Translating preclinical insights into early psychopharmacology trials: application of the IB-Derisk analyser tool

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CHAPTER III

ADMINISTRATION OF OXATHRIDINE, A FIRST-IN-CLASS HISTAMINE-3 RECEPTOR PARTIAL AGONIST IN HEALTHY MALE VOLUNTEERS: CENTRAL NERVOUS SYSTEM DEPRESSION AND PSEUDO-HALLUCINATIONS

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British Journal of Clinical Pharmacology (2023), 90(1), 321–335.
https://doi.org/10.1111/bcp.15910

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ABSTRACT

AIMS To characterise the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of single ascending doses of oxathridine, a first-in-class histamine-3 receptor partial agonist, in healthy male volunteers.

METHODS A randomised, double-blind, placebo-controlled study including the NeuroCart, consisting of a battery of drug sensitive neurophysiological tests, was performed. Oxathridine was administered orally as an aqueous solution. After dosing, safety and NeuroCart tests (adaptive tracking [AT], body sway [BS], saccadic peak velocity [SPV], smooth pursuit [SP] eye movements, VAS according to Bond and Lader, VAS according to Bowdle [VAS B&L, Bowdle], pharmaco-electroencephalogram [PEEG], Sustained Attention to Response Task [SART]) were performed at set times.

RESULTS Forty volunteers completed the study. Given doses were: 0.5, 2.5, 5, 0.25 and 1.5 mg. At 5 mg, unacceptable and unanticipated adverse events (AEs) of (orthostatic) hypotension and pseudo-hallucinations were reported. Statistically significant effects ([CI]; p-value) of 2.5 mg and 5 mg oxathridine were observed on AT ([-8.28, -1.60]; p=0.0048), ([-8.10, -1.51]; p=0.00530), BS ([0.6, 80.2]; p=0.0455), ([5.9, 93.1]; p=0.0205) and SPV ([-59.0, -15.9]; p=0.0011), ([-43.9, -1.09]; p=0.0399), respectively. Oxathridine 5 mg significantly increased all three VAS Bowdle subscale scores; VAS external ([0.183, 0.476]; p=<.0001), VAS internal ([0.127, 0.370]; p=0.0001) and VAS feeling high ([0.263, 0.887]; p=0.0006).

CONCLUSIONS NeuroCart tests indicated central nervous system (CNS) depressant effects. Oxathridine also unexpectedly caused pseudohallucinations. Although this led to the decision to stop further development of oxathridine, these observations suggest that the H3R system could be an interesting new target for the development of novel antipsychotics.

INTRODUCTION

Since its discovery in 1983, the histamine-3 receptor (H3R), has been a target of interest for central nervous system (CNS) drug development.[2-4] The H3R is an autoreceptor modulating histamine synthesis and release.[2,3] It also functions as a heteroreceptor regulating the release of important other neurotransmitters, such as serotonin, acetylcholine, noradrenaline and dopamine.[4] H3Rs are primarily expressed in the central nervous system (CNS) in brain regions associated with cognition, pain, sleep and homeostatic regulation, such as the cerebral cortex, hippocampal formation, basal ganglia and hypothalamus.[4] Although there have been several studies with investigational compounds targeted at the H3R, such as cipralisant, to date pitolisant, an H3R antagonist/inverse agonist, is the only drug targeted at the H3R that the EMA and FDA have approved.[5,6] Pitolisant is registered for treating excessive daytime sleepiness and cataplexy in adults with narcolepsy and to improve wakefulness and reduce excessive daytime sleepiness in adults with obstructive sleep apnoea (OSA).[5,7]

Oxathridine or 4-(1*H*-imidazol-4-ylmethyl)-pyridine sesquioxalate, is a highly selective partial agonist of the H3R with high potency (EC50=1.5nM) and intrinsic activity (0.7). Oxathridine behaves as a full agonist *in vivo*, inhibiting brain histaminergic neuron activity at low oral doses, and is therefore regarded as first-in-class. Preclinical studies demonstrated that oxathridine easily crosses the blood brain barrier (BBB) (data on file, supplementary material). In animal models of sleeping disorders, oxathridine had sleep promoting effects, without overt sedative reactions or anxiolytic properties as observed with GABAergic compounds. Preclinical safety experiments demonstrated a favourable effect profile. At high dose levels, undesired effects were observed starting with reduced arterial blood pressure and at higher doses piloerection and increased reactivity to touch and at the highest given doses initial decreased activity followed by increased activity, increased reactivity to touch and stereotypies. It was further noticed that at relatively low exposure levels monkeys demonstrated a change in behaviour with accepting and looking for human contact. The preclinical data supported further development of oxathridine, and a first in human (FIH) study was set up.

The starting dose for the FIH study was set at 0.5 mg (0.007 mg/kg for a 70 kg individual), which was more than 70 times lower than the no observed adverse effect level (NOAEL) in the most sensitive species (dogs) (data on file). This NOAEL was based on a telemetry study which demonstrated increased heart rate and decreased arterial blood pressure at a dose of 1 mg/kg with a maximum exposure (C_{max}) of 609 ng/mL/h, corresponding to a human equivalent dose (HED) of 0.54 mg/kg. Although a minimum anticipated biological effect level (MABEL) or pharmacologically active dose (PAD) was not formally established, at HED values of 0.024 mg/kg brain

histamine neuron activity in mice was significantly reduced as measured by decreased levels of the main metabolite of histamine, Nτ-methylhistamine (data on file). In cats, desirable effects on sleep were observed from C_{max} values of 30 ng/mL/h and higher. Therefore, pharmacological activity could be expected from HED values of 0.024 mg/kg (1.68 mg for a 70 kg individual) and C_{max} values of 30 ng/mL/h. A dose range was selected, which was expected to show significant pharmacological and functional effects, and to explore the large safety window that was also observed preclinically.

In addition to assessing tolerability, safety and pharmacokinetics in this FIH study, pharmacodynamics were explored using the NeuroCart.[8] The NeuroCart consists of a battery of drug-sensitive neurophysiological tests and has been applied to a broad spectrum of CNS active drugs, making it possible to compare the effect profile of a novel compound on the different NeuroCart tests, to known profiles of other compounds.[8] By doing so, the pharmacological characteristics of a novel compound can be mapped and held against predictions based on preclinical data in an early phase of clinical drug development.[8] Oxathridine was the first partial H3R agonist to be tested on the NeuroCart, so it was not yet known which NeuroCart test would be sensitive to oxathridine. Therefore NeuroCart tests sensitive to the sedative effects of GABAergic agonists were selected, such as visual analogue scales, saccadic eye movement measurements and adaptive tracking.[9-11]

Overall, this FIH study aimed to assess the pharmacokinetics, safety, tolerability and pharmacodynamic effects of single ascending doses of oxathridine in healthy male volunteers.

METHODS

GENERAL

The study was performed according to ICH GCP guidelines as laid down in the Declaration of Helsinki and its latest amendments. The Stichting Beoordeling Ethiek Biomedisch Onderzoek (BEBO), Assen, the Netherlands approved the study, and the study was registered at ToetsingOnline under number NL44541.056.13. Bioprojet Pharma sponsored the study, and the study was conducted at the Centre for Human Drug Research (CHDR), Leiden, the Netherlands. All volunteers gave written informed consent prior to the study start.

STUDY POPULATION

Healthy male volunteers between 18 and 45 years of age at screening were included. Health status was assessed by medical history, laboratory assessments and physical examination. Volunteers with a history or clinical evidence of alcohol or drug abuse within the 3 years prior to screening were excluded. Volunteers were not allowed to use any prescribed medications or over-the-counter medications within two weeks prior to the first study drug administration except for paracetamol (maximum 1g/day).

