A pharmacokinetic evaluation of five H₁ antagonists after an oral and intravenous microdose to human subjects

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Human microdosing studies with novel drug candidates offer an opportunity to evaluate their pharmacokinetic (PK) behaviour early in drug development and before committing to the expense of clinical Phase I-enabling activities.
- Such studies assume linearity of exposure with dose all the way down to a subtherapeutic dose.
- Previous studies have reported partial success in the use of this technique for assessing human PK of marketed drugs.

WHAT THIS STUDY ADDS

- The present study describes the application of the microdosing concept in early drug development for an H₁ antagonist programme where having good estimates of human PK and information about the shape of the concentration–time curve was critical for compound selection.
- Microdosing data were generated for four novel compounds and one reference compound, and the data were used for advancing the compound with the most favourable PK properties.
- To our knowledge, this is the first example of the use microdosing technique for compound selection.

AIMS

To evaluate the pharmacokinetics (PK) of five H₁ receptor antagonists in human volunteers after a single oral and intravenous (i.v.) microdose (0.1 mg).

METHODS

Five H₁ receptor antagonists, namely NBI-1, NBI-2, NBI-3, NBI-4 and diphenhydramine, were administered to human volunteers as a single 0.1-mg oral and i.v. dose. Blood samples were collected up to 48 h, and the parent compound in the plasma extract was quantified by high-performance liquid chromatography and accelerator mass spectroscopy.

RESULTS

The median clearance (CL), apparent volume of distribution (V_d) and apparent terminal elimination half-life (t_{1/2}) of diphenhydramine after an i.v. microdose were 24.7 l h⁻¹, 302 l and 9.3 h, and the oral C_{max} and AUC_{0-∞} were 0.195 ng ml⁻¹ and 1.52 ng h ml⁻¹, respectively. These data were consistent with previously published diphenhydramine data at 500 times the microdose. The rank order of oral bioavailability of the five compounds was as follows: NBI-2 > NBI-1 > NBI-3 > diphenhydramine > NBI-4, whereas the rank order for CL was NBI-4 > diphenhydramine > NBI-1 > NBI-3 > NBI-2.

CONCLUSIONS

Human microdosing provided estimates of clinical PK of four structurally related compounds, which were deemed useful for compound selection.

Introduction

Insomnia affects approximately 10–30% of the US population at any given time. If left untreated, insomnia may result in disturbances in metabolism and overall body function, reduced productivity, and significant changes in mood, behaviour and psychomotor function. Benzodiazepines, non-benzodiazepines and small-molecule melatonin agonists are currently approved prescription medications for insomnia in the US. In addition, over-the-counter antihistamines are widely used as sleep aids [1].

The sedating property of so-called 'first generation' antihistamines has been attributed to their ability to penetrate the central nervous system and block neuronal histamine 1 (H₁) receptors [2, 3]. Several of these agents (e.g. diphenhydramine) are available over-the-counter and marketed for their sleep-inducing properties; however, clinical studies demonstrating their efficacy and safety in treating insomnia are lacking, and such use of these agents has therefore not been approved by the Food and Drug Administration (FDA). Currently available, brain-penetrating, over-the-counter antihistamines, however, also exhibit functional antagonism of muscarinic receptors, a property thought to contribute to the undesirable effects of antihistamines such as dry mouth, blurred vision, constipation, tachycardia, urinary retention, and memory deficits. In addition, next-day impairment seen with existing antihistamines is attributed to continued plasma exposure due to extended apparent terminal elimination half-lives ($t_{1/2}$) [3–5]. Novel, selective and shorter-acting antagonists of the H₁ receptor may provide a potential alternative to current medications for the treatment of insomnia. The pharmacokinetic (PK) profile of novel compounds will probably be an important determinant of their utility as a sleep agent. An ideal compound will be rapidly absorbed, have a short duration of action (6–8 h) and will exhibit low PK variability in humans. Traditional approaches for predicting human PK, such as allometry and *in vitro* methodologies, have been applied for the estimation of systemic clearance (CL), apparent volume of distribution (V_d) and the size of the first human dose. However, these approaches provide no information on the shape of the concentration–time curve, which is a major determinant of duration of drug action. For an insomnia drug, the duration of action is key to the utility of the drug, and the traditional approaches for predicting PK were therefore considered inadequate for compound selection.

