Microdosing of the human protein therapeutic hRESCAP in healthy volunteers to predict its clinical pharmacokinetics

Maria LH Vlaming[1], Esther van Duijn[1], Ruud Brands[2], Albert D Windhorst[3], N. Harry Hendrikse[3], Sieto Bosgra[1], Marlous R. Dillingh[4], Martijn C. de Koning[1], Alex Fidder[1], Johannes A.J. Mocking[1], Hugo Sandman[1], Joost Westerhout[1], Babs O. Fabriek[1], Wilrike J. Pasman[1], Steven LA Erpelinck[1], Willem Seinen[2], Pierre A. Peeters[4], Wouter H. Vaes[1]

[1]TNO, Zeist, The Netherlands, [2]Alloksys Life Sciences B.V., Utrecht, The Netherlands, [3]VU University Medical Center, Amsterdam, The Netherlands, [4]Centre for Human Drug Research, Leiden, The Netherlands

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ABSTRACT

Purpose: For the development of New Biological Entities (NBEs) such as human protein therapeutics, considerable costs and time are spent on preclinical animal studies, which are often not predictive for the situation in humans. Microdosing, combined with the ultrasensitive analytical technique Accelerator Mass Spectrometry (AMS), provides a safe and reliable approach to obtain first-in-human pharmacokinetic data, after limited animal testing. We investigated whether microdosing can be used as a safe tool to study pharmacokinetics of NBEs in humans. This was done using human recombinant Placental Alkaline Phosphatase (hRESCAP), a protein currently under investigation for clinical applications.

Methods: hRESCAP was produced and ^{14}C-labelled under GMP-compliance. hRESCAP and [^{14}C]-hRESCAP were extensively characterized by *in vitro* methods, and ethical approval for a first-in-human phase 0 / phase 1 study was obtained. A microdose (53 µg, 0.5 nmol) of [^{14}C]-hRESCAP was intravenously (i.v.) administered as a safe starting dose to healthy volunteers (n=3), for safety and pharmacokinetics assessment. Subsequently, doses of 414, 1240 and 5300 µg (including 53 µg [^{14}C]-hRESCAP), or placebo (n=3 per group) were i.v. administered according to a single ascending design. [^{14}C]-hRESCAP plasma levels were determined with AMS and total hRESCAP levels with an enzymatic assay. Plasma concentration-time data were analyzed by nonlinear mixed-effects modeling.

Results: GMP-compliant manufacturing of hRESCAP and [^{14}C]-hRESCAP, and subsequent *in vitro* characterization was successfully performed. Single doses of 53 (microdose), 414, 1240 and 5300 µg hRESCAP (including 53 µg [^{14}C]-hRESCAP) were well tolerated in healthy subjects. Pharmacokinetic analyses showed a tri-phasic elimination, which was modeled as a linear model with three distinct elimination phases. The terminal half-life of hRESCAP was ~5 days. No differences in pharmacokinetics across dose groups and individuals was observed, indicating dose linearity from microdose to therapeutic doses.

Conclusions: A microdose of [^{14}C]-hRESCAP could be used as a safe starting dose for a first-in-human study, and to predict the pharmacokinetics of hRESCAP at therapeutic doses. hRESCAP was well tolerated in healthy subjects up to 5300 µg, and had a favorable half-life in humans. Microdosing of ^{14}C-labelled biotherapeutics preceded by a rationally designed preclinical package can substantially reduce the time and costs of drug development.

METHODS

GMP production of hRESCAP and [^{14}C]-hRESCAP.

hRESCAP was GMP-produced in a human amnion-derived production CAP 9 cell line (CEVEC Pharma, Köln, Germany) by Genlbet (Oeiras, Portugal). A radiolabelling procedure for production of [^{14}C]-hRESCAP by reductive amination of lysine residues was developed by TNO. The method was optimized to obtain [^{14}C]-hRESCAP with ~1 ^{14}C-label per hRESCAP dimer molecule, and an enzymatic activity of ≥70% of the starting material.

The labelling method was transferred to the VU University Medical Center for GMP-compliant radiolabelling. After GMP production, various batches of ^{14}C-hRESCAP (86-128 Bq/mL, 9-11 µg/mL) corresponding to ~0.4-0.6 ^{14}C-label per hRESCAP dimer were produced and analyzed ~48 hrs before injection.

METHODS

Preclinical analysis of hRESCAP and [^{14}C]-hRESCAP.

After extensive *in vitro* analysis of hRESCAP and [^{14}C]-hRESCAP (including stability, (radio)chemical purity, enzymatic activity, SDS-PAGE, mass spectrometry of the glycosylated and deglycosylated product, and determination of protein aggregation by SEC-UV) and limited animal testing (2-week repeated dose toxicity test in human Alkaline Phosphatase tolerized mice), approval from the Dutch Medical Ethical committee for a clinical Phase 0 / Phase 1 first-in-human study with [^{14}C]-hRESCAP and hRESCAP was obtained.

Clinical study. The clinical study with GMP-produced [^{14}C]-hRESCAP and hRESCAP was performed at the Centre for Human Drug Research as described in Figure 1. Safety was assessed after each dose and pharmacokinetics were determined after analysis of plasma samples.

