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# Pharmacokinetic properties of the clinical drug candidate PRI-002 with regard to genotype, sex, age, dose-dependence and food effects in mice

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ABSTRACT

The D-enantiomeric peptide RD2 (named Contraloid or PRI-002 in clinical trials) was developed for the direct disassembly of toxic amyloid-β (Aβ) oligomers, which are the most neurotoxic aggregate species and play a key role in the development and progression of Alzheimer's disease (AD). PRI-002/RD2 already demonstrated its safety and tolerability in three phase I clinical trials in young healthy volunteers and patients with mild neurocognitive impairment (MCI) or mild dementia due to AD. Results from a phase II clinical trial with PRI-002/RD2 are expected this year, which was designed to demonstrate safety and efficacy of PRI-002/RD2 in patients at an early stage of AD.

The objective of this study was to evaluate the effect of age, sex, genotype and food on the pharmacokinetics of intravenous or orally administered RD2 in male and female transgenic APPswe/PS1ΔE9 (APP PS1) (n = 62) or wild type (wt) mice (n = 169) under fed and fasted conditions. Age and concomitant food intake influenced the pharmacokinetics of RD2. Slightly higher plasma and brain levels were observed in young compared to old wt mice. However, the effect of food on plasma levels depended on the RD2 dose administered. While at lower doses (200 mg/kg) the effect was substantial, plasma levels were 15 times higher in fasted than in non-fasted animals, the effect was only minor at higher RD2 doses (600 mg/kg).

The results of this study indicate the important influence of prior food intake on the bioavailability of the compound, which may also apply to patients and is therefore of interest in further clinical development.

## Introduction

Alzheimer's disease (AD) is the most common form of dementia, with over 30 million patients worldwide (Gustavsson et al., 2023). Although AD still has no cure, three disease-modifying monoclonal antibodies (aducanumab (Cummings et al., 2021; Dunn et al., 2021), lecanemab (van Dyck et al., 2023) and donanemab (Sims et al., 2023)), have been approved by the US Food and Drug Administration (USFDA) in the last years, demonstrating that removal of amyloid-β (Aβ) aggregates from the brain slows cognitive and functional decline in patients with early AD.

The D-enantiomeric peptide RD2, also named Contraloid or PRI-002 in clinical trials and regulatory documents, is also aiming to remove toxic Aβ aggregates. In contrast to the monoclonal antibodies, however, it was developed to disassemble toxic Aβ oligomers into non-toxic Aβ monomers (Kass et al., 2022), rather than relying on the immune system to remove them. Previous preclinical studies demonstrated target engagement *in vitro* (van Groen and al., 2017; Zhang et al., 2019), *in vivo* (Schemmert et al., 2019) and *ex vivo* (Kass et al., 2022) by quantifying the reduction of Aβ oligomers in the presence of RD2. *In vitro* RD2 eliminated toxic Aβ assemblies by stabilizing Aβ monomers in their native intrinsically disordered conformation shown by analytical ultracentrifugation (Zhang et al., 2019). *Ex vivo* treatment of brain tissue of former AD patients with RD2 resulted in a disassembly of Aβ oligomers into Aβ monomers (Kass et al., 2022) and *in vivo* target engagement could be proven by showing a significant reduction of Aβ oligomers in

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the brains of APPswe PS1ΔE9 (APP PS1) mice, which were treated orally for 12 weeks with RD2, compared to placebo-treated mice (Schemmert et al., 2019). The atypical shape remarkable resistance to metabolism in simulated gastrointestinal fluids, blood plasma, and liver microsomes. It is not a substrate for human D-amino acid oxidase (hDAAO), and hDAAO does not influence the activity of the main cytochrome P450 (CYP) isoforms present in human liver microsomes (CYP1A, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) (Elfgen et al., 2019). In addition, proof of concept with regard to robust improvement of memory and cognitive deficits was demonstrated in three different transgenic mouse models and in cognitively impaired beagle dogs, a non-transgenic model for sporadic AD (van Groen and al., 2017; Schemmert et al., 2019; Kutzsche et al., 2023; Kutzsche et al., 2017). Furthermore RD2/PRI-002 has already demonstrated safety and tolerability in healthy volunteers and patients with mild neurocognitive impairment (MCI) or mild dementia due to AD with suitable pharmacokinetic characteristics to support further clinical development (Kutzsche et al., 2020; Kutzsche et al., 2025).

Since sex differences are already known in AD - women have a higher lifetime risk of developing AD (1 in 5) than men (1 in 10) and two-thirds of patients with AD are women (Lopez-Lee et al., 2024), it is of interest to determine whether the underlying biological mechanisms responsible for the sex-dependent effects also have an impact on the pharmacokinetic profile of RD2. In addition, pharmacokinetic changes occur with increasing age, including a decrease in hepatic and renal clearance and an increase in the volume of distribution of some drugs, which prolongs the elimination half-life (Mangoni and Jackson, 2004). The aging process causes changes in absorption in the intestine, e.g. due to a reduction in the surface area of the jejunum and slower drug absorption due to passive diffusion (Perrie et al., 2012). In addition other relevant physiological changes that occur in the small intestine during ageing are characterized by a decrease in gastric emptying (Evans et al., 1981), small intestinal transit time and splanchnic blood flow, which, in combination with decreased dissolution, further prolongs the time required for drug absorption (Perrie et al., 2012). Since AD patients are of advanced age, it is also important to investigate whether age-related changes occur in the pharmacokinetic profile of RD2.

Cerebrovascular impairment and a disrupted blood–brain barrier (BBB) were observed in APP PS1 mice (Ahn et al., 2018) and AD patients (Erickson and Banks, 2013; Zenaro et al., 2017; Sweeney et al., 2018), which could lead to increased uptake of compounds into the brain. In addition, also intestinal permeability and oral absorption of some compounds are reduced in APP PS1 mice (Jin et al., 2020) among other things, possibly due to altered abundance of some small intestinal drug transporters (Pan et al., 2018) or tightened paracellular junctions (Jin et al., 2020). To investigate, whether RD2 levels in the plasma and brain are altered due to age or pathological changes during disease, pharmacokinetic profile of old-age transgenic AD mice (APP PS1) and wild-type (wt) littermates was determined. Furthermore, a dose-dependency in pharmacokinetics most commonly results in an increase in half-life, a disproportionate increase in plasma concentration and in area under the drug concentration-time curve of the drug (Powis et al., 1981). In some cases, the rate of drug elimination increases with increasing dose. These non-linear alterations in drug concentrations with increasing dose have several implications also for clinical development such as higher steady-state-levels, variability and side effects. Therefore, in this study also different doses of RD2 were applied to investigate a possible dose-dependent effect of RD2 on pharmacokinetics.

In conclusion, the aim of this study was to investigate the pharmacokinetic profile of RD2 with regard to genotype, sex, age, dose-dependence and food effects in mice, in contrast to previous studies which were performed only in fasted young male mice and using radioactively labeled compound.

## Material and methods

### *Materials*

RD2 (ptlhthnrrrrr, all amino acid residues in D-enantiomeric conformation and C-term amidated) was purchased from CBL Patras (Patras, Greece) with a purity > 95% and with acetate as counter ion. The internal standard (ISTD), which has the same sequence as RD2 but with a substitution of leucine against valine at position three (ptvhthnrrrrr), was purchased from Peptides & Elephants GmbH (Potsdam, Germany) with a purity of 99.8%. All chemicals were acquired from Merck (Darmstadt, Germany), AppliChem (Darmstadt, Germany), VWR (Darmstadt, Germany) and Roth (Karlsruhe, Germany).