Human microdosing studies with novel drug candidates offer an opportunity to evaluate their PK behaviour early in drug development, before committing to the expense of clinical Phase I-enabling activities (toxicology and large-scale manufacturing and characterization of the active pharmaceutical ingredient). A microdose is defined by the FDA and European Medicines Agency as 'less than 1/100th of the dose calculated to yield a pharmacological

Figure 1

Chemical structures of diphenhydramine (DPH), NBI-1, NBI-2, NBI-3 and NBI-4. *The position of ¹⁴C. One of the two N-CH₃ groups was labelled with ¹⁴C

effect of the test substance to a maximum dose of ≤100 micrograms' [6, 7]. With the assistance of ultrasensitive analytical technologies, such as accelerator mass spectrometry (AMS), administration of sub-pharmacological and non-toxic doses of new chemical entities in humans is used to assess their preliminary clinical PK behaviour and intra-subject variability. For microdosing, the regulatory requirements for safety are relatively low and dose formulation is simple. Microdosing may therefore be a faster and less costly path to obtain human PK data early in drug development [8].

NBI-1, NBI-2, NBI-3 and NBI-4 (Figure 1) are small-molecule histamine H₁ receptor antagonists with similar chemical structures and physicochemical properties (with high solubility and membrane permeability). These compounds are analogues of the known selective antihistamine R-dimethindene, which has previously shown sedating properties of short duration of action, with similar PK characteristics as determined in animals [9]. *In vitro* pharmacology studies have shown that these compounds are potent and selective H₁ receptor antagonists with high-affinity binding [inhibition constant (K_i) of 1–4 nM] and functional activity (IC_{50} in the nanomolar range) at the cloned human H₁ receptor, with selectivity of >1000-fold for muscarinic receptors and >200-fold for other receptors [10, 11]. (The antihistamine diphenhydramine, for comparison, is a reasonably potent H₁ antagonist with K_i of 14 nM and approximately 20-fold selectivity for muscarinic receptors [4].) Three exploratory compounds, namely NBI-2, NBI-3 and NBI-4, had not been administered to humans before, whereas NBI-1 had been tested in Phase I clinical trials. The present study was designed to determine the PK profile of NBI-2, NBI-3 and NBI-4 in man, with the goal of identifying the most suitable compound for further development. NBI-1 and diphenhydramine, compounds for which PK data at pharmacological doses were available,

were used as reference compounds to add confidence to the PK data for the exploratory compounds at the sub-therapeutic dose. Necessary preclinical safety pharmacology and toxicology studies were performed with the three exploratory compounds to support dosing of a 100- μ g dose in humans.¹

Materials and methods

Chemicals and reagents

NBI-1 (NBI-75043; unlabelled, lot no. 03N BI050148, purity 99.7%; ¹⁴C-labelled, lot no. A270-040, radiopurity 98.4%), NBI-2 (NBI-79499; unlabelled, lot no. 1904389, purity 99.0%; ¹⁴C-labelled, lot no. A270-041, radiopurity 95.1%), NBI-3 (NBI-77025; unlabelled, lot no. 2051095, purity 99.0%; ¹⁴C-labelled, lot no. A270-042, radiopurity 93.3%), NBI-4 (NBI-82840; unlabelled, lot no. 2041556, purity 99.7%; ¹⁴C-labelled, lot no. A270-039, radiopurity 97.5%) and diphenhydramine (¹⁴C-labelled, lot no. A270-043, radiopurity 98.9%) were supplied by Neurocrine Biosciences (San Diego, CA, USA). All ¹⁴C-labelled compounds had a specific activity of 53 Ci mol⁻¹ and were synthesized by Girindus America, Inc. (Cincinnati, OH, USA) under contract from Neurocrine Biosciences. All compounds were ¹⁴C-labelled on the potentially metabolically unstable *N*-methyl carbon (Figure 1) because this required minimal synthetic effort for the radiosynthesis. This was acceptable because the primary purpose of the study was to determine PK of the parent compounds, and not the metabolites.

Pharmacokinetic studies in animals

Male CD-1 mice, Sprague-Dawley rats, Cynomolgus monkeys and Beagle dogs received intravenous (i.v.) administrations of NBI-1, NBI-2, NBI-3 and NBI-4, with approval from the respective Institutional Animal Care and Use Committees. The dose for monkey and dog was 2.5 mg kg⁻¹, whereas those for mice and rats ranged from 2.5 to 10 mg kg⁻¹. Blood samples were collected at predose, 0–48 h. All blood samples were collected in K₃ ethylenediamine tetraacetic acid tubes and plasma was harvested by centrifugation. Plasma samples were stored at –80°C in a freezer until analysis. Liquid–liquid extraction or protein precipitation method was used to prepare the samples for high-performance liquid chromatography (HPLC)–MS/MS analysis. The HPLC–MS/MS system consisted of an Agilent series HP1100 HPLC pump (Santa Clara, CA, USA) or Shimadzu LC10ADVP pump (Columbia, MD, USA) coupled to a CTC PAL autosampler (Leap Technologies, Carrboro, NC, USA) and an Applied Biosystem/MDS Sciex API3000 or 4000 triple quadrupole mass spectrometer