STUDY DESIGN

This was a randomised, double-blind, placebo-controlled, single ascending dose study. The study consisted of five cohorts of eight volunteers each (active/placebo ratio: 6:2). Before escalating to the next dose level, a blinded interim safety review, consisting of safety data (adverse events (AEs), ECGs, laboratory tests, vital signs), pharmacodynamic (PD) data and pharmacokinetic (PK) data of the first 24 hours after dosing was performed. In the first group, a sentinel scheme was used: two volunteers were dosed on the first day (active/placebo ratio: 1:1) and on the second day, the remaining six volunteers were dosed.

The study consisted of an inpatient study visit, a medical screening within three weeks prior to admission and a follow-up visit a week after discharge from the clinical unit. At check-in of the study visit an eligibility check consisting of concomitant medication use and AE review, body weight and height measurement, urine drug screening, alcohol breath test, 12-lead-ECG, vital signs and body temperature measurement was performed. During the dosing day (Day 1), volunteers were dosed in the morning, and throughout the day, safety, PK and PD measurements were performed at set times. Volunteers were discharged about 24 to 27 hours after dosing (Day 2).

TREATMENTS

Treatments consisted of 25 ml oxathridine solution or a matching placebo for oral administration. The solution was administered with purified water and blackcurrant syrup to a volume of 100 mL for masking purposes. Planned dose levels were 0.5, 2.5, 10, 25, 40, 60, 80 and 100 mg. Volunteers were in a fasted state from 10 hours prior to dosing and were allowed to eat from three hours after dosing. Volunteers were allowed to drink water ad libitum, except for one hour before and two hours after dosing when drinking water was not allowed.

PHARMACOKINETIC ASSESSMENTS

Blood samples for PK measurements were collected prior to dosing (1 sample) and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8, 12 and 24 hours after dosing. Serum oxathridine concentrations were measured using the validated analytical UPLC/MS-MS method in the Bioprojet Biotech Laboratory in accordance with the guideline EMEA/CMPH/EWP/192217/2009 Rev. 1 and the rules of Good Laboratory Practice. The lower limit of quantification (LLOQ) was 0.1 ng/mL. At the lower limit of quantification, the intraassay coefficient of variation (CV) was 10.8 % and the interassay CV was 12.4 %, respectively.

TOLERABILITY AND SAFETY ASSESSMENTS

After dosing, the following safety assessments were performed at set times throughout the study day: vital signs, physical examination, laboratory tests consisting of biochemistry, haematology and urinalysis and 12-lead ECG measurements.

PHARMACODYNAMIC ASSESSMENTS

NeuroCart measurements consisted of adaptive tracking, body sway, saccadic eye movements, smooth pursuit eye movements, VAS according to Bond and Lader, VAS according to Bowdle, and pharmaco-electroencephalogram (PEEG) and the Sustained Attention to Response Task (SART). The measurements were performed twice prior to dosing and were then repeated at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 22 hours after dosing. In addition, the Visual Verbal Learning Test (VVLT) was performed 3 hours after dosing (immediate recall) and 6 hours after dosing (recognition and delayed recall).

As part of the screening visit, volunteers underwent a NeuroCart test training to prevent learning effects during study conduct. During all test sessions, there was only one volunteer in each room, illumination settings were standardised between the rooms. When performing the tests, volunteers were comfortably seated behind a computer screen, except during body sway measurements, when they were standing.

ADAPTIVE TRACKING

The adaptive tracking test was performed as described initially by Borland and Nicholson,[12] using customised equipment and software (based on TrackerUSB hard-/software (Hobbs, 2004, Hertfordshire, UK)). During the test, a dot inside a circle is randomly moving on a screen. Volunteers are instructed to try to keep the dot inside the circle by operating a joystick. If the volunteer succeeds in this task, the speed of the moving circle increases, conversely, the speed of the circle decreases if the volunteer fails at the task. The outcome of the test is the average speed of the moving circle as a percentage of the maximum speed of the circle over a 3.5-minute period.

BODY SWAY

Body sway is a measure of postural stability, during measurements, volunteers are instructed to stand as still as possible with closed eyes. Body sway was performed as previously described by others.[13,14] The anteroposterior body sway was measured using a body sway meter based on the Wright ataxiameter.[15] All body movements over a 2-minute period were integrated and expressed as millimetres of sway and recorded.

SACCADIC EYE MOVEMENTS

The primary outcome of saccadic eye movement measurements was saccadic peak velocity (SPV) in degrees per second (deg/s). During the test, volunteers were instructed to follow a dot jumping approximately 15 degrees to either side on a computer screen with their eyes, while head movements were restrained using a fixed head support at 58 cm from the computer screen. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Saccadic eye movements were recorded using a computer-based system, customised Cambridge Electronics Design software for data sampling and analysis (Cambridge Electronics Design, Cambridge UK), and disposable surface electrodes for registration of the electro-oculographic signals (Medicotest N-00-S, Olstykke, Denmark).

SMOOTH PURSUIT EYE MOVEMENTS

For smooth pursuit eye movements, the target moves sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5 degrees eyeball rotation to both sides. Four cycles for each stimulus frequency were recorded. The time during which the eyes are in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as a parameter.

PHARMACO EEG (PEEG)

Each EEG measurement duration was two minutes. EEG recordings were made using four gold electrodes, fixed with EC2 paste at Fz, Cz, Pz and Oz, with the common ground electrode for the eye movement registration (international 10/20 system). The electrode resistance was kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements. The signals were amplified with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using customised CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artefacts were identified by visual inspection and excluded from the analysis. For each lead, a fast Fourier transform analysis was performed to obtain the sum of the amplitudes in the very low (0.5-2 Hz), delta- (2-4 Hz), theta (4-7.5 Hz), alpha (7.5-13.5Hz), beta (13.5-35 Hz) and gamma (35-48.9 Hz) frequency ranges.

VAS ACCORDING TO BOND AND LADER AND VAS BOWDLE

VAS in this study were used as originally described by Norris.[16] We used Dutch versions of the scales that have been frequently used at our research institute.[8] For VAS Bond and Lader, volunteers, indicate (with vertical marks) on sixteen horizontal

100-mm VAS how they feel. From these measurements, three main factors were calculated; subjective alertness' (from nine scores), 'contentedness or mood' (from five scores) and 'calmness' (from two scores).[17]

VAS Bowdle evaluates psychedelic effects with thirteen 10 cm VAS lines ranging from 0 (not at all) to 100 mm (extremely).[18] From these scores, three sum scores were calculated; 'internal perception' (reflects inner feelings that do not correspond with reality, including mistrustful feelings), 'external perception' (reflects a misperception of an external stimulus or a change in the awareness of the volunteer's surroundings) and 'feeling high.'[18]

VISUAL VERBAL LEARNING TEST

The VVLT tests the whole scope of learning behaviour, i.e. acquisition, consolidation, storage and retrieval.[19] Volunteers were presented with 30 words in three consecutive word trials. At each of these trials, an immediate recall was performed. Delayed recall was assessed six hours after dosing. Immediately after delayed recall, a recognition test was performed, consisting of 15 previously presented words and 15 new words in which the volunteer had to verbally indicate recognition of the word as quickly as possible. An operator behind a computer screen recorded the volunteer's response by clicking on the named word in a list with all 30 presented words and clicking on a bar stating 'different word' if an unlisted word was mentioned. Words mentioned twice or more were recorded as duplications. After three trials, a script automatically counted the scores per trial as: number correct, number incorrect, number double. If a correct word was mentioned twice, the overall score included 1 correct response and 1 double response. Similarly, an incorrect word, which was mentioned twice, was scored as 1 incorrect response and 1 double response