### *Animals*

All experimental procedures involving mice and their care were conducted in accordance with the *ARRIVE guidelines*. 169 C57BL/6 wt (Charles River Laboratories, Sulzfeld, Germany) and 62 APPswe/PS1ΔE9 tg mice (JAX) were used in this study. Male wt mice (age 11–12 weeks) and male and female APPswe/PS1ΔE9 and their wt litter mates (age 20–30 months) were used for this study. The animals were housed in groups of up to five mice in standard ventilated cages in our facility. Male mice were often single-caged at an advanced age. The facility was maintained on a 12/12 h light-dark cycle (7 a.m. – 7 pm.) with a temperature between 20 °C and 23 °C and a humidity of 50–60%. All animal experiments were conducted according to the German animal protection act (TierSchG §§ 7–9) and with permit of the local animal protection committee Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (AZ 84–02.04.2017.A029).

### *Pharmacokinetic studies*

For pharmacokinetic analyses different doses and time points of organ harvesting (plasma, brain, liver, and kidney) were chosen per route of administration: intravenous (i.v.) injection (into the tail vein) 3 mg/kg, 5 min, 20 min, 45 min, 120 min, 180 min, 240 min, 360 min; oral (p.o.) administration (via gavage) 200 mg/kg or 600 mg/kg, 30 min, 45 min, 75 min, 120 min, 180 min, 240 min and 360 min. The oral dose of 200 mg/kg was chosen, because it was used in one of the proof of concept studies (Schemmert et al., 2019). To achieve a higher concentration of RD2 in the brain, we increased the dose by a factor of 3, resulting in a dose of 600 mg/kg.

For each time point, as a standard, three mice per group; in exceptional cases, two or four mice per group were administered with the respective dose. For p.o. administration the physiological saline solution contained either 50mg/mL or 150 mg/mL RD2 and for i.v. administration 0.6 mg/mL RD2. Each mouse received water and food ad libitum, with the exception of the two fasted p.o. groups, which received no food 7 h and water 1 h before the RD2 administration. Approximately 2 min before each organ harvesting time point, the respective mouse was anesthetized with isoflurane (cp-pharma, Germany) inhalation anesthesia and blood was taken by cardiac puncture with a $K_3$-EDTA containing needle before the mouse was sacrificed by cervical dislocation. Blood was centrifuged at 4 °C, 1200 g for 15 min and the plasma was collected. Both brain hemispheres, a part of the liver and the left kidney were taken for each timepoint in order to acquire pharmacokinetic data in all of these organs. Organ samples were weighed and all samples were frozen and stored at – 80 °C until analysis.

### *Extraction and quantification of RD2*

First tissue (brain both hemispheres, kidney or liver) was homogenized 2 × 20 s at 6500 rpm in Precellys tissue homogenizer tubes (Precellys®24, Bertin Instruments, Montigny-le-Bretonneux, France) in 1 ml Tris buffer (pH 8.8, 20 mM Tris, 250 mM NaCl, protease inhibitors

(both Roche, Basel, Switzerland).

To correct the loss of analytes during sample preparation 50 ng/mL ISTD was added. For the extraction of RD2 and the ISTD out of plasma, brain-, kidney- or liver-homogenate 3% TCA were added while vortexing the solution for 10 s. Precipitated proteins were removed by centrifugation at 14,000 g for 5 min at 4 °C. The supernatants containing the peptides were transferred to a low binding autosampler vial and stored at −20 °C.

RD2 plasma and brain concentrations were quantified using a validated liquid chromatography method with mass spectrometric detection (Hupert et al., 2018; Jonas et al., 2026). The method had been qualified in terms of specificity, reproducibility, precision, and robustness according to ICH M10 guideline. RD2 kidney and liver concentrations were quantified using a non-validated liquid chromatography method with mass spectrometric detection. The lower limit of quantification (LLOQ) for plasma had been set to 0.5 ng/mL and for brain to 2 ng/mL. The lower limit of detection (LLOD) for plasma had been set to 2.65 ng/mL and for brain to 0.68 ng/mL. And the upper limit of quantification (ULOQ) for plasma and brain had been set to 500 ng/mL.

In short, an Agilent UHPLC-ESI-QTOF-MS system was used for separation of RD2 from the ISTD and the remaining plasma or brain homogenate components. The UHPLC (Agilent 1290 Infinity series) system consisted of a binary pump, an autosampler, a thermostatted column compartment and a 6550 iFunnel Q-TOF MS with an electrospray ionization (ESI) interface with a resolution of 20,000. Chromatographic separation was performed on an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm particle size; Waters, Milford, USA). Column temperature was kept at 50 °C. Flow rate was 500 µL/min. For plasma separation the mobile phase consisted of solvent A, which was 0.025% HFBA and 0.1% FA in water, and solvent B, which was 0.025% HFBA and 0.1% FA in ACN and for brain homogenate separation the mobile phase consisted of solvent A, which was 0.015% HFBA and 0.2% FA in water, and solvent B, which was 0.015% HFBA and 0.2% FA in ACN.

### *Pharmacokinetic parameters*

For calculation of pharmacokinetic parameters of RD2 for plasma, brain, kidney and liver, concentration- time profiles were analyzed. To determine the RD2 concentrations in kidney and liver, the concentration of RD2 in the residual capillary plasma was taken into account. The literature value of residual capillary plasma for the liver 20.2% and for the kidney 19.1% were used (Kaliss and Pressman, 1950) (Rathi et al., 2024; Dai et al., 2003; Leithold et al., 2016). As animals were sacrificed by heart puncture, only little blood was left in the brain after finalization. Thus, we considered the residual volume of blood in the brain and bound to brain vessels as negligible. Data analysis was performed wherever possible with a two-compartment model using Phoenix WinNonlin (Pharsight, a Certara Company; Radnor, USA) to calculate the maximum concentration ($C_{max}$), time to maximum concentration ($t_{max}$), the area under the curve from the first to the last measured data pair ($AUC_{0\text{-}last}$), the $AUC_{0\text{-}inf}$ (AUC from zero to infinity) represents the total RD2 exposure across time, Clearance (CL) and mean residence time (MRT). The rationale for selecting a two-compartment model was that the plasma concentration-time profile of RD2 exhibited a biphasic decline, reflected by a measurable distribution phase followed by an elimination phase. Missing values (marked "n.a.") could not be calculated by Phoenix WinNonlin, because the two-compartment model did not yield a valid result. If two-compartment model was not applicable, the analysis was performed with Graphpad Prism Software, Inc., (La Jolla, CA, USA). A Grubbs' outlier test was performed with Graphpad Prism Software, Inc., (La Jolla, CA, USA) and one brain and two plasma values were excluded from the analysis (see supplement Table 1). The analyses of the half-life ($t_{1/2}$) are additionally divided into $t_{1/2}$ of the absorption phase ($t_{1/2\ abs}$) and $t_{1/2}$ of the elimination phase of RD2 ($t_{1/2\ elim}$). Brain-to-plasma partition coefficient $K_p$ [%] was determined by following the equation: $K_p[\%] = \frac{AUC_{brain}}{AUC_{plasma}} * 100$. Bioavailability F(%) was determined by following the equation: $: F[\%] = \frac{AUC_{0-inf\ (p.o.)}}{AUC_{0-inf(i.v.)}} \cdot \frac{dose_{iv}}{dose_{po}}$ and $F_{obs}[\%] = \frac{AUC_{obs\ (p.o.)}}{AUC_{obs\ (i.v.)}}$

Due to the small number of animals per time point (n = 2 - 4), the pharmacokinetic data are presented descriptively as mean ± standard deviation (SD). Although the study design -with its very small group sizes of n = 2–4 per time point -was not intended to allow for comprehensive statistical analyses, the total number of animals was large enough and the $C_{max}$ values were normally distributed, so that a two-way ANOVA analysis could be performed (GraphPad Software, Inc., La Jolla, CA, USA).