(Toronto, Canada). The mobile phases used for chromatographic separation were either water and methanol or water and acetonitrile supplemented with either 0.1% formic acid or 0.01 M ammonium formate at pH 8.0 depending on the analyte of interest monitored. The MDS Sciex API-3000/4000 triple quadrupole mass spectrometer was equipped with either an electro ion spray interface or atmospheric pressure chemical ionization interface. The mass spectrometer was operated in the positive ion multiple reaction monitoring (MRM) mode. It was programmed to select protonated [M + H]⁺ molecules of analytes-of-interest via a Q1 mass analyser, which were further fragmented in Collision cell Q2. The product ions yielded from Q2 were monitored at Q3, the second mass analyser. The following MRM transitions were monitored for the analytes: 311.2 → 58.2 for NBI-1, 298.2 → 253.2 for NBI-2, 294.2 → 249.0 for NBI-3 and 329.2 → 58.2 or 329.2 → 104.1 for NBI-4. Plasma quantification was performed by fitting area peak ratios of the analyte-of-interest to internal standard to a linear least-square regression with reciprocal of concentration (1/X) or concentration squared (1/X²) as a weighting factor. Sciex Analyst software (version 1.4.1 or 1.4.2) was used for instrumentation control and data collection. Due to the exploratory nature of these studies, these analytical methods were not validated. The performance of the assays was determined by assessing the accuracy (% difference from the nominal) and/or precision [% coefficient of variation (CV)] for standard and quality control samples, and the acceptance criteria were ± 20%.

Human microdosing study

Volunteers and study design The human microdosing study was approved by the Institutional Review Board (PRA International, Zuidlaren, the Netherlands) and consisted of an eligibility screening period, two study periods involving administration of a single i.v. dose and a single oral dose of each ¹⁴C-labelled drug to a cohort of four healthy male subjects according to an open-label, randomized, two-period cross-over design with a wash-out period of 7 days between treatments. Subjects were randomized to treatment and sequence of oral/i.v. dosing within treatment. All subjects provided informed consent prior to screening and entry into the study. Seventeen subjects in the study were White men and of the remainder, one was Euro-African, one Asian and one was of mixed White origin. Overall, subjects ranged from 19 to 55 years old (median 24.5 years). The body mass index ranged from 19.6 to 29.5 kg m⁻².

Dose formulation and administration This part of the study was conducted at PRA International. Subjects received a single oral microdose of ¹⁴C-labelled drug on day 1 of one treatment period and a single i.v. microdose of the same drug on day 1 of the other treatment period. Study medication was administered in the upright (oral) or semi-supine (i.v.) position, between 07.15 and 08.00 h, after

¹A preliminary account of this work was presented at the 2007 AAPS meeting and exposition in San Diego, CA.

a 10-h overnight fast. Fasting was continued until lunch. There was a minimum of 2 h between dosing and lunch. No fluids were taken in from 2 h prior to and until 2 h after drug administration (lunch time), with the exception of water. Water was allowed until 1 h prior to and from 2 h after drug administration. For each cohort (study drug) there were two treatments: treatment A consisted of i.v. infusion of 4 ml solution in 5.0% glucose for injection containing 100 µg of diphenhydramine, NBI-1, NBI-2, NBI-3 or NBI-4 containing 7400 Bq (200 nCi) of ¹⁴C-labelled drug as a 10-min infusion via an infusion pump at a rate of 0.4 ml min⁻¹; treatment B consisted of oral intake of 100 ml drinking solution with 100 µg of diphenhydramine, NBI-1, NBI-2, NBI-3 or NBI-4 containing 7400 Bq (200 nCi) of ¹⁴C-labelled drug. At the start of the i.v. infusion, 96 ml of tap water was taken by the subject to match the fluid intake for the oral dosing (100 ml). Blood samples of 5 ml each were taken via an indwelling catheter or by direct venepuncture into sodium heparin-containing glass tubes at the following time points: predose and 0.17, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36 and 48 h postdose. The specimens were immediately cooled in ice water and processed within 1 h by centrifugation for 10 min at 4°C and 1500 g. Each tube was labelled, immediately frozen and stored at -70°C and then subsequently transferred to Xceleron (York, UK) for AMS analysis, where it was stored at -70°C.

Nonspecific binding Test preparations of the i.v. and oral dosing solutions of each compound were manufactured, which were passed through the dosing apparatus during mock administrations, and radioactive recovery was measured by liquid scintillation counting. Recoveries were >90% in all cases, suggesting little or no nonspecific binding to the dosing apparatus.