SUSTAINED ATTENTION TO RESPONSE TASK (SART)

This test was performed as a measurement of improved cognitive functioning, as H3R antagonists are hypothesised to have beneficial effects on cognition.[4] The SART is similar in many respects to a standard vigilance task, in that a single infrequent target is presented amongst a background of frequent non-targets. Unlike a traditional vigilance task, however, the volunteer is required to push the space bar to the non-target and inhibit their response to the target. To perform this task correctly, the volunteer must remain sufficiently attentive to their responses, such that at the appearance of a target they can substitute the directly antagonistic response.[20]

STATISTICAL ANALYSIS

Statistical analyses were performed using the SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA). The repeatedly measured PD endpoints were analysed separately by mixed model analyses of covariance (ANCOVA) with treatment-and-dose, time, and treatment-and-dose by time as fixed effects, subject as random effect, and with the (average) baseline value as covariate. Baseline was defined as the average of the two measurements performed prior to dosing. Measurements of VAS Bowdle, all EEG parameters, body sway and SART total omission errors were logarithmically transformed (after 0 was changed to 0.01). Log-transformed parameters were back-transformed after analysis where the results may be interpreted as percentage change.

Contrasts for all dose levels of oxathridine versus placebo were calculated within the model up to the 3-hour measurement after dosing. This timepoint was based on post hoc inspection of the time profiles of the concentrations in this clinical study, showing that for all doses a 3-hour period covered most of the exposure. Assuming that the PD effects would be closely related to the PK profile, this analysis was considered most relevant.

The VVLT endpoints were analysed separately by mixed model analyses of variance (ANOVA) with treatment-and-dose as fixed effect. Treatment effects are reported as the contrasts specified below where the average of the measurements up to last time point will be calculated within the statistical model.

For all NeuroCart tests contrasts are reported along with 95% confidence intervals and analyses are two-sided with a significance level of 0.05.

PK calculations were performed using R (v2. 12.0, R Core Team). Standard non-compartmental methods were used in the calculations. Data below the limit of quantification before t_{max} was replaced with zero. Data below the limit of quantification after t_{max} was replaced with not applicable, i.e., excluded from analysis.

RESULTS

SUBJECT DISPOSITION

In total 40 healthy male volunteers participated; all completed the study. The cohorts were comparable with regards to age, weight, height, and BMI (Table 1).

PHARMACOKINETICS

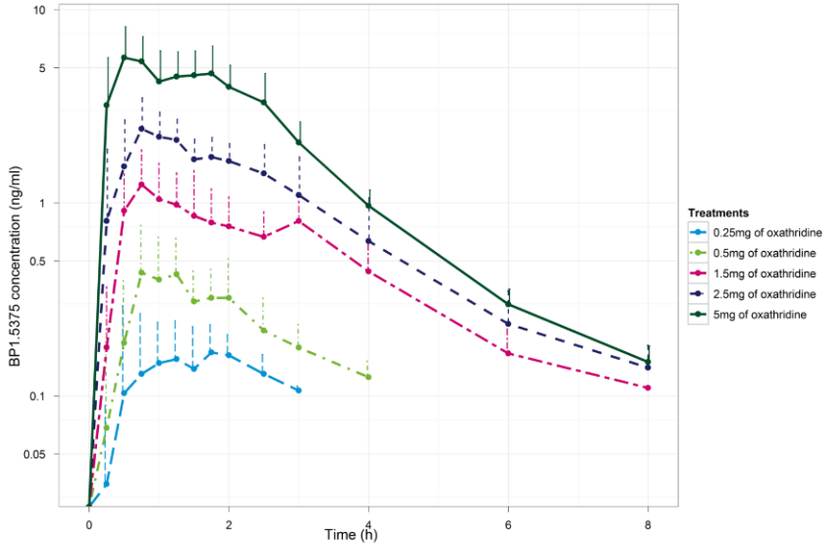
Maximum median serum concentrations were reached at about 1.00 to 1.26 hours after dosing across the dose levels (Table 2, Figure 1). The median half-life of oxathridine varied between 1.20 hours and 1.45 hours across the dose levels. Exposure to oxathridine increased more than dose proportionally in the higher dose range.

Table 1 Demographics

Demographic variables	Placebo N=10	Oxa 0.25 mg N=6	Oxa 0.5 mg N=6	Oxa 1.5 mg N=6	Oxa 2.5 mg N=6	Oxa 5 mg N=6
AGE, YEARS						
Mean	23.7	21.0	22.5	24.0	23.3	22.8
SD	3.9	3.1	2.6	4.6	3.9	2.4
Min, max	20-32	18-25	18-26	19-31	19-28	20-26
HEIGHT (CM)						
Mean	183.6	181.1	182.7	183.8	186.0	186.8
SD	5.0	9.2	6.7	8.7	11.2	4.0
Min, max	175.8 – 192.3	171.8 – 195.0	175.9 – 191.4	170.0 – 191.7	174.4 – 204.2	181.6 – 191.1
WEIGHT (KG)						
Mean	77.7	73.1	79.4	71.9	73.8	76.8
SD	8.4	10.9	11.3	9.0	11.1	5.6
Min, max	67.7 – 94.9	61.7 – 84.9	68.4 – 96.5	61.2 – 86.2	60.2 – 85.8	68.5 – 82.8
BMI (KG/M^2)						
Mean	23.1	22.4	23.7	21.3	21.2	22.0
SD	1.9	3.7	2.3	1.6	1.3	1.0
Min, max	19.9 – 26.8	18.3 – 27.2	21.5 – 26.8	19.6 – 24.1	19.8 – 22.9	20.8 – 23.0

Abbreviations: Oxa =oxathridine, SD = standard deviation, Min, max =minimum, maximum

Figure 1 Pharmacokinetics of oxathridine



Abbreviations: BP1.5375 = oxathridine

Table 2 Pharmacokinetics of oxathridine

Oxathridine													
	C_{max} (ng/mL)			AUC_{0-24} (ng/mL*h)			$AUC_{0-\infty}$(ng/mL*h)			T_{max} (h)		$T_{½}$ (h)	
Dose (mg)	Mean range	SD	Mean/ dose	Mean range	SD	Mean/ dose	Mean range	SD	Mean/ dose	Median	Range	Median	Range
0.25 (n=6)	0.22 (0.12; 0.47)	0.13	0.88	0.29 (0.04; 0.65)	0.23	1.16	0.57 (0.35; 0.79)	0.21	2.28	1.13	0.12 – 0.47	1.22	0.93 – 1.50
0.5 (n=6)	0.5 (0.23; 0.92)	0.28	1.00	0.92 (0.37; 1.78)	0.51	1.84	1.20 (0.64; 1.95)	0.46	2.40	1.26	0.75 – 1.78	1.45	0.94 – 2.33
1.5 (n=6)	1.4 (0.73; 1.81)	0.48	0.93	3.55 (2.42; 4.54)	0.77	2.37	3.83 (2.66; 4.76)	0.77	2.55	1.15	0.75 – 3.00	1.20	1.14 – 1.42
2.5 (n=6)	2.73 (1.22; 3.69)	0.87	1.09	6.54 (3.44; 9.63)	2.21	2.62	6.84 (3.69; 9.96)	2.24	2.74	1.12	0.75 – 2.00	1.29	0.83 – 1.47
5 (n=6)	7.12 (4.32; 10.05)	1.99	1.42	14.97 (12.81; 19.45)	2.33	2.99	15.26 (13.07; 19.79)	2.36	3.05	1.00	0.50 – 2.00	1.31	1.15 – 1.51

Abbreviations: C_{max}: maximum concentration, AUC: area under the curve, SD: standard deviations

TOLERABILITY AND SAFETY

An overview of AEs by dose group is provided in Table 3. All observed AEs were self-limiting. There were no serious AEs in the study. Doses could not be escalated as planned due to safety concerns about subjective effects. In cohort 1 (0.5 mg), one AE of somnolence was reported and classified as mild. As this dose was well tolerated and the next planned dose of 2.5 mg was still more than 10 times lower than the HED of the NOAEL and had a predicted C_{max} which was far below the exposure observed at the NOAEL in the most sensitive species, it was decided to escalate the dose to 2.5 mg as initially planned. In cohort 2 (2.5 mg), all volunteers who received oxathridine reported one or more AEs. The most frequently reported AEs were nausea, fatigue, somnolence, blurred vision, dizziness, and orthostatic hypotension. All these AEs were mild, whereas two AEs of balance disorder and dizziness, were reported as moderate. Because of these observations, the dose for the next cohort was escalated to 5 mg instead of the planned 10 mg.