## Results and discussion

### *Food effect in young wt mice*

Concomitant food intake can influence the pharmacokinetic profile of an orally administered drug through various mechanisms, e.g. by delaying gastric emptying and thereby delaying the absorption, altering the pH in the gastrointestinal tract or changing the luminal metabolism of a drug (Welling, 1977; Welling, 1989). To examine whether the profile of RD2 is altered by food intake, fasted and non-fasted young wt mice were treated orally with two different doses (200 mg/kg and 600 mg/kg) of RD2 and the concentration-time profile of brain, plasma, kidney and liver were determined.

Under non-fasted condition the absorption of the low dose of RD2 into the plasma is delayed ($t_{max}$: 75 min) and reduced ($C_{max}$: 225 ng/mL; $AUC_{0\text{-}inf}$: $26\times10^3$ min*(ng/mL)) in comparison to fasted conditions ($t_{max}$: 30 min, $C_{max}$: 3401 ng/mL; $AUC_{0\text{-}inf}$: $138\times10^3$ min*(ng/mL)), thus leading to lower RD2 concentrations in liver and kidney (Fig. 1, Table 1). However, the kidney remains the main excretory organ of RD2. This is in agreement with an earlier pharmacokinetic study using radioactively-labelled RD2 (Leithold et al., 2016). Although concomitant food intake had a strong effect on RD2 plasma concentration in the low-dose group ($C_{max}$ values were 15 times higher in the fasted ($3.4 \times 10^3$ ng/mL) than in the non-fasted (225 ng/mL) animals), the differences in brain levels were only marginal (fasted: $C_{max}$: 105.9 ng/g vs. non-fasted: $C_{max}$: 128.6 ng/g) (Fig. 2, Table 1).

Interestingly, these differences are no longer present in the mice treated with a high dose (600 mg/kg RD2). RD2 plasma and brain levels are even higher in the non-fasted (plasma $C_{max}$: $6.4\times 10^3$ ng/mL, brain $C_{max}$: 230.3 ng/g), than fasted animals (plasma $C_{max}$: $4.9 \times 10^3$ ng/mL, brain $C_{max}$: 175 ng/g) (Fig. 2, Table 1). Based on the data obtained in this study, we can only speculate as to why concomitant food intake had only an effect on RD2 plasma concentration in lower doses and not in higher doses. Further experiments are needed to clarify the exact mechanism.

### *Dose effect in young wt mice*

In the fasted groups we observed a linear pharmacokinetic profile, since a 3-fold increase in dose resulted in a 3-fold increase in drug exposure (200mg/kg $AUC_{0\text{-}inf}$: $138\times10^3$ min* (ng/mL), 600mg/kg $AUC_{0\text{-}inf}$: $401\times10^3$ min* (ng/mL)). In contrast, the increase in drug exposure in the non-fasting groups is not linearly related to the increase in the administered dose (200mg/kg $AUC_{0\text{-}inf}$: $26\times10^3$ min* (ng/mL), 600mg/kg $AUC_{0\text{-}inf}$: $951\times10^3$ min* (ng/mL)) (Fig. 3, Table 1). A 3-fold increase in dose resulted in a 37-fold increase in drug exposure. However, this difference may be due to the very low plasma concentration in the non-fasted low dosed mice (200mg/kg $C_{max}$: 225 ng/mL; $AUC_{0\text{-}inf}$: $26\times10^3$ min* (ng/mL)), since the plasma concentrations in the high dose are similar in the non-fasted (600mg/kg $C_{max}$: 6.4 $*10^3$ ng/mL; $AUC_{0\text{-}inf}$: $951\times10^3$ min* (ng/mL)) and fasted mice (600mg/kg $C_{max}$: 4.9 $\times 10^3$ ng/mL; $AUC_{0\text{-}inf}$: $401\times10^3$ min* (ng/mL)) (Fig. 3, Table 1). A two-way ANOVA analysis also revealed a significant difference between the

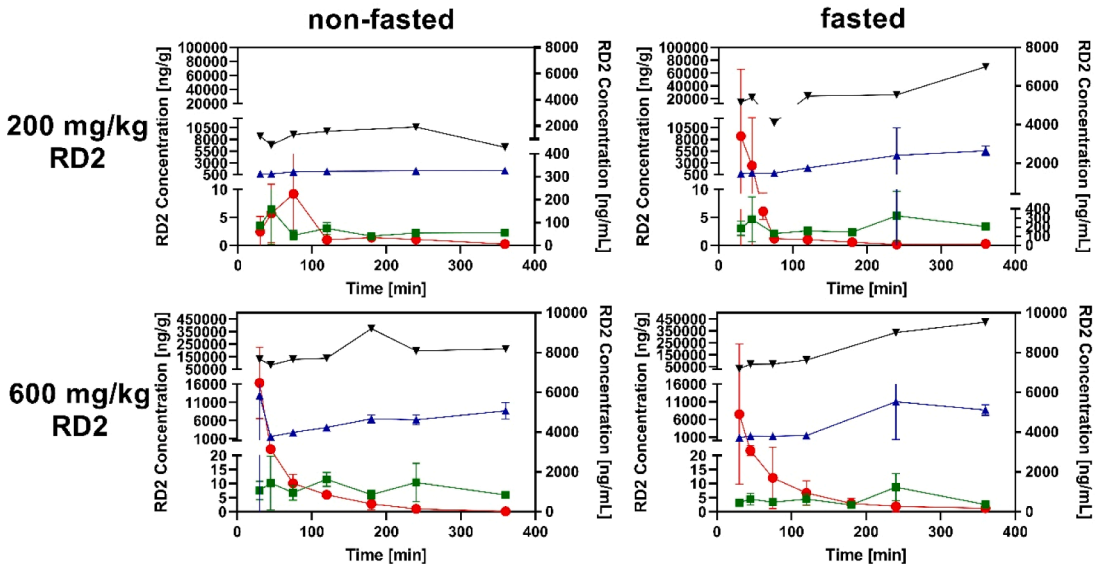


**Fig. 1.** Pharmacokinetic profiles of different doses of RD2 in fasted and non-fasted conditions. Pharmacokinetic concentration-time profiles of RD2 were investigated in brain (green squares), liver (blue triangles), kidney (black triangles) (left y-axis: RD2 concentration in ng/g) and plasma (red circles) (right y-axis: RD2 concentration in ng/ml), after oral (p.o. 200 mg/kg (A, B) and 600 mg/kg (C, D)) administration to wild type mice (male C57Bl/6 N, 11–12 weeks, 3 mice/time point) under non-fasted (A, C) and fasted conditions (B, D).