Quantification of ¹⁴C-labelled parent drug by HPLC and AMS

The AMS analysis was conducted by Xceleron by a method described in detail by Lappin *et al.* [12]. A brief description of the method is given here. The concentration of parent drug in each plasma sample obtained from the clinical study was determined by HPLC-AMS by way of a calibration curve. Calibration curves, for each of the five drugs, were constructed from control plasma into which varying concentrations of ¹⁴C-drug were added, along with a constant amount of nonlabelled drug, which acted as an internal standard. No correction was made for radiochemical purity as the error (maximum 6.4%, see Chemicals and reagents) was considered insignificant with respect to the precision of the analysis and any possible intrasubject variability. The samples were extracted as described below and analysed by HPLC-AMS. The ultraviolet (UV) response (relating to the nonlabelled drug being used as an internal standard) was also measured. The AMS response for fractions corresponding to the parent drug, divided by the UV

peak area, was plotted on the ordinate of the calibration curve. To aliquots of the clinical samples, an exact and known amount of nonlabelled parent drug was added (as an internal standard). The plasma samples were extracted and analysed by HPLC-AMS, whilst also recording the UV response. The AMS response for the analyte was divided by the UV peak area and was correlated to the ordinate of the calibration curve to give the plasma concentration on the abscissa. Because AMS measures ¹⁴C-drug, the addition of the nonlabelled drug did not affect the results. The addition of the nonlabelled drug, however, did have the effect of increasing the overall concentration of the drug in the sample and therefore reduced any possible nonspecific binding effects. This technique (subject to UK patent application 07140040.3) enabled the quantification of very low levels of drug while actually maintaining much higher total drug concentrations. Also, all five compounds were ¹⁴C-labelled on the N-methyl moiety, which is metabolically unstable. As discussed in Results, this does not affect the quantitative analysis of the parent.

Plasma extraction A volume (600 µl) of extraction solvent (methanol for NBI-1 and acetonitrile for others) containing a known amount of nonradiolabelled parent compound was added to a Waters Oasis protein precipitation plate (Waters Ltd., Elstree, UK). Thawed aliquots (100 µl for NBI-1 and 200 µl for others) of plasma were added to the wells. The plate was agitated for 1 min and then sealed, and the vacuum applied. The extract was collected and taken to dryness under N₂. The residue was reconstituted in 200 µl of mobile phase at the relevant HPLC starting conditions. The reconstituted extract (50 µl) was analysed by HPLC followed by AMS.

HPLC separation of the parent and fraction collection Generic conditions for all five compounds were as follows: HPLC system, Agilent 1200 series with fraction collector (Agilent Technologies, Stockport, UK); column, Waters XTerra MS C18, 3.5 µm, 4.6 × 150 mm (Waters Ltd.); guard column, Waters Xterra MS Guard Cartridge 4.6 × 10 mm (Waters Ltd.); column temperature, 20°C; UV detector, Agilent 1200 series UV detector (Agilent Technologies) set at 260 nm; injection volume, 50 µl; flow rate, 0.8 ml min⁻¹; mobile phase A, ammonium formate 0.01 M, pH 8.0; mobile phase B, methanol (100%). The mobile phase A and B composition and linear gradient applied for each compound were optimized to separate the parent compound from their putative N-demethyl metabolites using authentic standards. For diphenhydramine, mobile phase composition of 53%A and 47%B was maintained from 0 to 50 min, which was adjusted linearly to 10%A and 90%B during 50–53 min, and maintained from 53 to 58 min, which was then linearly adjusted back to 53%A and 47%B from 58 to 65 min, and maintained from 65 to 70 min. For NBI-1 and NBI-4, mobile phase composition of 50%A and 50%B was linearly adjusted to 25%A and 75%B from 0 to 32 min and

maintained from 32 to 35 min, which was linearly adjusted again to 5%A and 95%B from 35 to 36 min and maintained from 36 to 39 min, which was then linearly adjusted back to 50%A and 50%B from 39 to 44 min, and maintained from 44 to 47 min. For NBI-2, mobile phase composition of 60%A and 40%B was maintained from 0 to 50 min, which was adjusted linearly to 10%A and 90%B during 35–45 min and maintained from 45 to 50 min, which was then linearly adjusted back to 60%A and 40%B from 50 to 52 min, and maintained from 52 to 57 min. For NBI-3, mobile phase composition of 55%A and 45%B was maintained from 0 to 45 min, which was adjusted linearly to 10%A and 90%B during 45–53 min, and maintained from 53 to 58 min, which was then linearly adjusted back to 55%A and 45%B from 58 to 65 min, and maintained from 65 to 70 min. In each case, the eluant was collected as a series of fractions every 30 s for 6 min over the parent peak area. Since each sample had been fortified with nonradiolabelled parent compound, the retention times were identified from the UV response for each chromatogram. The fractions corresponding to the UV chromatographic peak for the parent drug were pooled and analysed by AMS. The fractions for each chromatographic analysis corresponding to the parent peak were assessed individually.

