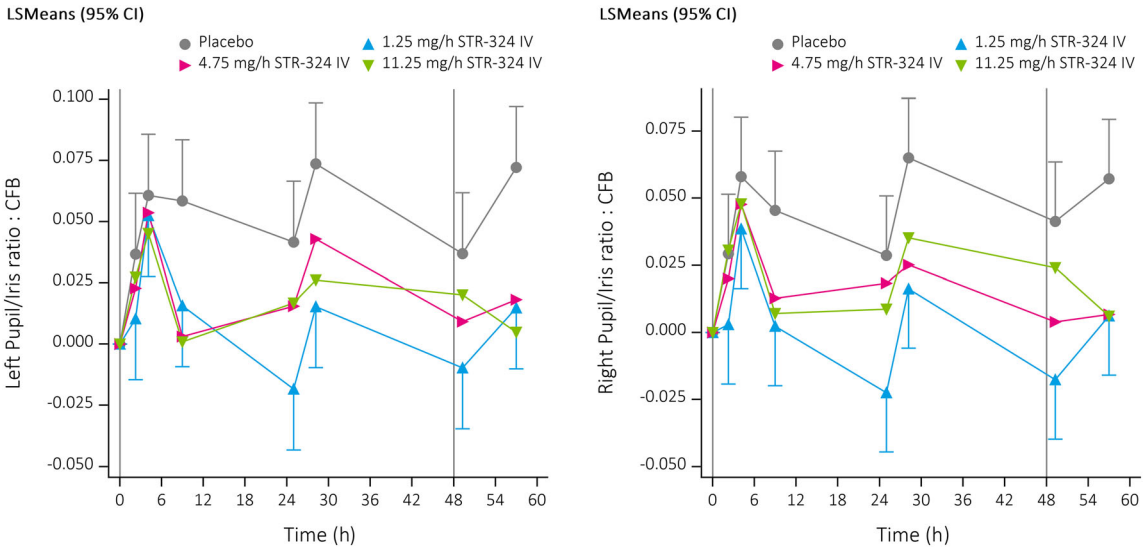

FIGURE 4 Least square means (LSmeans) change from baseline (CFB) in pupil/iris ratio of the right eye in part 2. The 95% confidence interval (CI) is shown for the outer lines in each graph. Vertical grey lines indicate the start and stop of 48 h infusion

a brief period before being rapidly metabolised, which results in a short half-life of approximately 12 minutes.[5,24,25] The effect of DENKIs can be expected to occur shortly after a painful stimulus is applied, as it will only be active as long as enkephalins are released. As no dose-dependent effects were observed in any of the evoked pain tests performed, the observed effects are unlikely to be due to the pharmacological effects of the compound and are most likely due to chance. No alpha correction for multiple testing was performed in this study. In preclinical studies, STR-324 showed strong analgesic activity in murine models simulating postoperative pain and neuropathic pain, but also without dose dependency. The highest dose level in this study was approximately 20-fold higher than the human equivalent of the lowest pharmacological active dose in murine models, suggesting that the lack of observed effect is not due to insufficient exposure. It is important to note that the parent compound could also not be measured in animal models, and the comparison is therefore based on allometric scaling and not on actual pharmacokinetic data. Because of the short action of enkephalins, a longer time of exposure to the study drug at the dose levels explored in this study is not expected to yield different results.

The fact that no consistent analgesic effect was observed in this proof-of-concept study might be due to limited involvement of enkephalinases in the acute nociceptive pain models that were used. Possibly, an effect of a DENKI only becomes clinically measurable after acute or prolonged exposure to a nociceptive stimulus or can be best demonstrated in evoked pain models simulating neuropathic pain. It could also be hypothesised that pain intensity may modulate the quantity of released enkephalins, thus modulating the extent of efficacy of DENKIs; human-evoked pain models possibly do not allow sufficient pain intensity to be reached to model real-world painful stimuli that activate a measurable enkephalin response.