**Table 1**
Pharmacokinetic parameters of RD2 under fasted and non-fasted conditions and different doses in murine plasma and brain.

| PLASMA<br>**administration route** | | **p.o.** | **p.o.** | **p.o.** | **p.o.** | **i.v.** |
|---|---|---|---|---|---|---|
| **dosis**<br>**condition**<br>**parameter** | **unit** | **200 [mg/kg]**<br>**non-fasted** | **200 [mg/kg]**<br>**fasted** | **600 [mg/kg]**<br>**non-fasted** | **600 [mg/kg]**<br>**fasted** | **3 [mg/kg]**<br>**non-fasted** |
| $C_{max}$ | [ng/mL] | 225 | $3.4 \times 10^3$ | $6.4 \times 10^3$ | $4.9 \times 10^3$ | $5.7 \times 10^3$ |
| $t_{max}$ | [min] | 75 | 30 | 30 | 30 | 5 |
| $AUC_{0\text{-}inf}$ | [min*(ng/mL)] | $26\times10^3$ | $138\times10^3$ | $951\times10^3$ | $401\times10^3$ | $219\times10^3$ |
| $AUC_{0\text{-}last}$ | [min* (ng/mL)] | $18\times10^3$ | $69\times10^3$ | $260\times10^3$ | $299\times10^3$ | $213\times10^3$ |
| CL | [mL/(min*kg] | $7.8 \times 10^3$ | $1.6 \times 10^3$ | 631 | $1.5 \times 10^3$ | 13.7 |
| MRT | [min] | n.a. | n.a. | n.a. | n.a. | 49.8 |
| $t_{1/2(abs)}$ | [min] | 2.1 | 3.5 | 1.8 | 2.4 | n.a. |
| $t_{1/2\ (elim)}$ | [min] | 120.9 | 3.8 | 94.9 | 33.8 | 23.6 |
| F | [%] | 0.2 | 0.9 | 2.2 | 0.9 | n.a. |
| BRAIN | | | | | | |
| $C_{max}$ | [ng/g] | 128.6 | 105.9 | 230.3 | 175 | 114.1 |
| $t_{max}$ | [min] | 45 | 240 | 120 | 240 | 5 |
| $AUC_{0\text{-}last}$ | [min*(ng/g)] | $17\times10^3$ | $24\times10^3$ | $57\times10^3$ | $25\times10^3$ | $18\times10^3$ |
| $K_P$ | [%] | 94.4 | 34.8 | 21.9 | 8.4 | 8.4 |

$C_{max}$: maximum concentration, $t_{max}$: time to maximum concentration, $AUC_{0\text{-}inf}$: area under the curve from zero to infinity, $AUC_{0\text{-}last}$: area under the curve from the first to the last measured data pair, CL: Clearance, MRT: mean residence time, $t_{1/2\ (elim)}$: half-life of the elimination phase, F: Bioavailability, Kp :Brain-to-plasma partition coefficient. Missing values (marked "n.a.") for MRT, could not be calculated for these groups.

200 mg/kg and 600mg/kg doses, in the non-fasted animals, but not in the fasted animals (p:0.004). Fasting results in greater absorption at 200 mg/kg, resulting in a smaller difference compared to 600 mg/kg. Since these differences cannot be explained by physiological changes caused by food consumption, such as fluctuations in the gastric and intestinal pH, delayed gastric emptying, increased bile secretion, and increased splanchnic and hepatic blood flow (Cheng and Wong, 2020), the interaction/binding of RD2 with food itself could be a possible explanation. At lower doses, the free fraction of RD2 may be reduced in the presence of food. At higher doses, unspecific binding sites may become saturated, resulting in a much higher free fraction of RD2 and leading to a disproportionately higher plasma level.

Furthermore, comparison of the $C_{max}$ values of the low and high doses in the fasted animals, indicates a saturation of uptake at early time points. Passive uptake mechanisms are typically not saturable, because they depend solely on the available surface area of the gastrointestinal (GI) tract and the strength of the concentration gradient between the GI tract lumen and the portal vein (Zhang et al., 2021). However, occasionally, with very large doses, absorption can slow down due to the large quantity of the drug present in a confined space.

Another possible explanation for the saturated uptake of RD2 in 600 mg/kg treated fasted animals, could be that RD2 uptake is transporter mediated. If the substrate levels are sufficiently high, drug transporters can become saturated. Once this occurs, no additional drug can be absorbed from the GI tract, even if it is available for transport, and increasing the dose will not increase exposure. This is only a hypothesis that could potentially explain the observed effects and that must be verified in future experiments.

In addition, it should be noted that the $C_{max}$ was reached at the first time point in both the high-dose group (fasted and non fasted) and the fasted low-dose group. An earlier time point would have been required to examine the absorption phase more accurately.

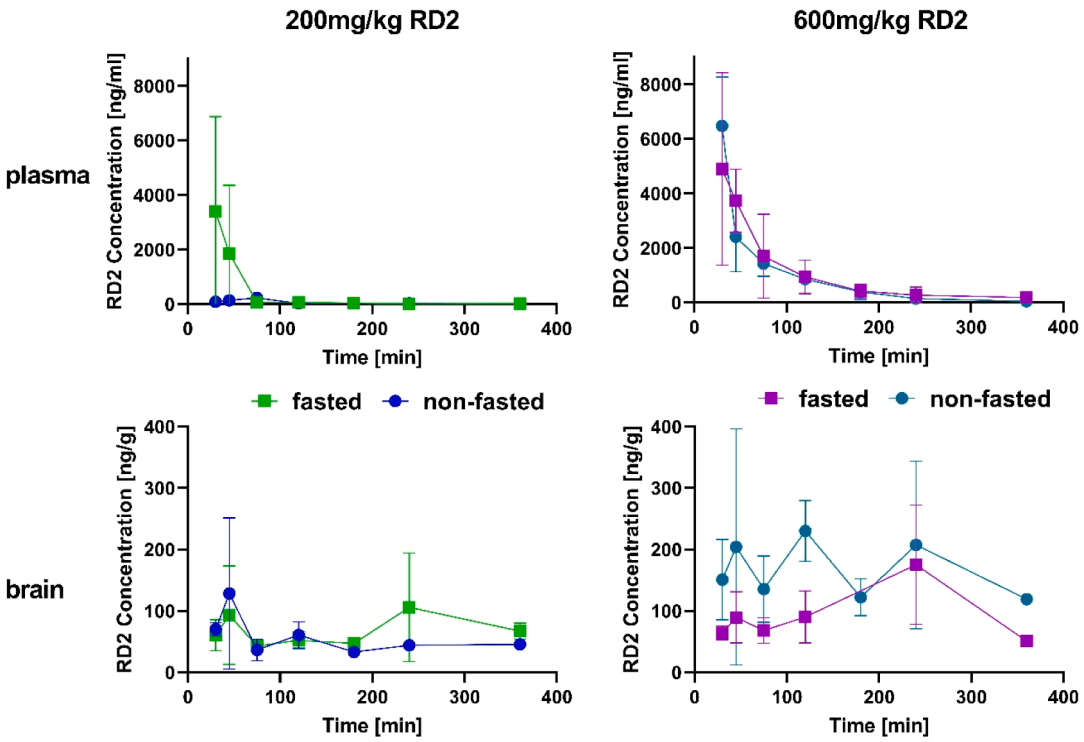


**Fig. 2.** Same data as in Fig. 1 but pairwise comparison of RD2 concentration time profiles in plasma and brain of fasted and male C57Bl/6 N non-fasted animals 11–12 weeks old, 3 mice/time point.