AMS analysis The sample preparation for AMS analysis including combustion, graphitization, and packing of cathodes with graphite, the AMS analysis itself and the AMS data processing were the same as previously reported [12, 13]. The limit of quantification was 1 pg mL⁻¹ plasma. The precision of the measurement on the AMS is based upon 5% on triplicate analyses of the same cathode. Variability throughout the assay was measured on the response of the internal standard (UV response for nonradiolabelled parent) and had a CV of <15%.

Pharmacokinetic analysis PK parameters were calculated via a noncompartmental model with WinNonlin software version 4.1 (Pharsight, Mountain View, CA, USA) as described previously [12].

Results

Human microdosing study

Overall, administration of diphenhydramine, NBI-1, NBI-2, NBI-3 and NBI-4 as an i.v. or oral solution using the microdosing technique appeared to be safe and well tolerated in healthy male subjects. During the observation period of 48 h after dose administration of diphenhydramine, NBI-1, NBI-2, NBI-3 and NBI-4, no clinically relevant abnormalities or trends were observed as measured by electrocardiogram, vital signs, and clinical laboratory and physical examinations (data not shown).

Figure 2

Mean concentration–time curves of total ¹⁴C and diphenhydramine after a 0.1-mg i.v. and oral dose of diphenhydramine to human volunteers. Each data point is an average of data from four human volunteers. Error bars represent standard deviations. Both ¹⁴C and diphenhydramine levels were determined using accelerator mass spectrometry as described in Materials and Methods. IV-0.1 mg (—■—); Oral-0.1 mg (—□—)

Diphenhydramine PK

The concentration–time curves for total radioactivity (¹⁴C) and diphenhydramine in the plasma of subjects receiving 0.1 mg i.v. and oral dose of diphenhydramine are shown in Figure 2. After i.v. administration of diphenhydramine, the median systemic CL, apparent volume of distribution at steady state (V_{ss}) and $t_{1/2}$ were calculated to be 24.7 L h⁻¹, 158 L and 9.3 h, respectively (Table 1). After a 0.1-mg oral dose, diphenhydramine appeared to be rapidly absorbed with a median peak concentration (C_{max}) of 0.195 ng mL⁻¹, area under the curve from zero to infinity ($AUC_{0-\infty}$) of 1.52 ng h mL⁻¹, and a moderate oral bioavailability of 34% (Table 1). The extrapolation from area under the curve from zero to the last time point (AUC_{0-t}) to $AUC_{0-\infty}$ was small, indicating that the experimental period was adequate for the study (Table 1). In addition to the plasma concentration for parent drug, the total ¹⁴C content was

Table 1 Mean PK parameters for five H₁ antagonists after i.v. and oral dose to humans

(A) I.v. dosing							
Drug	AUC _{0-t} (ng h ml ⁻¹)	AUC _{0-∞} (ng h ml ⁻¹)	t _{1/2} (h)	CL (l h ⁻¹)	V (l)	V _{ss} (l)	MRT _(inf) (h)
DPH	4.37 (3.93–5.17)	4.43 (4.19–5.20)	9.3 (5.7–12)	24.7 (20.7–25.9)	302 (214–433)	158 (99.6–264)	6.4 (4.8–10)
NBI-1	4.80 (4.12–6.75)	4.95 (4.13–6.80)	9.1 (6.5–13)	22.3 (16.1–27.0)	308 (170–361)	128 (103–175)	6.8 (3.8–8.0)
NBI-2	11.2 (7.62–12.6)	11.3 (7.79–12.8)	9.0 (7.5–10)	9.79 (8.58–14.0)	134 (96.5–176)	77.6 (46.9–117)	7.9 (5.3–8.9)
NBI-3	6.97 (6.67–9.01)	7.43 (6.69–9.42)	11 (6.0–16)	14.3 (10.7–16.7)	198 (144–327)	130 (113–248)	10 (7.8–17)
NBI-4	2.47 (1.49–6.03)	2.66 (1.65–6.54)	15 (8.0–23)	43.7 (16.4–66.7)	685 (538–1060)	401 (201–565)	10 (4.7–17)
(B) Oral dosing							
Drug	T _{max} (h)	C _{max} (ng ml ⁻¹)	AUC _{0-t} (h ng ml ⁻¹)	AUC _{0-∞} (h ng ml ⁻¹)	t _{1/2} (h)	MRT _(inf) (h)	%F
DPH	2.5 (1.0–3.0)	0.195 (0.153–0.223)	1.36 (1.11–1.60)	1.52 (1.39–1.89)	12 (7.2–26)	15.2 (11.9–16.5)	34 (29–42)
NBI-1	1.0 (0.75–2.0)	0.625 (0.532–1.20)	2.78 (1.90–4.23)	2.86 (1.94–4.28)	6.7 (3.6–10)	6.9 (4.5–7.5)	52 (41–88)
NBI-2	1.0 (0.75–3.0)	0.704 (0.525–0.887)	6.54 (5.05–7.53)	6.63 (5.17–7.71)	9.5 (5.7–9.8)	11 (8.7–12)	60 (50–75)
NBI-3	2.0 (1.0–2.0)	0.349 (0.279–0.482)	3.05 (2.31–5.17)	3.42 (2.57–5.56)	11 (7.4–17)	13 (9.3–20)	52 (33–53)
NBI-4	1.5 (1.0–2.0)	0.106 (0.040–0.203)	0.636 (0.210–1.86)	0.778 (0.229–1.87)	13 (7.6–15)	12 (9.6–25)	26 (11–48)

