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DRUG METABOLISM & DISPOSITION

Disposition into Adipose Tissue Determines Accumulation and Elimination Kinetics of the Cholesteryl Ester Transfer Protein Inhibitor Anacetrapib in Mice

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ABSTRACT

The cholesteryl ester transfer protein (CETP) inhibitor anacetrapib exhibits a long terminal half-life ($t_{½}$) in humans; however, the dispositional mechanisms that lead to this long $t_{½}$ are still being elucidated. As it is hypothesized that disposition into adipose tissue and binding to CETP might play a role, we sought to delineate the relative importance of these factors using a preclinical animal model. A multiple-dose pharmacokinetic study was conducted in C57BL6 wild-type (WT) lean, WT diet-induced obese (DIO), natural flanking region (NFR) CETP-transgenic lean, and NFR-DIO mice. Mice were dosed orally with 10 mg/kg anacetrapib daily for 42 days. Drug concentrations in blood, brown and white adipose tissue, liver, and brain were measured up to 35 weeks postdose. During dosing, a 3- to 9-fold accumulation in 72-hour postdose blood concentrations of anacetrapib was observed. Drug concentrations in white adipose tissue accumulated ~20- to 40-fold, whereas 10- to 17-fold accumulation occurred in brown adipose and approximately 4-fold in liver. Brain levels were very low (<0.1 μM), and a trend of accumulation was not seen. The presence of CETP as well as adiposity seems to play a role in determining the blood concentrations of anacetrapib. The highest blood concentrations were observed in NFR DIO mice, whereas the lowest concentrations were seen in WT lean mice. In adipose and liver tissue, higher concentrations were seen in DIO mice, irrespective of the presence of CETP. This finding suggests that white adipose tissue serves as a potential depot and that disposition into adipose tissue governs the long-term kinetics of anacetrapib in vivo.

Introduction

Cholesterol-lowering drugs are currently the most widely used therapeutics for the treatment of atherosclerosis and dyslipidemia. In addition to low-density lipoprotein (LDL)-lowering agents such as statins, novel therapeutics are being developed that target the cholesterol balance and turnover through alternate mechanisms. The cholesteryl ester transfer protein (CETP) appears to be a promising pharmacologic target, as it governs the equilibrium between LDL and high-density lipoprotein (HDL) cholesterol, and inhibition of CETP is thought to provide an effective mechanism that is able to shift the cholesterol balance toward the "good" HDL cholesterol (Linsel-Nitschke and Tall, 2005; Tall, 2007).

The validity of HDL as a surrogate for cardiovascular risk has recently been questioned (Tuteja and Rader, 2014), and initial efforts to develop CETP inhibitors for increasing HDL levels clinically have been hampered either by unwanted side effects, such as blood pressure increases caused by Pfizer's torcetrapib (Barter et al., 2007), or by lack of efficacy, as was the case for Roche's dalcetrapib (Schwartz et al., 2012) and Lilly's evacetrapib (Nicholls et al., 2015). Nonetheless, pharmaceutical companies continue to invest significant resources into the development of safe and effective CETP inhibitors. To assess whether patients treated with CETP inhibitors will have a long-term benefit (e.g., by lowering the risk of cardiovascular events and thus decreasing mortality), long-term outcome trials of Merck's investigational compound anacetrapib (MK-0859, Fig. 1) are ongoing.

Anacetrapib thus far has been shown to have a favorable safety and efficacy profile in humans. In addition to lowering LDL and raising HDL, the phase 3 Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib trial demonstrated that CETP inhibition by anacetrapib was well tolerated and that anacetrapib was devoid of torcetrapib-like liabilities such as blood pressure increases (Krishna et al., 2008; Bloomfield et al., 2009; Gotto et al., 2014 a,b); however, anacetrapib exhibits complex accumulation and washout kinetics in circulation in humans. As the clinical development of anacetrapib progressed and patients were treated for longer duration, the $t_{½}$ appeared to be longer than had been predicted from initial studies with shorter treatment duration. (Gutstein et al., 2012; Gotto et al., 2014a; Merck internal data)

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ABBREVIATIONS: AUC, area under the concentration-time curve; CETP, cholesteryl ester transfer protein; DIO, diet-induced obese; HDL, high-density lipoprotein; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LDL, low-density lipoprotein; NFR, natural flanking region (CETP transgene); P-gp, P-glycoprotein; $t_{½}$, half-life; WT, wild-type.