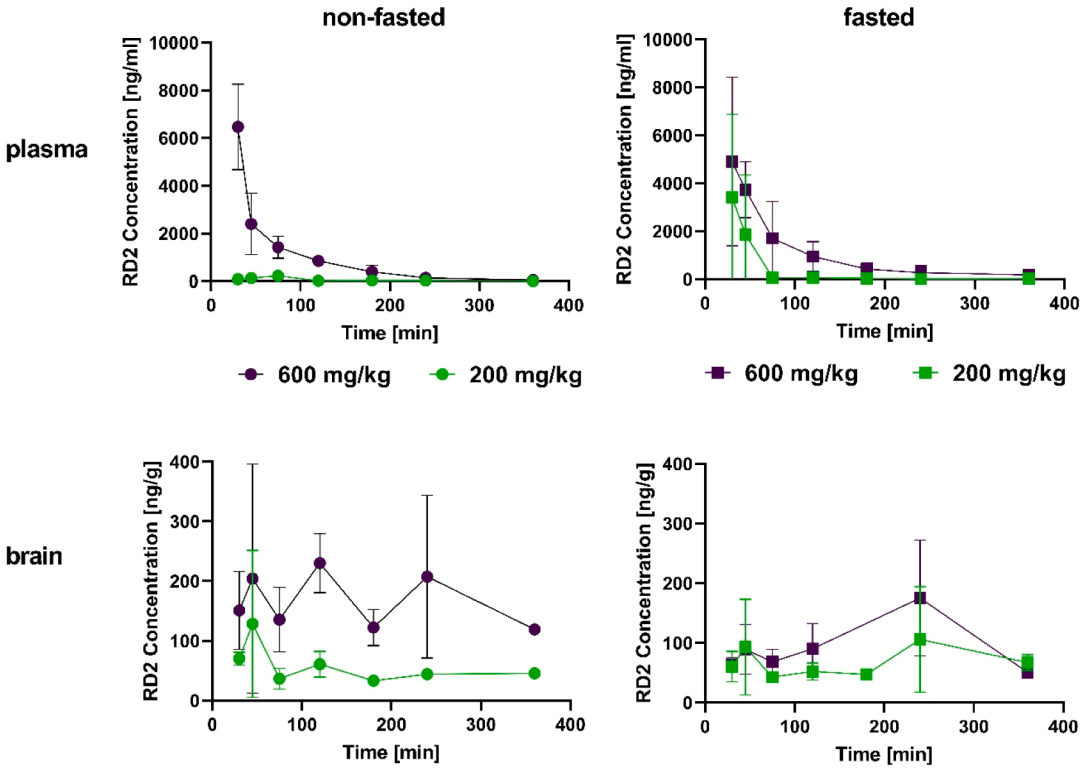


**Fig. 3.** Same data as in Fig. 1 but pairwise comparison of RD2 concentration time profiles in plasma and brain of different RD2 doses under fasted and non-fasted conditions. (male C57Bl/6 N, 11–12 weeks, 3 mice/time point).

*Genotype*

In APP PS1 mice (Ahn et al., 2018) and AD patients (Erickson and Banks, 2013; Zenaro et al., 2017; Sweeney et al., 2018), cerebrovascular impairments and a disturbed BBB were observed, which could lead to increased uptake of compounds into the brain. In addition, also gut

alterations occur at multiple levels in AD, including structural, cellular and functional changes. These include increased intestinal barrier permeability, also known as a 'leaky gut', and loss of tight junctions (Palermo et al., 2025), which could influence the bioavailability of a drug. Based on the results displayed in Fig. 4, it is not possible to make a clear statement as to whether the genotype has an effect on the plasma level of RD2, as the number of individuals in each group is too small. A two-way ANOVA analysis also revealed no statistical differences between gender or genotype. While RD2 $C_{max}$ and $AUC_{0\text{-last}}$ levels ($3.4 \times 10^3$ ng/mL; $310 \times 10^3$ min* (ng/mL)) are increased in male tg mice after oral administration compared to wt mice ($3 \times 10^3$ ng/mL; $151 \times 10^3$ min* (ng/mL)), the opposite is true for female mice (tg $2.3 \times 10^3$ ng/mL; $219 \times 10^3$ min* (ng/mL) vs wt $5.3 \times 10^3$ ng/mL; $417 \times 10^3$ min* (ng/mL)) (Table 2). RD2 brain levels with regard to $C_{max}$ are slightly higher in tg mice compared to wt mice, irrespective of sex or application route (p.o. or i.v.) (Fig. 5) (Table 2). Whereas $AUC_{0\text{-last}}$ values were slightly lower in tg mice after p.o. application than in wt mice and they were higher after i.v. application (Table 2). These small differences, however, may be explained by interindividual variability rather than by an effect of the genotype on the uptake of RD2 into the plasma and brain. This result is in agreement with earlier results comparing the brain uptake of [$^{18}$F]-labelled RD2 in a transgenic AD mouse model in comparison to wt littermates that showed a tendency of higher brain retention in transgenic mice after i.v. administration (Willuweit et al., 2023).

The atypical shape of the curve of the orally treated female wt mice, with its double peak, is probably not a true effect caused by two-phase absorption due to e.g. two absorption sites in the upper and lower intestinal tract, but rather by the high interindividual variability.

Missing values (marked "n.a.") AUC $_{0\text{-inf}}$, CL, $t_{1/2\ (elim,}$ $F_{obs}$ and MRT could not be calculated for these groups. Note the implications (e.g., total exposure may be underestimated by $AUC_{0\text{-last}}$ in these cases).

*Sex differences*

Although APP/PS1 mice are known to have sex differences in brain Aβ concentration, spatial learning ability and memory function, since soluble and insoluble Aβ levels in the brains of female APP/PS1 mice were higher than those of males and the learning and memory abilities of female APP/PS1 mice were poorer than those of males (Li et al., 2016), these differences do not seem to have an impact on plasma levels of RD2, as the pharmacokinetic profile is very similar between male and female APP/PS1 mice after p.o. and i.v. administration. In wt mice oral administration of RD2 resulted in different $t_{max}$ and $C_{max}$ values for male and female. One possible explanation could be the sex differences in upper gastrointestinal motility (Soni et al., 2019), which is slower in female mice than in male mice. This effect is also present in humans, as gastric emptying, small-bowel transit and colonic transit are significantly slower in woman compared to men (Sadik et al., 2003). Alternatively, these deviations could result from interindividual variability between the mice. The lack of differences in RD2 plasma concentrations between male and female wt mice after i.v. administration possibly suggests that there are no sex differences in distribution of RD2. However, the small number of individuals in each group (a consequence of destructive PK sampling) limits the ability to detect differences in sex and genotype, therefore these findings should be considered preliminary rather than definitive.

*Age*

Pharmacokinetic changes occur with increasing age. In this study higher plasma and brain levels were observed in young wt mice (plasma $C_{max}$: $4.9 \times 10^3$ ng/mL; brain $C_{max}$: 175 ng/g) (Table 1) compared to old wt mice (plasma $C_{max}$: $3 \times 10^3$ ng/mL; brain $C_{max}$: 94.4 ng/g) (Table 2). Since this effect is also present after i.v. administration (young wt mice plasma $C_{max}$: $5.7 \times 10^3$ ng/mL; brain $C_{max}$: 114.1ng/g, compared to old