Decrease of the pupil/iris ratio (miosis) is a well-known opioidergic effect. In this study a statistically significant treatment effect was observed compared to placebo, although the effect was greatest at the lowest dose. The treatment effect is the result of an increase in the pupil/iris ratio (mydriasis) in the placebo group, while the ratio remained quite stable in all three (active) treatment groups during infusion. The observed decrease in pupil/iris ratio in this study (<10% ratio relative reduction) was minor compared to the miosis induced by opioids such as buprenorphine that show a decrease in ratio of up to 50%.[26] Although it is possible that the relative static pupils were a pharmacodynamic effect of the compound, the observed treatment effect was most likely a result of unexpected placebo group behaviour, not a true opioidergic effect. An increase in pupil size (mydriasis) could hypothetically be caused by other factors, such as nociceptive tasks or circadian rhythm. However, pupillometry measurements were not (immediately) preceded by nociceptive tests.

Several parameters of the CNS test battery showed a statistically significant effect. Although pharmacological effects cannot be excluded, this is unlikely due to inconsistency of the effects regarding time of measurement and dose level.

We hypothesised that, given the expected mode of action and kinetics of the enkephalin degradation, administration of STR-324 would lead to a decrease in activity of APN and BigET-1 during and shortly after infusion. However, no changes in BigET-1 were observed and an unexpected increase in activity of APN at the highest tested dose was measured. It is possible that STR-324-induced modification of the levels of APN and NEP may be only measured locally instead of systemically, and should therefore be measured in a different compartment than blood. Although the pharmacokinetic profile in this study supports the hypothesis that STR-324 remains in the plasma in its active form at least temporarily, preclinical data demonstrated that STR-324 and opiorphin distribute to other compartments within seconds after administration, which may also explain the absence of measurable clinical changes related to other biological interactions that APN and NEP have. Alternatively, variability might have been too large with respect to a limited effect size. It is important to emphasise the exploratory nature of these biomarkers and the (pre)clinically unconfirmed relation with the mode of action of STR-324. In addition, the analytical methods were qualified but not validated thus there is no absolute certainty regarding the accuracy of the obtained results for these exploratory biomarkers.

Because intrasubject variability of evoked pain models is lower than intersubject variability, a partial crossover design was used where each participant could serve as his own control while keeping the ascending dose fashion of a typical first-in-human study. This approach benefits the efficient assessment of the effects of a novel compound despite limitations on study size. Although the number of included participants is common for phase I first-in-human studies and despite the innovative study design, smaller analgesic effects possibly remain undetected due to group sizes. Given that only male participants were included in this trial, this limits generalisability.

Finally, the evoked pain models that were included can profile a drug to a certain extent, but not all types of pain were evaluated. A phase IIa proof-of-concept study on postoperative pain to confirm the analgesic potential of STR-324 is ongoing.

In conclusion, this study suggests that STR-324 was observed to be generally well tolerated at all dose levels and infusion regimens tested in this healthy male population. Pharmacokinetic profiling of STR-324 was limited. However, dose proportionality can be assumed based on metabolite data. No clear dose-dependent effects were observed on the nociceptive thresholds or on tests related to CNS or bowel functioning.

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CONTRIBUTORS

Substantial contributions to the conception or design of the work, the acquisition, analysis, and/or interpretation of data for the work: all authors. Drafting the work or revising it critically for important

intellectual content: all authors. Final approval of the version to be published: all authors. Agreement to be accountable for all aspects of the work: all authors.

AUTHOR DISCLOSURES

L.M.M., C.L.B., E.M.J.vB., I.M.C.K., E.K., K.B. and G.J.G.: No conflict of interest to report. V.V., V.J.-P. and A.C.B. are employed by Stragen.

DATA AVAILABILITY STATEMENT

The authors will not make data collected for the study available to others, including individual participant data and a data dictionary defining each field in the set.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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