At a dose of 5 mg, all volunteers who received oxathridine reported one or more adverse events. The most frequent events were dizziness and visual (pseudo)-hallucinations. For this study, it was chosen to use the term 'pseudo-hallucination' to describe the AEs experienced by the volunteers, as in contrast to 'typical hallucinations' the volunteers reported that they were aware that the perceived images were not real and that they could change the content of the visual phenomena.[21]The pseudo-hallucinations started on average 40 minutes after dosing and lasted 50 minutes up to two hours, in all volunteers, the effects disappeared completely without treatment and did not reoccur. One volunteer had orthostatic hypotension and fainted about 30 minutes after dosing and felt light-headed for the following three hours. Three single AEs of nausea, dizziness and syncope were reported as moderate, and the others were reported as mild.

The occurrence of these AEs led to the decision to halt further dose escalation. Two additional cohorts with low dose levels of 0.25 mg and 1.5 mg to better characterise the PD and PK of oxathridine were performed instead.

At a dose level of 0.25 mg, two of the volunteers receiving oxathridine reported AEs. AEs consisted of dizziness, somnolence, feeling of relaxation and paraesthesia, which all were of mild severity.

All volunteers receiving a dose of 1.5 mg oxathridine reported one or more AEs. The most frequent reported AEs were nausea, vision blurred, dizziness and hypotension. Four events of dizziness, two events of hypotension and one of orthostatic hypotension were reported as moderate, and the others were reported as mild. No pseudo-hallucinations were reported with these additional low doses.

As expected with AEs of dizziness, hypotension, and orthostatic hypotension, decreases in supine and standing blood pressure, mostly diastolic, were observed with dose levels of 1.5 mg oxathridine or higher (supplementary material). A compensatory increase in standing heart rate was observed.

There were no clinically relevant changes in laboratory assessments (clinical chemistry, haematology, and urinalysis). Also, there were no clinically relevant observations on any ECG parameters.

PHARMACODYNAMICS

A dose-dependent decrease in performance on adaptive tracking was observed, which was statistically significant for the two highest dose levels (Table 4). At these dose levels, a statistically significant increase in body sway was observed as well (Table 4). Additionally, a decrease in SPV and increased saccadic inaccuracy were observed with these dose levels (Table 4). In the highest dose group of 5 mg oxathridine, statistically significant effects were observed on all three VAS Bowdle subscales (VAS external, VAS internal and VAS feeling high) (Table 5, Figure 2. In the lowest dose levels (0.25 and 0.5 mg), oxathridine decreased the reaction time of correct responses of the delayed word recognition task of the VVLT (Table 5). No statistically significant effects were observed on the other NeuroCart measurements.

Table 3 AEs per dose group

System Organ Class / Preferred Term	Number (%) of subjects					
	Placebo n = 10	Cohort 4 Oxa 0.25 n = 6	Cohort 1 Oxa 0.5 n = 6	Cohort 5 Oxa 1.5 n = 6	Cohort 2 Oxa 2.5 n = 6	Cohort 3 Oxa 5.0 n = 6
Any event	2 (20)	2 (33)	1 (16.7)	6 (100)	6 (100)	6 (100)
EAR AND LABYRINTH DISORDERS						
Tinnitus					1 (16.7)	
EYE DISORDERS						
Asthenopia					1 (16.7)	1 (16.7)
GASTROINTESTINAL DISORDERS						
Nausea	1 (10.0)			3 (50.0)	2 (33.3)	2 (33.3)
Vomiting				1 (16.7)		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS						
Asthenia				1 (16.7)		
Fatigue	1 (10.0)				3 (50.0)	1 (16.7)
Feeling abnormal				1 (16.7)		1 (16.7)
Feeling of relaxation		1 (16.7)				
NERVOUS SYSTEMS DISORDERS						
Balance disorder					1 (16.7)	
Disturbance in attention				1 (16.7)		
Dizziness		1 (16.7)			1 (16.7)	
Dysarthria					1 (16.7)	
Headache	1 (10.0)			1 (16.7)	1 (16.7)	
Paraesthesia		1 (16.7)				
Somnolence	2 (20.0)	1 (16.7)	1 (16.7)	1 (16.7)	2 (33.3)	1 (16.7)
Tremor				1 (16.7)		
Vision blurred				2 (33.3)	2 (33.3)	2 (33.3)
PYCHIATRIC DISORDERS						
Disturbance in attention						1 (16.7)
Euphoric mood					1 (16.7)	2 (33.3)
Hallucination, visual						4 (66.7)
VASCULAR DISORDERS						
Dizziness		1 (16.7)		4 (66.7)	3 (50.0)	6 (100.0)
Flushing				1 (16.7)		
Hypotension				3 (50.0)		
Orthostatic hypotension				1 (16.7)	2 (33.3)	
Pallor				1 (16.7)		
Syncope						1 (16.7)