Data are median values of four individual subjects. Data in parentheses are the range for the four subjects. Percent bioavailability (%F) was calculated by multiplying by 100 the ratio of AUC_{0-t} after oral and i.v. administration, correcting for the actual dose received by each subject. MRT, mean residence time.

Table 2 Comparison of total ¹⁴C and parent drug after i.v. and oral administration of a microdose of five H₁ antagonists

Drug	After i.v. dose			After oral dose		
	AUC _{0-t} total ¹⁴ C (ng-eq h ml ⁻¹)	AUC _{0-t} parent (ng h ml ⁻¹)	Percent of AUC as parent	AUC _{0-t} total ¹⁴ C (ng-eq h ml ⁻¹)	AUC _{0-t} parent (ng h ml ⁻¹)	Percent of AUC as parent
DPH	20.5	4.46	22%	18.6	1.36	7.3%
NBI-1	33.5	5.12	15%	32.0	2.92	9.1%
NBI-2	18.7	10.6	57%	18.1	6.41	36%
NBI-3	19.4	7.41	38%	23.6	3.39	14%
NBI-4	28.0	3.11	11%	28.0	0.835	3.0%

Data are average of four subjects.

also measured (Figure 2). Provided that the ¹⁴C label remained in the core structure of the drug, the total ¹⁴C concentration represents the total equivalent concentration of the drug and any metabolites in the plasma. In the current study, however, some caution should be adopted in the interpretation of the data as the compounds were labelled in an N-¹⁴C-methyl moiety, which could be readily cleaved during metabolism. Therefore, 'percent of total ¹⁴C AUC as parent' was likely to be overestimated. Additionally, extrapolation from the AUC_{0-t} to AUC_{0-∞} for the ¹⁴C was relatively high (up to 76%; data not shown). Nevertheless, both limitations would tend to underestimate total ¹⁴C. Assuming that the total ¹⁴C concentrations were representative of drug-related compounds (parent plus metabolites), diphenhydramine underwent a fair degree of metabolism (Figure 2). It is evident from the AUC representing parent drug as a percent of total ¹⁴C (7.3%; Table 2) that diphenhydramine was extensively metabolized and that the plasma t_{1/2} of total radioactivity was longer than

for the parent drug. At least in part, this should be ascribed to ongoing formation of metabolites as long as parent compound is present.

NBI-1, NBI-2, NBI-3 and NBI-4 PK

The concentration–time curves for NBI-1, NBI-2, NBI-3 and NBI-4 concentration in the plasma of subjects receiving 0.1 mg i.v. and oral dose of each compound are shown in Figure 3, and the PK parameters calculated are shown in Table 1. The rank order of oral bioavailability of the four compounds was as follows: NBI-2 > NBI-1 > NBI-3 > diphenhydramine > NBI-4. Following i.v. administration, the median t_{1/2} of diphenhydramine, NBI-1, NBI-2 and NBI-3 were similar in the 9–11 h range, and that of NBI-4 was slightly higher (approximately 15 h) owing to its high median V_d (685 l). The median CL values of NBI-2 and NBI-3 were lower than of diphenhydramine and NBI-1, which in turn were lower than NBI-4. Of all the compounds examined, NBI-4 appeared to show the highest

Figure 3

Mean concentration–time curves of NBI-1, NBI-2, NBI-3 and NBI-4 after a 0.1-mg i.v. and oral dose of each compound to human volunteers. Each data point is an average of data from four human volunteers. Error bars represent standard deviations. NBI-1, NBI-2, NBI-3 and NBI-4 levels were determined using high-performance liquid chromatography followed by accelerator mass spectrometry as described in Materials and Methods. IV-0.1 mg (—■—); Oral-0.1 mg (—□—)

variability in oral C_{max} , AUC, and bioavailability and i.v. CL, probably due its lower bioavailability (Figure 4). For all compounds, the oral plasma AUC of the parent compound represented low to moderate percent of total ^{14}C ranging from 3.0% for NBI-4 to 36% for NBI-2 (Table 2). Limitations regarding the metabolic instability of the ^{14}C notwithstanding, NBI-4 appeared to be most extensively metabolized, which is also consistent with its higher CL and lower bioavailability.