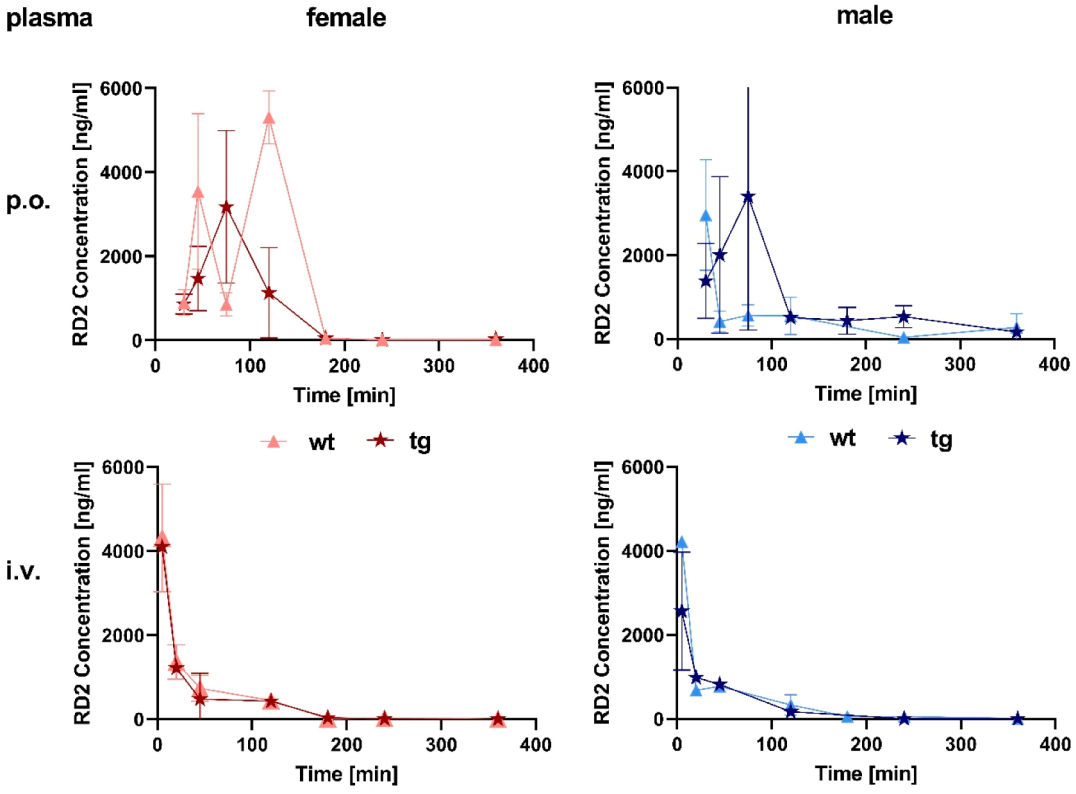


**Fig. 4.** Pairwise comparison of RD2 concentration time profiles in plasma of RD2 in tg APPswe/PS1ΔE9 and age matched wt mice of both gender tg after p.o. (600 mg/kg) and i.v. (3 mg/kg) administration. (age 20–30 months, p.o.: 2–4 mice/time point; i.v. 1–3 mice/time point; under fasted conditions).

**Table 2**
Pharmacokinetic parameters of RD2 in plasma and brain of both sexes of tg APPswe/PS1ΔE9 and age matched wt mice.

| PLASMA | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **administration route** | | **p.o.** | **p.o.** | **p.o.** | **p.o.** | **i.v.** | **i.v.** | **i.v.** | **i.v.** |
| **dosis** | | **600 [mg/kg]** | **600 [mg/kg]** | **600 [mg/kg]** | **600 [mg/kg]** | **3 [mg/kg]** | **3 [mg/kg]** | **3 [mg/kg]** | **3 [mg/kg]** |
| **sex** | | **male** | **male** | **female** | **female** | **male** | **male** | **female** | **female** |
| **genotype** | | **tg** | **wt** | **tg** | **wt** | **tg** | **wt** | **tg** | **wt** |
| **parameter** | **unit** | | | | | | | | |
| $C_{max}$ | [ng/mL] | $3.4 \times 10^3$ | $3 \times 10^3$ | $2.3 \times 10^3$ | $5.3 \times 10^3$ | $3 \times 10^3$ | $3.6 \times 10^3$ | $5 \times 10^3$ | $3.6 \times 10^3$ |
| $t_{max}$ | [min] | 75 | 30 | 75 | 120 | 5 | 5 | 5 | 5 |
| $AUC_{0\text{-}inf}$ | [min*(ng/mL)] | n.a. | n.a. | n.a. | n.a. | $121 \times 10^3$ | n.a. | $112 \times 10^3$ | $119 \times 10^3$ |
| $AUC_{0\text{-}last}$ | [min* (ng/mL)] | $310 \times 10^3$ | $151 \times 10^3$ | $219 \times 10^3$ | $417 \times 10^3$ | $99 \times 10^3$ | $116 \times 10^3$ | $112 \times 10^3$ | $136 \times 10^3$ |
| CL | [mL/(min*kg] | n.a. | n.a. | n.a. | n.a. | 24.8 | n.a. | 26.8 | 25.3 |
| MRT | [min] | n.a. | n.a. | n.a. | n.a. | 36.9 | n.a. | 32.7 | 47.5 |
| $t_{1/2\ (elim)}$ | [min] | n.a. | n.a. | n.a. | n.a. | 4.5 | n.a. | 8.9 | 24.4 |
| $F_{obs}$ | [min] | 1.6 | 0.7 | 1.0 | 1.5 | n.a. | n.a. | n.a. | n.a. |
| BRAIN | | | | | | | | | |
| $C_{max}$ | [ng/g] | 109.1 | 94.4 | 107 | 81.3 | 138:3 | 60.8 | 62.8 | 50.7 |
| $t_{max}$ | [min] | 240 | 75 | 75 | 75 | 120 | 45 | 20 | 5 |
| $AUC_{0\text{-}last}$ | [min*(ng/g)] | $22 \times 10^3$ | $20 \times 10^3$ | $17 \times 10^3$ | $16 \times 10^3$ | $10 \times 10^3$ | $7.2 \times 10^3$ | $14 \times 10^3$ | $8.8 \times 10^3$ |
| $K_P$ | [%] | 7.1 | 13.3 | 7.8 | 3.8 | 10.1 | 6.2 | 12.5 | 5.3 |

$C_{max}$: maximum concentration, $t_{max}$: time to maximum concentration, $AUC_{0\text{-}inf}$: area under the curve from zero to infinity, $AUC_{0\text{-}last}$: area under the curve from the first to the last measured data pair, CL: Clearance, MRT: mean residence time, $t_{1/2\ (elim)}$: half-life of the elimination phase, $F_{obs}$: Bioavailability, Kp :Brain-to-plasma partition coefficient.