Abbreviations: Oxa: oxathridine

Table 4 PD effects

		Contrasts up to 3 hours Estimate of difference (confidence interval) p-value				
Parameter	**Treatment P-value**	**OXA-0.25 vs Placebo**	**OXA-0.5 vs Placebo**	**OXA-1.5 vs Placebo**	**OXA-2.5 vs Placebo**	**OXA-5 vs Placebo**
Adaptive tracking (%)	0.6422	-.873 (-4.16, 2.409) p=0.5939	-1.28 (-4.58, 2.017) p=0.4368	-2.51 (-5.83, 0.817) p=0.1357	-4.94 (-8.28, -1.60) p=0.0048	-4.81 (-8.10, -1.51) p=0.0053
Body sway (mm)	0.4571	-7.4% (-31.4%, 25.1%) p=0.6099	5.7% (-21.1%, 41.4%) p=0.7047	29.2% (-4.2%, 74.2%) p=0.0915	34.6% (0.6%, 80.2%) p=0.0455	43.0% (5.9%, 93.1%) p=0.0205
Saccadic inaccuracy (%)	0.1670	0.41 (-0.60, 1.42) p=0.4207	-0.19 (-1.20, 0.81) p=0.7043	0.63 (-0.54, 1.79) p=0.2877	2.00 (0.97, 3.04) p=0.0003	1.49 (0.47, 2.52) p=0.0050
Saccadic Peak Velocity (deg/s)	0.3217	3.07 (-18.3, 24.41) p=0.7733	-7.08 (-29.4, 15.25) p=0.5252	-10.8 (-33.8, 12.13) p=0.3487	-37.4 (-59.0, -15.9) p=0.0011	-22.5 (-43.9, -1.09) p=0.0399
Smooth Pursuit (%)	0.4627	2.29 (-2.23, 6.82) p=0.3113	-0.01 (-4.07, 4.04) p=0.9954	0.89 (-3.34, 5.12) p=0.6742	0.27 (-3.77, 4.31) p=0.8928	-0.77 (-4.99, 3.45) p=0.7135
VAS Alertness (mm)	0.1270	-1.89 (-8.73, 4.95) p=0.5812	2.44 (-4.36, 9.25) p=0.4738	-4.43 (-11.6, 2.77) p=0.2235	-0.70 (-8.04, 6.64) p=0.8482	-5.83 (-13.2, 1.50) p=0.1167
VAS Calmness (mm)	0.1938	6.80 (-0.37, 13.98) p=0.0626	-1.05 (-8.93, 6.84) p=0.7897	3.07 (-4.34, 10.49) p=0.4075	2.32 (-5.36, 10.00) p=0.5445	4.82 (-2.56, 12.19) p=0.1944
VAS Mood (mm)	0.2423	2.60 (-3.25, 8.46) p=0.3740	5.38 (-0.46, 11.21) p=0.0700	2.32 (-3.67, 8.31) p=0.4389	1.40 (-4.81, 7.61) p=0.6513	4.51 (-1.53, 10.55) p=0.1394
VAS External log(mm)	0.0031	-.020 (-.168, 0.128) p=0.7850	0.016 (-.127, 0.159) p=0.8213	0.096 (-.049, 0.241) p=0.1914	-.014 (-.156, 0.127) p=0.8376	0.330 (0.183, 0.476) p=<.0001
VAS Internal log(mm)	0.0226	-.006 (-.127, 0.116) p=0.9249	0.075 (-.044, 0.194) p=0.2084	0.087 (-.033, 0.207) p=0.1528	0.051 (-.064, 0.167) p=0.3751	0.249 (0.127, 0.370) p=0.0001
VAS feeling high log(mm)	0.0468	-.156 (-.471, 0.160) p=0.3249	0.049 (-.258, 0.355) p=0.7488	0.013 (-.295, 0.322) p=0.9310	0.014 (-.286, 0.314) p=0.9242	0.575 (0.263, 0.887) p=0.0006
EEG Alpha Fz-Cz (uV)	0.6320	-8.5% (-28.6%, 17.3%) p=0.4739	-14.8% (-33.4%, 9.2%) p=0.1996	0.1% (-23.5%, 31.0%) p=0.9934	-8.5% (-28.8%, 17.5%) p=0.4761	-2.2% (-24.2%, 26.1%) p=0.8581
EEG Alpha Pz-Oz (uV)	0.4700	-17.5% (-45.1%, 24.1%) p=0.3462	-35.2% (-56.4%, -3.6%) p=0.0331	-6.0% (-38.8%, 44.6%) p=0.7753	-26.9% (-51.3%, 9.6%) p=0.1255	-20.0% (-46.5%, 19.5%) p=0.2675
EEG Beta Fz-Cz (uV)	0.4216	10.0% (-14.3%, 41.2%) p=0.4424	-15.3% (-34.0%, 8.5%) p=0.1822	7.4% (-17.5%, 39.9%) p=0.5868	5.4% (-18.0%, 35.5%) p=0.6751	16.6% (-10.0%, 51.0%) p=0.2372
EEG Beta Pz-Oz (uV)	0.6551	-12.6% (-38.5%, 24.2%) p=0.4430	-29.4% (-50.1%, -0.0%) p=0.0498	-13.3% (-40.7%, 26.7%) p=0.4535	-14.2% (-39.8%, 22.3%) p=0.3873	-12.9% (-38.7%, 23.6%) p=0.4296
EEG Delta Fz-Cz (uV)	0.5773	-1.4% (-22.7%, 25.7%) p=0.9072	-10.0% (-29.3%, 14.5%) p=0.3807	13.7% (-12.1%, 47.1%) p=0.3206	-5.3% (-26.0%, 21.3%) p=0.6589	1.9% (-20.7%, 31.0%) p=0.8776
EEG Delta Pz-Oz (uV)	0.5890	-4.1% (-34.0%, 39.4%) p=0.8222	-27.6% (-50.1%, 4.9%) p=0.0858	1.6% (-31.7%, 51.1%) p=0.9366	14.0% (-21.9%, 66.4%) p=0.4887	-0.1% (-31.7%, 45.9%) p=0.9941
EEG Gamma Fz-Cz (uV)	0.6472	14.9% (-9.8%, 46.4%) p=0.2519	-10.6% (-29.8%, 13.7%) p=0.3500	5.8% (-17.8%, 36.2%) p=0.6523	6.6% (-16.5%, 36.2%) p=0.5979	9.0% (-15.2%, 40.2%) p=0.4906
EEG Gamma Pz-Oz (uV)	0.6894	2.7% (-31.3%, 53.6%) p=0.8938	-24.7% (-49.5%, 12.2%) p=0.1581	-2.8% (-36.7%, 49.3%) p=0.8945	8.2% (-27.8%, 62.2%) p=0.6946	14.6% (-24.2%, 73.2%) p=0.5092

		Contrasts up to 3 hours Estimate of difference (confidence interval) p-value				
Parameter	**Treatment P-value**	**OXA-0.25 vs Placebo**	**OXA-0.5 vs Placebo**	**OXA-1.5 vs Placebo**	**OXA-2.5 vs Placebo**	**OXA-5 vs Placebo**
EEG Theta Fz-Cz (uV)	0.6453	11.6% (-13.5%, 43.9%) p=0.3876	-11.9% (-31.6%, 13.4%) p=0.3157	11.9% (-14.3%, 46.2%) p=0.4007	5.4% (-18.3%, 36.1%) p=0.6777	10.5% (-15.3%, 44.0%) p=0.4517
EEG Theta Pz-Oz (uV)	0.5617	-13.2% (-41.5%, 28.6%) p=0.4689	-31.0% (-53.3%, 1.9%) p=0.0612	-4.3% (-36.7%, 44.9%) p=0.8332	10.9% (-25.2%, 64.3%) p=0.5978	-7.6% (-38.1%, 38.1%) p=0.6939
SART total commission errors	0.4500	-3.57 (-8.01, 0.88) p=0.1122	-0.46 (-4.64, 3.71) p=0.8228	-0.47 (-4.76, 3.82) p=0.8267	-3.68 (-8.45, 1.09) p=0.1261	-3.69 (-7.90, 0.52) p=0.0840
SART mean RT correct	0.9940	18.80 (-67.2, 104.8) p=0.6596	-0.97 (-82.2, 80.23) p=0.9807	6.87 (-77.3, 91.00) p=0.8692	53.86 (-38.4, 146.1) p=0.2434	11.66 (-70.8, 94.06) p=0.7755
SART total omission errors	0.2723	-71.9% (-95.3%, 68.9%) p=0.1609	-64.6% (-93.6%, 96.4%) p=0.2283	-7.7% (-84.1%, 436.5%) p=0.9277	-72.6% (-96.0%, 88.5%) p=0.1832	8.5% (-80.5%, 503.8%) p=0.9242
SART post error slowing	0.7256	-.052 (-.177, 0.072) p=0.4037	-.008 (-.122, 0.106) p=0.8897	-.077 (-.194, 0.040) p=0.1943	-.079 (-.212, 0.053) p=0.2332	-.038 (-.155, 0.079) p=0.5192
SART RT variability	0.4898	-8.87 (-19.1, 1.352) p=0.0869	-.664 (-10.3, 8.969) p=0.8895	4.566 (-5.27, 14.41) p=0.3532	-4.23 (-15.2, 6.787) p=0.4412	-3.83 (-13.7, 6.011) p=0.4351
SART total error score	0.6362	-6.87 (-15.5, 1.72) p=0.1135	-1.83 (-9.92, 6.25) p=0.6477	1.87 (-6.42, 10.15) p=0.6506	-6.07 (-15.4, 3.24) p=0.1941	-4.91 (-13.1, 3.26) p=0.2310