Understanding the causes underlying the long $t_{½}$ is crucial to fully characterizing the pharmacokinetic properties of anacetrapib. Given the neutral lipophilic properties of anacetrapib, the high affinity to adipose tissue might contribute to the accumulation and retention of the drug in the body, such that a "drug depot" might be formed in the adipose upon multiple dosing, which would ultimately drive systemic pharmacokinetics and be responsible for the prolonged terminal half-life ($t_{½}$) in plasma. There is a considerable knowledge gap regarding how adipose disposition correlates to the long $t_{½}$, and the question remains whether binding to plasma CETP would also be a determinant of plasma $t_{½}$. To gain further insight into the long terminal $t_{½}$ of anacetrapib, we established a preclinical model to delineate the impact of adiposity and CETP binding on the accumulation and washout kinetics of anacetrapib.

Since rodents naturally lack CETP, it was necessary to use a transgenic animal model to examine the impact of CETP. To that end, a preclinical in vivo experiment was conducted using wild-type (WT) mice (lacking human CETP) and CETP transgenic mice (expressing human CETP transgene) that were fed either a normal or a high-fat diet to increase adiposity. Mice bearing the CETP transgene of the natural flanking region (NFR) have been well characterized (Masucci-Magoulas et al., 1995). Furthermore, diet-induced obese (DIO) mice (fed on high-fat diet for 2 months) have previously been described as animal models to investigate the impact of adiposity on animal physiology (for a representative example, see Li et al., 2010). Our hypothesis was that pharmacokinetic and tissue distribution kinetics (in particular into the adipose tissue) in the lean and DIO WT and NFR mice would help define the relative roles of CETP binding and adipose accumulation in the long $t_{½}$ phenomenon.

Material and Methods

Chemicals and Dose Preparation. Anacetrapib (MK-0859) was provided by the GMP Group, Process Chemistry, Merck Research Laboratories. The oral dose formulation was prepared as an emulsion as follows. The compound was dissolved in Imwitor-742:Tween-80 (1:1, w/w) at a starting concentration of 10 mg/ml and sonicated for 25 minutes. The preparation was diluted 1:4 with water to yield a final concentration of 2 mg/ml, vortexed, sonicated for 5 minutes, then stirred before dosing.

Animal Studies. All experiments were performed according to procedures approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. Male C57BL/6 mice (obtained from Taconic) of different genotypes and treatment conditions (henceforth termed *subgroups*) were used in this study: WT-lean, WT diet-induced obese (WT-DIO), CETP NFR-transgenic lean (NFR-lean), and CETP NFR-transgenic diet-induced obese (NFR-DIO). Obesity was induced in mice by feeding Rodent Diet with 60% kcal% fat (product no. D12492; Research Diets Inc., New Brunswick, NJ) for 12 weeks starting at 8 weeks of age. All mice were 20 weeks old at the start of dosing, and the average body weights of lean and DIO mice were ~30 and ~45 g, respectively.

A diagram of the study design is depicted in Fig. 2. Anacetrapib was administered orally (via gastric gavage) once daily at a dose of 10 mg/kg for 6 weeks (42 days) to the aforementioned subgroups of mice under fasted conditions ($n = 50$ per subgroup at the time of initial dosing). A dose of 10 mg/kg per day was selected, as it was anticipated to attain systemic exposures comparable to a therapeutic human dose of 100 mg per day. On days 1, 15, 28, and 42 during the daily dosing period, five mice per subgroup underwent serial blood sampling (1, 2, 4, 7, 24, 48, and 72 hours after oral dosing) for a full blood pharmacokinetic profile on the respective sampling day. At the terminal time point (72 hours), the mice were sacrificed, and liver, brain, brown adipose tissue, and epididymal white adipose tissue were collected and snap-frozen in liquid nitrogen. Whole blood was also collected for measurement of trough drug concentrations (C_{72h}).

At 4, 10, 16, 24, and 35 weeks after cessation of dosing (henceforth termed *postdose* period, corresponding to 70, 112, 154, 210, and 287 days since the beginning of daily dosing), five mice per subgroup were sacrificed, and whole blood, liver, brain, brown adipose tissue, and epididymal white adipose tissue were collected at a single time point and processed as described already.