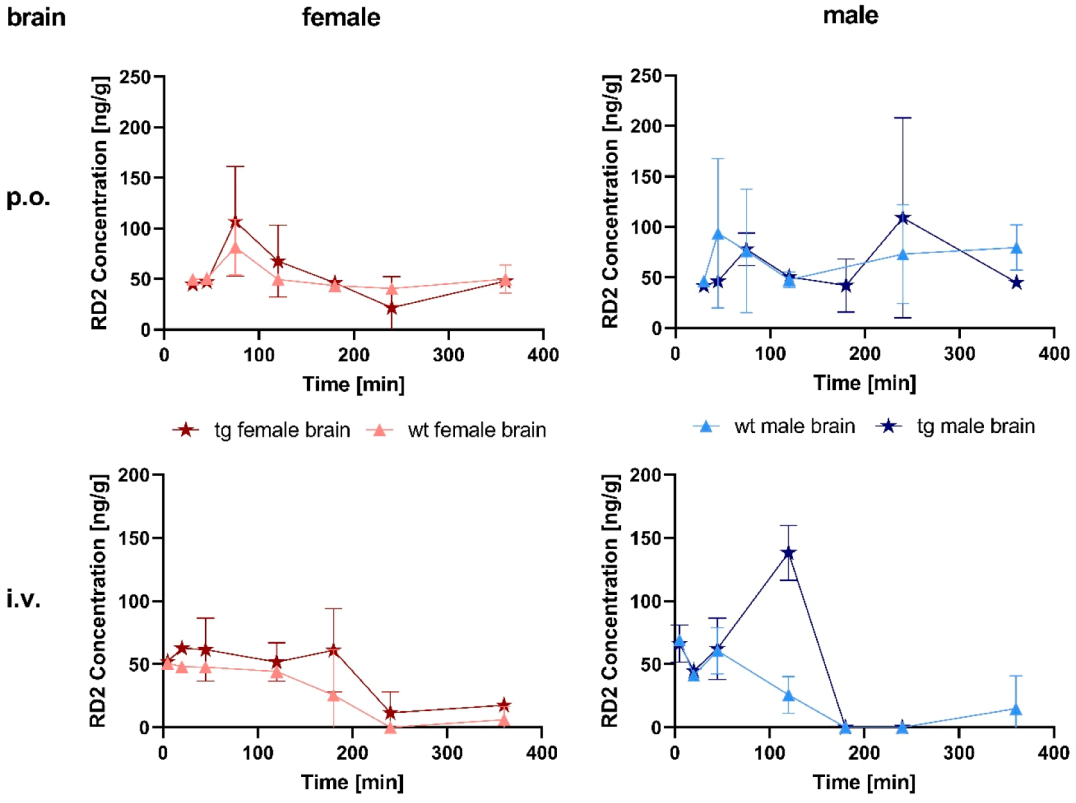


**Fig. 5.** Pairwise comparison of RD2 concentration time profiles in brain of RD2 in tg APPswe/PS1ΔE9 and age matched wt mice of both sexes after p.o. (600 mg/kg) and i.v. (3 mg/kg) administration. (age 20–30 months, p.o.: 2–4 mice/time point; i.v. 1–3 mice/time point; under fasted conditions).

wt mice plasma $C_{max}$: $3.6 \times 10^3$ ng/mL; brain $C_{max}$: 60.8 ng/g), an altered absorption cannot be the only cause (Table 1, Table 2, Fig. 6, Fig. 7, Fig. 8).

Age-related changes in the amount of plasma proteins and thus in the plasma protein binding capacity, which influences the free plasma fraction of a drug, could be one possible explanation for these effects. Since human serum albumin (HSA) levels generally decrease in the elderly, while human α1-acid glycoprotein (AGP) levels are not altered per se by age (Grandison and Boudinot, 2000; Veering et al., 1990), a change in the free fraction could occur for RD2, which mainly binds to MSA (supplement Figure 1) or HSA, rather to human (Leithold et al., 2016; Leithold et al., 2016; Veering et al., 1990; Veering et al., 1990) or murine AGP (supplement Figure 1).

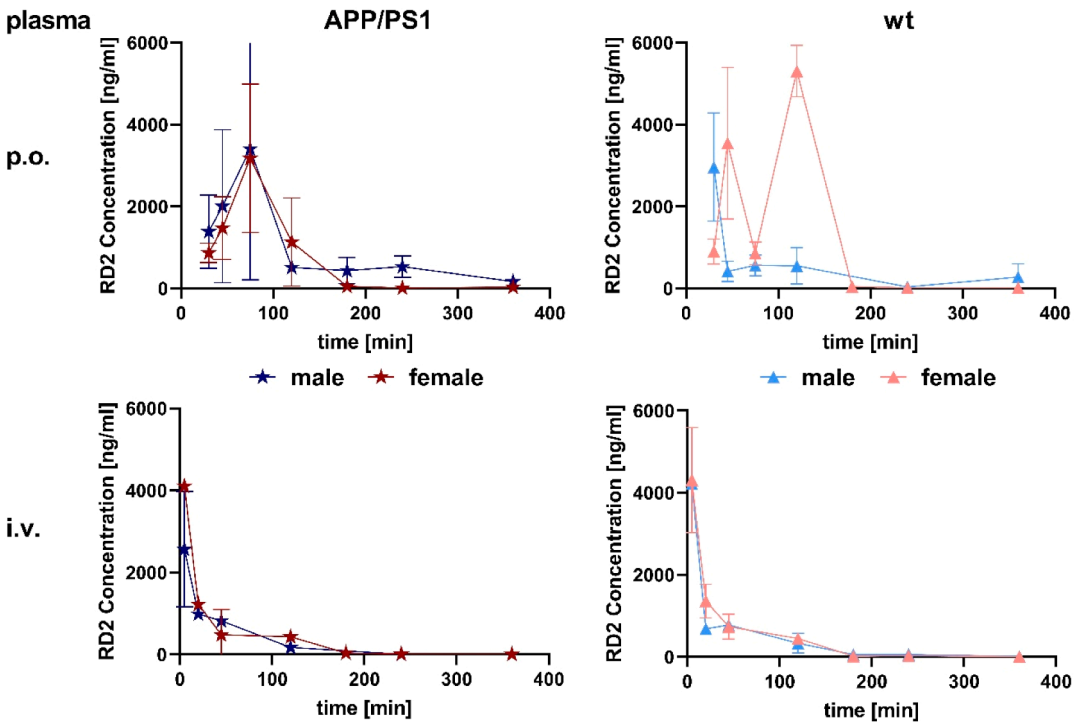


**Fig. 6.** Same data as in Fig. 4 but pairwise comparison of RD2 concentration time profiles in plasma of RD2 in male and female tg APPswe/PS1ΔE9 and age matched wt mice after p.o. (600 mg/kg) and i.v. (3 mg/kg) administration. (age 20–30 months, p.o.: 2–4 mice/time point; i.v. 1–3 mice/time point, under fasted conditions).