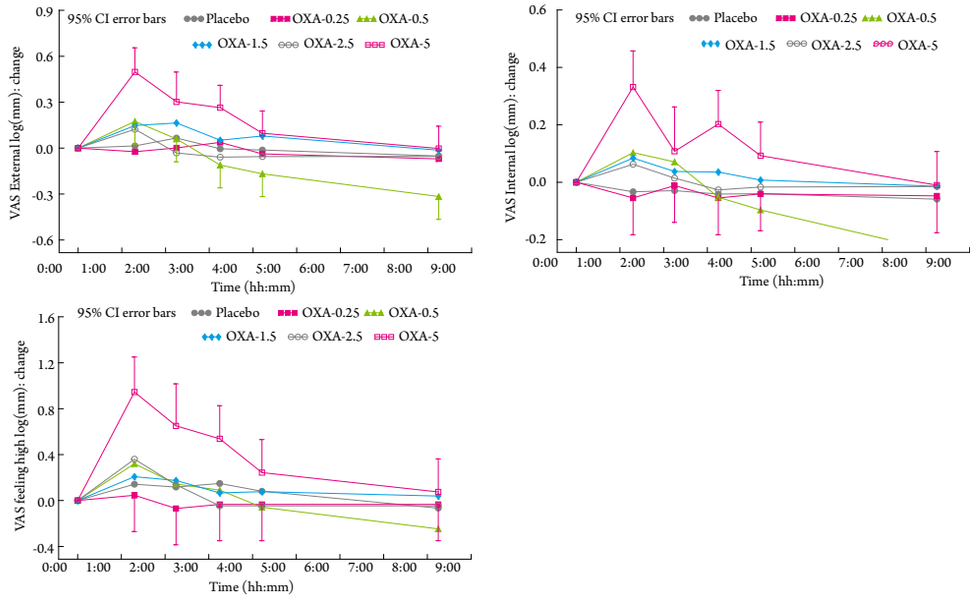
Abbreviations: EEG, electroencephalogram; Oxa, oxathridine; SART, Sustained Attention to Response Task; VAS, visual analoque scale.

Table 5 VVLT

		Contrasts all measurements				
Parameter	**Treatment P-value**	**OXA-0.25 vs Placebo**	**OXA-0.5 vs Placebo**	**OXA-1.5 vs Placebo**	**OXA-2.5 vs Placebo**	**OXA-5 vs Placebo**
Word recall correct 1	0.5126	-0.90 (-4.33, 2.53) p=0.5975	-0.73 (-4.17, 2.70) p=0.6668	-0.90 (-4.33, 2.53) p=0.5975	-1.57 (-5.00, 1.87) p=0.3601	-3.40 (-6.83, 0.03) p=0.0520
Word recall correct 2	0.6855	2.17 (-2.03, 6.37) p=0.3017	-0.50 (-4.70, 3.70) p=0.8102	1.00 (-3.20, 5.20) p=0.6315	-1.00 (-5.20, 3.20) p=0.6315	-1.17 (-5.37, 3.03) p=0.5760
Word recall correct 3	0.7095	2.53 (-2.48, 7.55) p=0.3117	-1.63 (-6.65, 3.38) p=0.5123	0.87 (-4.15, 5.88) p=0.7275	-0.63 (-5.65, 4.38) p=0.7989	-0.97 (-5.98, 4.05) p=0.6976
Delayed word recall correct	0.8847	1.93 (-3.62, 7.48) p=0.4837	0.10 (-5.45, 5.65) p=0.9710	0.77 (-4.78, 6.32) p=0.7806	-1.90 (-7.45, 3.65) p=0.4912	-0.23 (-5.78, 5.32) p=0.9324
Delayed word recognition correct	0.8967	0.33 (-2.74, 3.41) p=0.8268	-0.50 (-3.57, 2.57) p=0.7428	-0.83 (-3.91, 2.24) p=0.5850	0.83 (-2.24, 3.91) p=0.5850	-0.83 (-3.91, 2.24) p=0.5850
Delayed word recognition RT correct (msec)	0.0235	-102 (-202, -1.43) p=0.0470	-172 (-273, -71.6) p=0.0014	-24.6 (-125, 75.90) p=0.6224	-32.4 (-133, 68.07) p=0.5166	-30.1 (-131, 70.40) p=0.5471

Abbreviation: Oxa, oxathridine.

Figure 2 VAS Bowdle sum scores (internal, external, feeling high)



Abbreviations: Oxa = oxathridine, VAS = visual analoque scale

DISCUSSION

This FIH, randomised, double-blind, placebo-controlled study investigated the safety, pharmacokinetics, and pharmacodynamics of the first-in-class H_3 agonist oxathridine. As expected, the effects of 2.5 and 5 mg oxathridine on the NeuroCart tests of adaptive tracking, body sway and saccadic eye movements were indicative of CNS-depressant effects. However, the safety profile of oxathridine in healthy volunteers was different than expected based on the preclinical data, with mild to moderate orthostatic hypotension and pseudo-hallucinations at the two highest administered dose levels. This led to the decision to halt further dose escalation at 5 mg and to expand the dose range with 0.25 and 1.5 mg of oxathridine to characterise its safety, PK and PD profile more thoroughly.

In retrospect, the planned dose range of 0.5-100 mg was too large, especially when taking into account the pharmacologically active (PAD) and anticipated therapeutic doses (ATD) as recommended by current European Medicines Agency (EMA) guidelines.[1] Preclinical experiments demonstrated pharmacological effects from HED levels of 1.68 mg and C_{max} values of 30 ng/mL and higher. However, predictions of sleep-inducing doses were mainly based on cats, which is an unusual species in preclinical research. Moreover, the NOAEL was about 20-fold higher, and determined by cardiovascular effects in dogs which can be adequately monitored in humans. Since CNS-depression can also be measured accurately in healthy volunteers, a large dose range was selected for the FIH study.

The occurrence of pseudo-hallucinations was entirely unexpected. The time courses of the occurrence and resolving of pseudo-hallucinations and the increased scores on all three subscales of the VAS Bowdle (VAS internal, VAS external, VAS feeling high – Figure 2) closely followed the pharmacokinetic profile (Figure 1), providing a strong argument that pseudo-hallucinations are a pharmacological effect of oxathridine. In view of the high selectivity of oxathridine, this effect is likely related to stimulation of H_3R. However, to our knowledge there are no other reports of clinical studies with H_3R agonists, so without replication we cannot be absolutely certain that the pseudo-hallucinations are a class-effect. Due to their nature, pseudo-hallucinations could not be observed preclinically, although some non-specific behavioural symptoms such as increased reactivity to touch and monkeys accepting and looking for human contact, were observed in the animal studies, which retrospectively could have been indicative of cognitive, behavioural or perceptive dysregulations. These behavioural effects were observed at considerably higher exposure levels than for pseudo-hallucinations in humans. This could be due to different sensitivities, to detect spontaneous behavioural observations in animals, compared to subjective VAS-scores in humans. It could also be that humans are more sensitive to H_3R-agonism, since cardiovascular effects of (orthostatic) hypotension also occurred at lower exposure levels in healthy volunteers than observed preclinically, indicating that humans are more sensitive to the effects of H_3R agonists than animals.