Allometric scaling from nonclinical species and human microdosing

Another aim of this study was to understand how well the allometric scaling predicted human CL compared with the human microdosing. This methodology is frequently utilized by pharmaceutical companies to predict exposure in humans, which in turn is useful for selecting the compound for further development and to calculate the starting dose for first-time-in-human clinical trials. Mean i.v. CL

for mouse, rat, dog and monkey were available for NBI-1, NBI-2, NBI-3 and NBI-4 from i.v. PK studies conducted in these animal species (Table 3; only the pertinent nonclinical PK data are shown in this study). Allometric scaling was performed and the human CL was predicted using the rule of exponents [14]. As is frequently observed in allometric scaling, the coefficient of determination (R^2) values were high. However, as has been noted previously [15] a high R^2 value does not necessarily have good predictive value. In each case the CL predicted from allometry was higher than that obtained in the current microdosing study, suggesting a systematic error in prediction for this class of compounds.

Discussion

In the current study, three compounds that were candidates for further development (NBI-2, NBI-3, NBI-4) and two

Figure 4

An assessment of variability in selected pharmacokinetic (PK) parameters after a 0.1-mg i.v. and oral dose of diphenhydramine (DPH), NBI-1, NBI-2, NBI-3 and NBI-4 to human volunteers. PK parameters were calculated from concentration–time curves from each of the four human volunteers as described in Materials and Methods, and are plotted to show the extent of variability observed (see Table 1 for summary data). Oral C_{max} , AUC_{0-t} and bioavailability, and CL after i.v. dosing are shown. Horizontal lines are mean data for four subjects

comparators (diphenhydramine and NBI-1) were administered to human volunteers as a microdose (100 µg) by both the oral and i.v. routes of administration. The i.v. and oral human PK of diphenhydramine in young and healthy Whites has been studied previously at pharmacologically active doses [16–21]. In these previously published studies, a range of PK parameters (assuming an average 70 kg body weight) after a 50-mg i.v. dose of diphenhydramine were reported, and the data are summarized in Table 4. Normalizing the data from the 0.1-mg subtherapeutic oral dose in the current study to a 50-mg dose gives C_{max} and AUC

values of 95 ng mL⁻¹ and 790 ng h mL⁻¹, values that are remarkably similar to those published previously (Table 4). Similarly, the dose-independent PK parameters calculated after i.v. dose, namely V_d , CL and $t_{1/2}$, were similar at 0.1 mg (current study) and at 50 mg (previously published studies) (Table 4). These comparisons suggest that diphenhydramine exhibits linear PK in the dose range 0.1–50 mg, and compounds of similar structural class and physico-chemical properties would be expected to do the same, adding confidence to the data obtained with the remaining compounds used in this study.

Table 3

Comparison of human systemic CL predicted from nonclinical species (allometric scaling with rule of exponents) with human microdosing

Drug	Systemic CL (ml min ⁻¹ kg ⁻¹)				Allometric equation coefficient and exponent (a, b)	Human Coefficient of determination R ²	Human Systemic CL (ml min ⁻¹ kg ⁻¹)	
	Mouse	Rat	Monkey	Dog			Predicted	Microdosing
NBI-1	70.0	98.2	26.7	29.3	(1.16, 429)	0.998	9.03	5.22
NBI-2	159	58.0	16.8	37.8	(1.06, 422)	0.977	5.72	2.51
NBI-3	108	73.7	32.2	36.1	(1.15, 489)	0.998	9.74	3.33
NBI-4	252	95.6	30.0	33.5	(0.639, 61.5)	0.987	13.2	10.0

The relationship between body weight and PK parameters is described by simple allometry according to the following equation: where, a and b are the coefficient and exponents of the allometric equation, respectively [14].