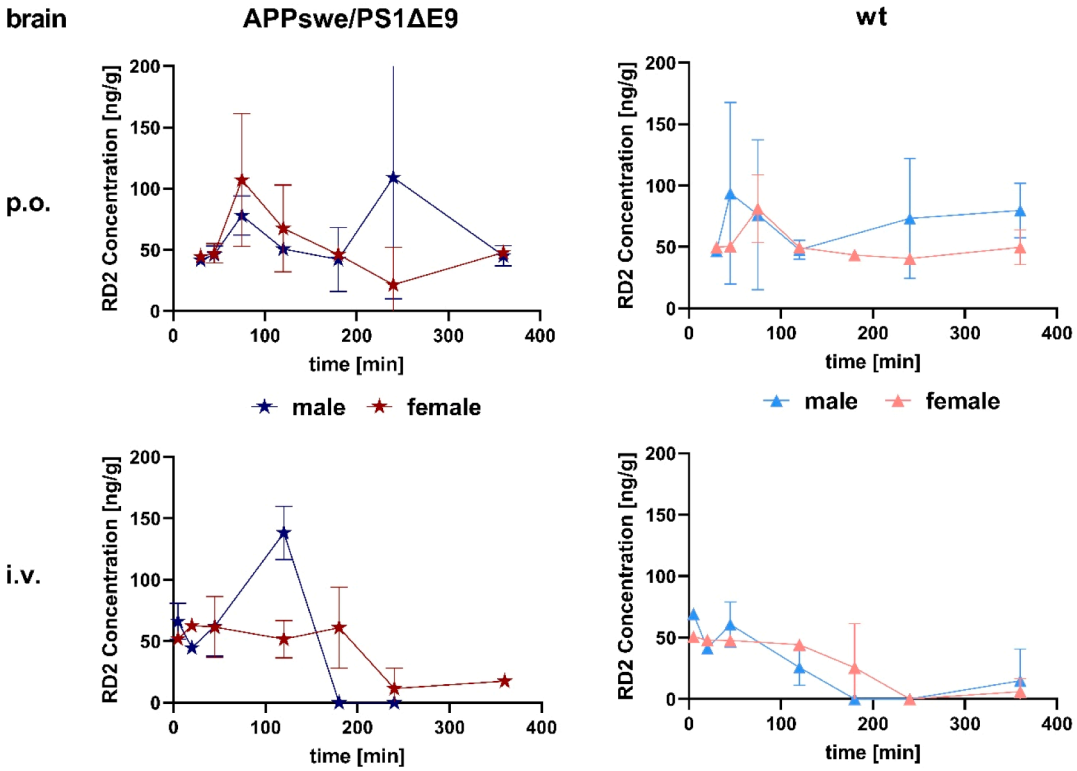


**Fig. 7.** Same data as in Fig. 5 but pairwise comparison of RD2 concentration time profiles in brain of RD2 in male and female tg APPswe/PS1ΔE9 and age matched wt mice after p.o. (600 mg/kg) and i.v. (3 mg/kg) administration. (age 20–30 months, p.o.: 2–4 mice/time point; i.v. 1–3 mice/time point; under fasted conditions).

## Discussion and conclusion

Different parameter including age, sex, genotype and food can have an effect on the pharmacokinetics of a compound (Stader and Marzolini, 2022; Mangoni and Jackson, 2004). For example, the simultaneous intake of a compound and food can strongly impact compound release, absorption, distribution, metabolism and/or elimination, and consequently, the efficacy and safety of the compound (Koziolek et al., 2019).

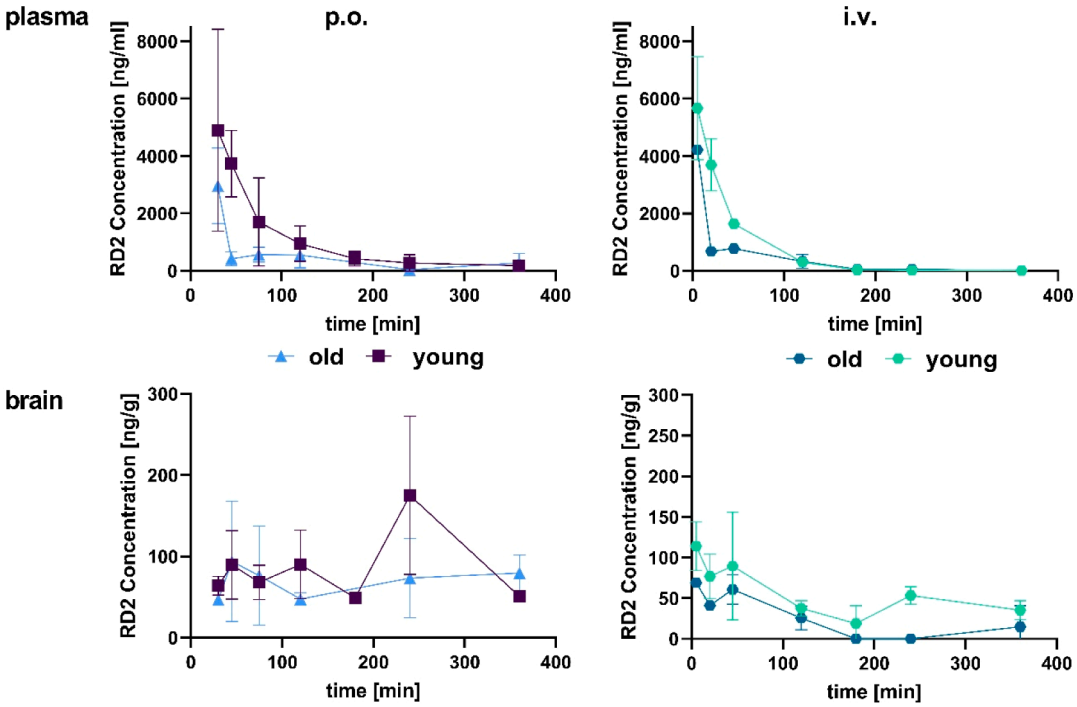


**Fig. 8.** Pairwise comparison of RD2 concentration time profiles in plasma and brain of RD2 in young (11–12 weeks) and old (20–30 month) wt male mice (p.o.: young 3 mice/time point; old: 2–3 mice/time point; i.v. young: 3 mice/time, old: 1–3 mice/time point) after p.o. (600 mg/kg) and i.v. (3 mg/kg) administration under fasted conditions. (Data are partly already shown in Fig. 3,6,7).

Therefore, food-drug interactions are one of the main challenges in the administration of oral drugs. To investigate whether the pharmacokinetics of RD2 are influenced by these parameters, this study examined male and female transgenic APPswe/PS1ΔE9 (APP PS1) and wild-type (wt) mice under fed and fasted conditions.

Due to the small number of individuals in each group (a consequence of destructive PK sampling) the ability to detect differences in sex and genotype was very limited and the results show no clear effect of sex or genotype; therefore, these findings should be considered preliminary rather than definitive. However, these results are in line with the results of a randomized, double-blind, single-center phase 1b trial with 20 mild neurocognitive impairment (MCI) patients aged between 50 and 80 years, which received 300 mg PRI-002/RD2 once daily (q.d.) or placebo for 28 days. In this study also no correlations were found between any pharmacokinetic parameter and sex, age, or weight of patients (Kutzsche et al., 2025). However, the comparison of the mean $C_{max}$ value (19.6 ng/ml) of the healthy male subjects (19–45 years of age), which received a single dose of 320 mg PRI-002/RD2 in the Single Ascending-dose Phase I Study (First-in-human) (Kutzsche et al., 2020) to the MCI patients from the phase 1b trial mean $C_{max}$ 4.46 ng/ml might probably indicate an effect of age on this parameter. Nevertheless, when making this comparison, it is important to take into account that first the dose was not exactly the same (300 mg/kg vs. 320 mg/kg) and - more importantly - the MCI patients were allowed to eat, whereas the healthy volunteers had to be fasting before taking PRI-002/RD2. This would fit very well to the major findings of this study that previous food consumption substantially impacts the bioavailability of RD2, whereas age has only a minor influence. Therefore, the effect of concomitant food may also be relevant in AD patients, as it possibly increases the variability of plasma and brain levels, this may also be of interest for the clinical development of the drug.

## Declaration of conflicting interests

DW and AW are cofounders and DW is co-owner of Priavoid GmbH which holds IP of RD2/PRI-002. DW, AW and SaS are employees of Priavoid GmbH. DW is co-inventor of RD2/PRI-002. This did not have any influence on the interpretation of data. All other authors have no conflict of interest to report.

## Ethics declaration

This study was conducted in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. This study was approved by the local animal protection committee Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (Approval No AZ 84–02.04.2017.A029).

## CRediT authorship contribution statement

**Luana Cristina Camargo:** Writing – review & editing, Methodology, Investigation. **Alissa Jonas:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Ian Gering:** Writing – review & editing, Methodology, Investigation. **Sarah Schemmert:** Writing – review & editing, Investigation. **Dominik Jacobs:** Writing – review & editing, Investigation. **Maja Schäfer:** Writing – review & editing, Investigation. **Dieter Willbold:** Writing – review & editing, Resources, Conceptualization. **Antje Willuweit:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Janine Kutzsche:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Formal analysis, Data curation, Conceptualization.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2026.107649.

## Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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