The mechanism of action underlying pseudohallucinatory effects is uncertain. A stage of dreaming without being asleep might be an explanation. Pseudo-hallucinations have also been reported after use of drugs targeted at the GABA-ergic system, the main inhibitory neurotransmitter system in the brain.[22,23] In some healthy volunteers, but not all, zolpidem (a $GABA_A$ agonist with high α_1 subtype selectivity) caused concentration-related pseudo-hallucinations.[24,25] In the current study, all volunteers reported being able to change their visual pseudo-hallucinations intentionally. This is reminiscent of hypnagogic hallucinations which occur in narcolepsy.[26] This condition is characterised by a deficiency of orexin (hypocretin), an important neuropeptide for -among others- the regulation of the sleep-wake cycle, in which histamine plays a prominent role.[26] Within this system, H_3R pharmacology is complex and involved in the regulation of many other neurotransmitter systems such as serotoninergic (5HT), dopaminergic and cholinergic systems.[4] Several CNS-penetrating compounds affecting these systems, like antimuscarinics and 5HT2a- or D2-agonists, are well-known

psychomimetics, suggesting that the pseudo-hallucinations may have been caused by dysregulation of these systems by oxathridine. Abnormal H3R expression is observed in the brain of patients with schizophrenia, which further points in the direction of a role for the H3R in the occurrence of hallucinations.[27] It therefore seems that pharmacological interference on homeostatic brain processes of sleep can lead to a disruption of different aspects of sleep onset, leading to phenomena of pseudo-hallucinations.

From dose levels of 2.5 mg and higher, oxathridine demonstrated CNS-depressant effects on the NeuroCart. In Table 6 the NeuroCart effects of oxathridine are compared with several other sleep-promoting and (anti)histaminergic compounds: lorazepam ($GABA_A$-agonist), diphenhydramine (H1R antagonist and some antimuscarinic/anticholinergic action) and CEP-26401 (investigational H3R antagonist/inverse agonist).[9,28-30]

Table 6 Summary NeuroCart effects for different CNS active compounds

	Lorazepam 2 mg ($GABA_A$-agonist)[9,28]	Diphenhydramine 50 mg (H1R antagonist, with some antimuscarinic/ anticholinergic action)[29]	CEP-26401 125 ug (H3R antagonist/ inverse agonist)[30]	Oxathridine 5 mg (H3R partial agonist)
Adaptive tracking (%)	-9.53 (-11.9; -7.21) P<0.0001	-2.64 (-3.92; -1.13) P=0.0001	1.20 (0.42; 1.98) P=0.0029	-4.81 (-8.10, -1.51) P=0.0053
Body sway (%)	89.0 (62.8; 119.6) P<0.0001	12.25 (-2.35; 29.03) P=0.1021	-28.72 (-61.94; 4.51) P=0.0895	43.0 (5.9; 93.1) P=0.0205
Saccadic peak velocity (deg/s)	- 59.23 (- 46.05;-72.41) P<0.001	-13.8 (-21.7; -5.9) P=0.0010	16.99 (9.73; 24.24) P<0.0001	-22.5 (-43.9; -1.09) P=0.0399
Smooth pursuit (%)	-10.8 (-14.2; -7.3) P<0.0001	-0.5 (-3.1; 2.1) P=0.7149	-0.31 (-2.10; 1.48) P=0.7310	-0.77 (-4.99; 3.45) P=0.7135
pEEG	All frequencies of EEG bands were statistically significantly affected	Not reported	No effect	No effect
VAS Bond & Lader alertness (mm)	-1.80 (-3.52;-0.08) P0.041	-1.0 (-4.4; 2,3) P=0.5377	No effect	-5.83 (-13.2; 1.50) P=0.1167
VAS Bond & Lader calmness (mm)	-0.10 (-0.41; 0.22) P=0.529	1.1 (-1.0; 3.2) P=0.3066	No effect	4.82 (-2.56; 12.19) P=0.1944
VAS Bond & Lader mood (mm)	-0.24 (-0.96; 0.49) P=0.510	0.4 (-0.8; 1.7) P=0.5059	No effect	4.51 (-1.53; 10.55) P=0.1394
VAS Bowdle internal	0.07 (0.03; 0.11) P=0.0007	Not reported	No effect	0.249 (0.127; 0.370) P=0.0001
VAS Bowdle external	0.10 (0.05; 0.16) P=0.0004	Not reported	No effect	0.330 (0.183; 0.476) P<0.0001
VAS Bowdle Feeling high	0.12 (0.02; 0.22) P=0.0168	Not reported	No effect	0.575 (0.263; 0.887) P=0.0006
VVLT	Not reported	Not reported	No effect	Effect on word recall correct 1 -3.40 (-6.83; 0.03) P=0.0520

Abbreviations: pEEG, pharmaco-electroencephalogram; VAS, visual analogue scale; VVLT, visual verbal learning test

The effect size of oxathridine on adaptive tracking was approximately half the effect size of 2 mg lorazepam, and twice as large as the effect of diphenhydramine. On body sway oxathridine's effect was approximately half the effect size of 2 mg lorazepam and 4 times larger than the effect size of 50 mg diphenhydramine. The effect size of oxathridine on SPV was approximately three times smaller than the effect size of 2 mg lorazepam, but larger than the effect size of diphenhydramine. Comparable to diphenhydramine, oxathridine did not affect smooth pursuit, while lorazepam did. Overall, the NeuroCart profile of oxathridine is indicative of a CNS depressant compound with less sedative capacity than 2 mg lorazepam, but more than 50 mg diphenhydramine. Furthermore, in line with their mechanisms of action, the effects of oxathridine on the NeuroCart tests are opposite to the effects observed with the H3R-inverse agonist CEP-26401.[30] Compared with placebo, the groups with the two lowest doses of oxathridine showed lower reaction times in the delayed word recognition task of the VVLT. This might be indicative of improved cognitive functioning. However, no CNS stimulating effects were observed on the other NeuroCart tests, which in contrast to the VVLT are baseline corrected. Therefore, these effects may well have been false positive findings.

Although further development of oxathridine was ceased based on the findings of this study, important lessons can be learned from it. This study demonstrates how the NeuroCart can be used in early phase clinical trials to measure important pharmacological characteristics of novel CNS compounds and compare these to existing compounds.[8] In line with recommendations by the EMA this study demonstrates the importance of considering the predictions of MABEL, PAD and Anticipated Therapeutic Dose when designing a study, particularly for compounds with a new mechanism of action.[1] This study also shows that there is inherent uncertainty in the translation from preclinical to human studies. Although the preclinical data of oxathridine indicates an appropriate 'therapeutic window' with desired effects at lower dose levels than undesired effects, in humans there was no therapeutic window as dose levels with desired effects were associated with unacceptable AEs. This study emphasises that not all AEs are translatable between animals and humans, as some psychiatric AEs, such as hallucinations, cannot be observed in animals. However, non-specific changes in behaviour observed preclinically might indicate that animals experience disruptive cognitive or perceptive symptoms and, therefore, should make investigators aware of potential psychiatric symptoms in humans. A final intriguing lesson of the clearly drug-related and fully reversible pseudo-hallucinations in this study was, that the H3R system could be an interesting new target for the development of novel antipsychotics. Moreover, there is an increasing interest in the therapeutic effect of psychomimetic agents targeted at other receptor systems, such as psilocybin (5HT2a-partial agonist) and esketamine (NMDA-antagonist).[31,32] It could be speculated that H3R-antagonists could serve a similar role in psychomimetic assisted psychotherapies.

In conclusion, this study demonstrates how the pharmacodynamics of a novel compound can be investigated in early phase clinical trials. The findings of this study contribute to the field of knowledge about H3R pharmacology and delineate its complexity as already described by others.[33] This knowledge can be used for the future development of compounds targeted at the H3R, including potential antipsychotics or therapeutic psychomimetics.