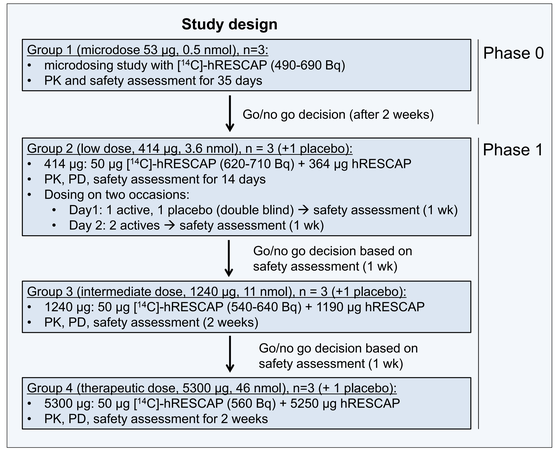

Figure 1. Clinical Phase 0 / Phase 1 study with [^{14}C]-hRESCAP. *The set up of the study is depicted in the scheme (left panel). In short, a microdose of [^{14}C]-hRESCAP alone or supplemented with hRESCAP was i.v. administered to healthy male volunteers and blood was collected at various time points. After collection, [^{14}C]-levels in plasma were determined with a 1 MV Tandetron Accelerator Mass Spectrometer by TNO (right panel). Total alkaline phosphatase (AP) concentrations in plasma were determined with an enzymatic AP assay on a clinical Olympus AU400 analyzer.*

CONCLUSIONS

- A microdose of [^{14}C]-hRESCAP could be used as a safe starting dose for a first-in-human study and predicted the pharmacokinetics of hRESCAP at therapeutically relevant doses
- hRESCAP was well tolerated in healthy individuals and had a terminal half life of about 4.8 days, which is encouraging for its potential use for treatment of chronic inflammation.
- Microdosing of ^{14}C-labelled biotherapeutics preceded by a rationally designed preclinical package can substantially reduce the time and costs of drug development, as well as the number of laboratory animals used for preclinical studies

RESULTS

GMP compliant manufacturing of [^{14}C]-hRESCAP was successfully performed at each dosing occasion. After administration of a microdose [^{14}C]-hRESCAP (53 µg, ~500 Bq), the radiolabel could be detected by AMS in plasma of individuals up to 35 days after dosing, and minor variation between subjects was observed (Figure 2A). Single ascending doses of hRESCAP (up to 5.3 mg) were well tolerated in healthy individuals. At the highest dose, total plasma AP levels were ~3-fold above endogenous AP levels until about 4-5 days after administration (not shown). Pharmacokinetic analysis of [^{14}C]-hRESCAP at various doses indicated a dose linear behavior of the protein (Figure 2B). The terminal half life of [^{14}C]-hRESCAP was calculated to be 4.8 days. Furthermore, data from the microdose could be used to accurately predict levels of the enzyme at higher doses (Figure 2C).

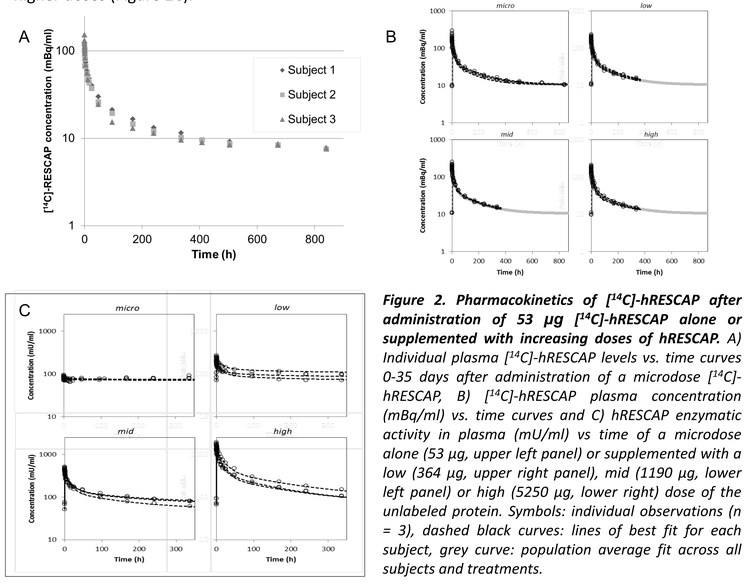

Figure 2. Pharmacokinetics of [^{14}C]-hRESCAP after administration of 53 µg [^{14}C]-hRESCAP alone or supplemented with increasing doses of hRESCAP. *A) Individual plasma [^{14}C]-hRESCAP levels vs. time curves 0-35 days after administration of a microdose [^{14}C]-hRESCAP, B) [^{14}C]-hRESCAP plasma concentration (mBq/ml) vs. time curves and C) hRESCAP enzymatic activity in plasma (mU/ml) vs time of a microdose alone (53 µg, upper left panel) or supplemented with a low (364 µg, upper right panel), mid (1190 µg, lower left panel) or high (5250 µg, lower right) dose of the unlabeled protein. Symbols: individual observations (n = 3), dashed black curves: lines of best fit for each subject, grey curve: population average fit across all subjects and treatments.*

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Contact details: Marijn Vlaming, PhD
marijn.vlaming@tno.nl
+31 888 66 1764