Sample Preparation and Bioanalysis. Anacetrapib and its [^{13}C-D$_3$]-stable labeled internal standard were extracted from all matrices via liquid-liquid extraction with 80:20 hexane/isopropanol after pH adjustment with pH 9.8 carbonate buffer. Extracts were dried under nitrogen and reconstituted in 50:50:0.1 acetonitrile/water/formic acid for injection to the high-performance liquid chromatography-MS/MS system. Blood was processed as received. White adipose and brown adipose tissue were homogenized with the addition of nine volumes of control CD-1 mouse plasma for an overall dilution factor of 10-fold. Brain tissue was homogenized with the addition of four volumes of control CD-1 mouse plasma for an overall dilution factor of 5-fold. Liver tissue was homogenized with 3 volumes of control CD-1 mouse plasma for an overall dilution factor of 4-fold.

The high-performance liquid chromatography system employed a Waters Acquity autosampler system, which included binary solvent manager, sample manager, and sample organizer. Separation was achieved on a Waters Acquity ultra-performance LC BEH Shield RP18 2.1 × 50-mm (1.7-μm particle size) column with an isocratic flow of 80/20/0.1 (v/v/v) acetonitrile/water/formic acid at 0.6 ml/min. Aliquots of 2–10 μl were injected.

Detection of the analytes and internal standard was achieved using a Sciex API 4000, 5000, or 5500 triple quadrupole MS/MS system equipped with a TurboIonSpray ionization source operated in the positive ion mode. Multiple reaction monitoring ion transitions at retention times unique to each compound were monitored as follows for the respective [M + H] + ions: anacetrapib (m/z 638.1 → 283.1) and internal standard (642.1 → 287.1). Both analytes had a retention time of 0.7–0.9 minutes.

Software and Calculations. Chromatographic data were acquired and peak areas integrated using the Sciex Analyst software (Version 1.4.2, AB Sciex, ON, Canada). Concentration data for the calibration standards, QC samples, and study samples were determined using Watson LIMS (Version 7.3; Thermo LabSystems Inc., Philadelphia, PA).The peak area ratios of analyte to IS were plotted as a function of the nominal concentrations of analyte. Standard calibration curves were established in each matrix with ranges from 0.25 to 1000 ng/ml for MK-0859 low-concentration matrices and 10 to 10,000 ng/ml for high-concentration matrices and were constructed using linear (weighted $1/\times^2$) regression. The equation of the calibration curve(s) was used to back calculate the concentrations of anacetrapib in QC and unknown samples.

Pharmacokinetic Calculations. For the full pharmacokinetic profiles obtained on days 1, 15, 28, and 42 during daily dosing, pharmacokinetic parameters were calculated using established noncompartmental methods. The area under the plasma concentration versus time curve (AUC) was determined using the WinNonlin Software (version 5.1; Certara, Princeton, NJ) whereby the linear trapezoidal method was used for ascending concentrations, and the log-trapezoidal method was used for descending concentrations. The portion of the AUC from the last measurable concentration to infinity was estimated by

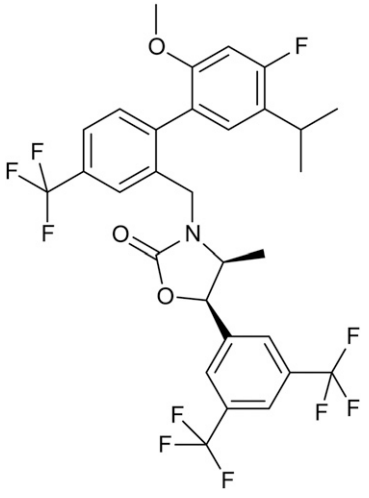

Fig. 1. Chemical structure of anacetrapib (MK-0859).

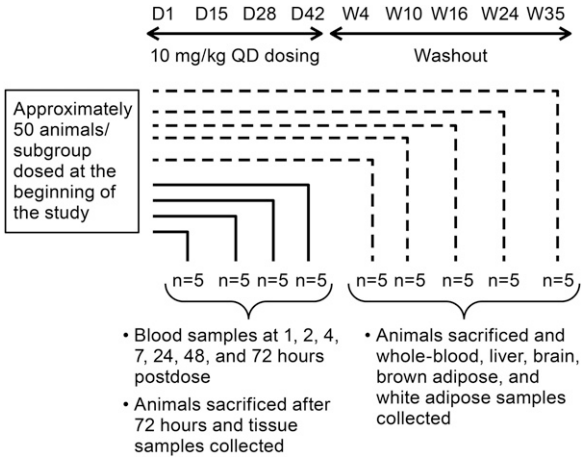

Fig. 2. Study design and sampling regimen for dosing (days 1–42) and washout period (postdose weeks 4–35).