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APPENDIX

IB-DERISK ANALYSER OVERVIEW OF OXATHRIDINE

Oxathridine or 4-(1H-imidazol-4-ylmethyl)-pyridine sesquioxalate is a first-in-class histamine-3 receptor (H3R) partial agonist, behaving as a full agonist *in vivo*. Since oxathridine was the first H3R agonist to be given to humans, the preclinical data was studied extensively before deciding to continue to a clinical study.

The data of the preclinical package was entered in the IB-Derisk analyser tool.[1] When sorting the preclinical data on C_{max} the compound demonstrated a favourable profile, with desired effects indicated by green at relatively low C_{max} values and occurrence of undesired effects starting at higher C_{max} values (Figure 1). In general, the of occurrence of adverse events (AEs) followed a predictable pattern with occurrence of relatively mild and transient AEs indicated in yellow at lower exposures and more severe AEs indicated in orange and severe AEs indicated in red at higher exposures (Figure 1).

Desired effects are indicated in green (Figure 1). In freely moving cats, oral administration of 1-3 mg/kg oxathridine, corresponding to C_{max} values between 30-150 ng/mL, statistically significant increased deep sleep, at the expense of arousal, without any significant effect on both light and REM sleep was observed. Additionally, oxathridine decreased the levels of the main metabolite of histamine, t-methylhistamine (t-MeHA), in the brains of mice at low oral doses of 0.3 mg/kg, corresponding to an interpolated C_{max} value of 116.5 ng/mL. Furthermore, at a dose of 0.3 mg/kg, corresponding to an interpolated C_{max} value of 591 ng/mL, oxathridine almost fully suppressed the deficit in sleep elicited by zolpidem withdrawal in rats.

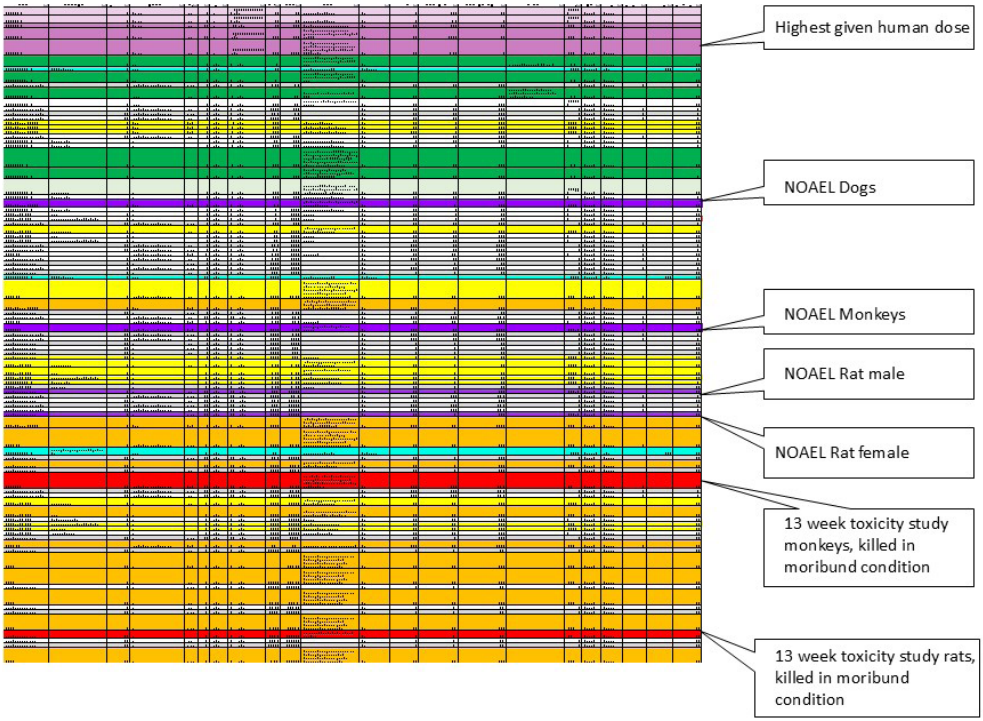
Dogs were the most sensitive species for oxathridine. The NOAEL in dogs was set at 1 mg/kg, corresponding to a C_{max} value of 609 ng/mL. At this dose a slightly reduced arterial blood pressure and slightly increased heart rate were observed. At dose levels below 1 mg/kg, some minor effects on arterial blood pressure and heart rate were observed in dogs as well, indicated in yellow in the IB-Derisk (Figure 1). At dose levels above the NOAEL, markedly increased arterial blood pressure and atrio-ventricular blocks of grade 2 and grade 3 were observed in dogs. For monkeys the NOAEL was set at 8 mg/kg, corresponding to a C_{max} of 2173 ng/mL, based on a 13-week toxicity study. At this dose, the principal clinical sign was a change of behaviour with monkeys accepting and looking for human contact. In rats the NOAEL was set at 15 mg/kg, corresponding to a C_{max} of 3440 ng/mL in males and 5385 ng/mL in females. At lower dose levels limited effects suggestive of respiratory stimulant and bronchodilatery properties, piloerection and increased reactivity to touch were observed.

The findings of the FIH study with oxathridine were entered in the IB-Derisk overview in the colour pink/purple (Figure 1). The starting dose for the FIH study of 0.5 mg was a factor 70 lower than the NOAEL in the most sensitive species (dogs). Based on the *in vivo* pharmacodynamic experiments, no pharmacological activity was expected at this dose level. In the first in human (FIH) study, this dose was well tolerated and therefore it was decided to continue to the next planned dose level of 2.5 mg. At this dose level, all volunteers reported one or more AEs, the most frequently reported being dizziness, hypotension, syncope and nausea.[2] In addition, decreases in supine and standing blood pressure were observed with a compensatory increase in heart rate. In animals slight increases in heart rate were only observed at an exposure level a factor 100 higher than the exposure associated with these effects in humans. For the next cohort, the dose was therefore escalated to 5 mg instead of the scheduled 10 mg. Even though the C_{max} value associated with 5 mg in humans was only 7.12 ng/mL, much lower than the C_{max} of approximately 273 ng/mL associated with slightly reduced arterial blood pressure in dogs (the most sensitive species), decreases in blood pressure with compensatory increases in heart rate were observed in the healthy volunteers. Furthermore, all volunteers reported the remarkable and unexpected AE of pseudo-hallucinations.[2] The volunteers reported that they were aware that the perceived images were not real and that they could change the content of the visual phenomena. Because of these AEs, it was decided to not escalate to higher dose levels, but to add two extra dose levels of 0.25 mg and 1.5 mg, to better characterise the pharmacodynamics and pharmacokinetics of oxathridine. Pharmacodynamic effects indicative of central nervous system depressant effects as measured by the NeuroCart were observed from dose levels of 2.5 mg and higher.[3]

In contrast to the preclinical findings demonstrating an appropriate 'therapeutic window' with the desired effects occurring at lower exposure levels than the undesired effects, in the clinical study the low doses at which no desired pharmacological effects were observed, were already associated with unacceptable adverse events. It seems therefore that humans are more sensitive to the effects of H3R agonism than animals as decreases in blood pressure were observed from exposure levels of 1.4 ng/mL in humans versus 273 ng/mL in animals. Due to the nature of the AEs of pseudo-hallucinations, these could not be observed preclinically. Possibly, the changes in behaviour consisting of monkeys looking for and accepting human contact from exposure levels of 2173 ng/mL and higher could be indicative of cognitive or perceptive symptoms in the animals.

This study demonstrates that not all AEs are translatable between animals and humans. This warns researchers performing FIH studies with central nervous system (CNS) active compounds, to pay special attention to psychiatric effects of new compounds, as these cannot be reliably predicted from animal experiments.

Figure 1 1B-Derisk overview oxathridine



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