Table 4

Comparison of DPH PK parameters obtained from the current microdose study with the published literature (normalized to 50-mg dose where applicable)

Parameter	Microdose study data	Published literature					
		Spector, 1980 [21]	Berlinger, 1982 [16]	Meredith, 1984 [18]	Blyden, 1986 [17]	Simons, 1990 [20]	Scavone, 1990 [19]
V _d (l)	313	480	295	462	317	NR	249
CL (l h ⁻¹)	24	79	42	41	26	NR	37
t _{1/2} (h)	9.0	4.3	4.9	9.3	8.5	9.2	4.7
Oral-%F	35	58	NR	NR	72	NR	69
Oral-C _{max} (ng ml ⁻¹)*	96	NR	NR	NR	66	76	48
Oral-AUC (ng h ml ⁻¹)*	675	NR	NR	NR	667	585	270

*Data were normalized to 50-mg dose where applicable. NR, data not reported.

Similar to diphenhydramine, the oral PK of 10 mg NBI-1 has also been studied in humans. At a 10-mg oral dose of NBI-1 (n = 6), a median C_{max} of 50.0 ng ml⁻¹ was attained at 1.0 h, median t_{1/2} was 8.4 h, and median AUC_{0-∞} was 328 ng h ml⁻¹ (unpublished data on file at Neurocrine Biosciences). Normalizing the data from 0.1-mg subtherapeutic dose in this study to a 10-mg dose gives median C_{max} and AUC values of 62.5 ng ml⁻¹ and 286 ng h ml⁻¹, respectively, which are similar to those observed at the 10-mg dose. These comparisons further corroborate that NBI-1 exhibits linear PK in the dose range 0.1–10 mg, and compounds of similar structural class and physicochemical properties would be expected to do the same, adding confidence to the data obtained with the exploratory compounds, i.e. NBI-2, NBI-3 and NBI-4.

The systemic CL values predicted from allometry were also compared with the data obtained from the current microdosing study (Table 3). Allometry tended to overpredict human CL for the four compounds with variable magnitude of error (Table 3). This is consistent with previous analysis of allometry data of several marketed compounds where a systematic bias toward overprediction of human CL has been documented [14]. However, it should be noted that for the present set of compounds, the error in prediction of CL by allometry was within a factor of 3, which suggests that there were no gross over- or underprediction of CL. This analysis assumes that the microdosing PK

data are in fact representative of PK data at therapeutic doses, an assumption that is valid for the two compounds (diphenhydramine and NBI-1) where data at both a micro and therapeutic dose were available.

An ideal drug to treat insomnia will have a rapid onset of action, short duration of action and exhibit low variability. All four NBI compounds appeared to be rapidly absorbed after an oral microdose, which suggests that all compounds are likely to be equivalent in terms of onset of action. Duration of action is determined by the time above which the concentration of the drug at receptor exceeds a certain threshold. In this respect, the shape of the concentration–time curve is more informative, whereas t_{1/2} may or may not be a good indicator of the duration of action. Since the PK–pharmacodynamic relationship for use of H₁ antagonists for the treatment of insomnia has not been established, one can only use t_{1/2} to predict which compounds will be likely to have a shorter duration of action. NBI-1 and NBI-2 had a somewhat shorter t_{1/2} after oral administration compared with NBI-3 and NBI-4. In terms of variability in AUC, C_{max}, CL and V_d, NBI-4 was the most variable, followed by NBI-1 and NBI-3, with NBI-2 being the least variable. Since only four subjects per compound were evaluated in this study, emphasis on variability can be misleading. However, if combined with mechanistic information derived from PK parameters, the observed data on variability and t_{1/2} can be more meaningful. In

other words, compounds with higher bioavailability and lower systemic CL will tend to exhibit lower variability in AUC and C_{max} and compounds with lower V_d will tend to have a shorter $t_{1/2}$. So, an ideal insomnia drug will have a high bioavailability and low CL (which will tend to make the compound less variable) and it will have low V_d (which will result in a shorter $t_{1/2}$). In this respect also, NBI-2 appeared to have the highest bioavailability, lowest CL, lowest V_d and a short $t_{1/2}$ compared with NBI-1, NBI-3 and NBI-4. Taking all of the data together, we selected NBI-2 to be the most suitable drug candidate.

In conclusion, human microdosing provided estimates of clinical PK of four structurally related compounds under development, which appeared to be reliable and were useful for compound selection. Microdosing is a useful and cost-effective technique for estimating human PK in drug discovery and development, which, if appropriately used complementarily to existing human PK prediction methods (i.e. *in vitro* and allometric scaling), may lead to a more efficient drug development process.

Competing interests

AM, ZO'B, JW, CO'B, GB and HB are employees of Neurocrine Biosciences. AM and HB hold stock in the company. RF was a full-time employee of Neurocrine Biosciences during the course of study conduct and manuscript preparation. PC was previously employed by Neurocrine Biosciences and holds stock in the company. RCG and GL are employees of Xceleron Ltd and hold stock in the company.

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