C_t/k_e, where C_t represents the last measurable concentration and k_e is the elimination rate constant. The latter was determined from the concentration versus time curve by linear regression at the terminal phase of the semi-logarithmic plot. The terminal $t_{½}$ was calculated as $0.693/k_e$.

For the period after cessation of daily dosing (postdose period), the washout profiles were obtained by plotting the mean blood and tissue concentrations from the single time-point collections on weeks 4, 10, 16 (for blood: 18), 24, and 35 postdose, corresponding to 70, 112 (for blood: 126), 154, 210, and 287 days total time from beginning of the daily dosing period. The estimated mean terminal half-lives from the postdose period were determined from the terminal slope estimated from the last three data points (unless otherwise specified).

Results

Pharmacokinetics of Anacetrapib during 42-Day Dosing. Anacetrapib was dosed orally for 42 days in WT lean, WT DIO, NFR lean, and NFR DIO mice. From the pharmacokinetic data collected over the dosing period, moderate accumulation was seen in the systemic exposures for all subgroups, with up to 1.8-fold increases in mean $AUC_{0–24h}$ and less than 1.5-fold increases in mean C_{max} (Fig. 3; Table 1). The average 72-hour blood concentrations were 3- to 9-fold higher on day 42 compared with day 1. On day 15 and day 28, the 72-hour blood concentrations were 2-fold to 7-fold higher compared with day 1 (data not shown).

Accumulation and Elimination Kinetics of Anacetrapib in Mouse Blood and Tissues. Upon daily dosing for 42 days, the subgroups of mice exhibited differences in terms of the accumulated 72-hour concentrations in blood and tissues (Fig. 4). In blood, mean anacetrapib 72-hour concentrations on day 42 were below 0.04 μM in WT lean mice, whereas WT DIO and NFR lean mice exhibited mean concentrations of 0.33 μM and 0.12 μM, respectively, and the combination of adiposity and presence of plasma CETP in the NFR DIO mice resulted in mean anacetrapib concentrations of 0.47 μM on day 42 (Fig. 4). In WT lean mice, blood concentrations in most animals were below the limit of quantitation by 24 weeks after cessation of treatment. Small but measurable amounts of drug were present in blood 35 weeks after cessation of treatment in the other three subgroups. Approximately 7%–11% of the day 42 concentrations remained in the blood of WT DIO and NFR lean mice, whereas 25% of day 42 levels remained in NFR DIO mice.

Substantial drug accumulation in adipose tissue was observed over the 42-day dosing period, with the highest levels observed in brown and white adipose tissues of WT DIO and NFR DIO mice (Fig. 5). Trough levels in these tissues were in the range of 400 and 100 μM, respectively. WT lean and NFR lean mice had about 3- to 4-fold lower levels than their DIO counterparts. Compared with day 1 of dosing, drug levels in white adipose tissue were accumulated approximately 20-fold in WT lean, WT DIO, and NFR lean mice and 40-fold in NFR DIO mice (Fig. 5).

Of note, the white adipose tissue concentrations in WT DIO and NFR DIO mice appeared to be initially increasing after cessation of dosing, such that the mean levels during 4–16 weeks postdose were approximately 20% higher than the mean trough levels on day 42 (Fig. 5). At the end of the 35-week postdose period, 40%–60% of the day 42 levels remained in the white adipose of WT lean and NFR lean mice. In white adipose of the WT DIO and NFR DIO mice, the residual levels were equivalent to 80% or more of the day 42 levels.

By comparison, the washout from brown adipose tissue was faster. After a 10- to 17-fold accumulation during dosing, a clear decrease in drug levels in the postdose period was seen in all groups of mice (Fig. 5), such that the residual levels at 35 weeks postdose were about 5% of the day 42 levels.

In liver, drug accumulation was in the range of 2- to 4-fold. Drug levels on day 42 in the livers of DIO mice were in the range of 29–42 μM, about 100-fold higher than those in livers of lean animals (Fig. 6).

As the PK profiles of NFR mice were overlapping with those of WT mice (which was the case for lean as well as DIO mouse groups), it appears that the presence of CETP (unlike the degree of adiposity) did not impact the accumulation of the drug in adipose and liver.

Levels of anacetrapib in brain were generally very low (<0.1 μM), and were subject to high interanimal variability, such that a clear trend of accumulation could not be seen (Fig. 7). The residual drug levels at

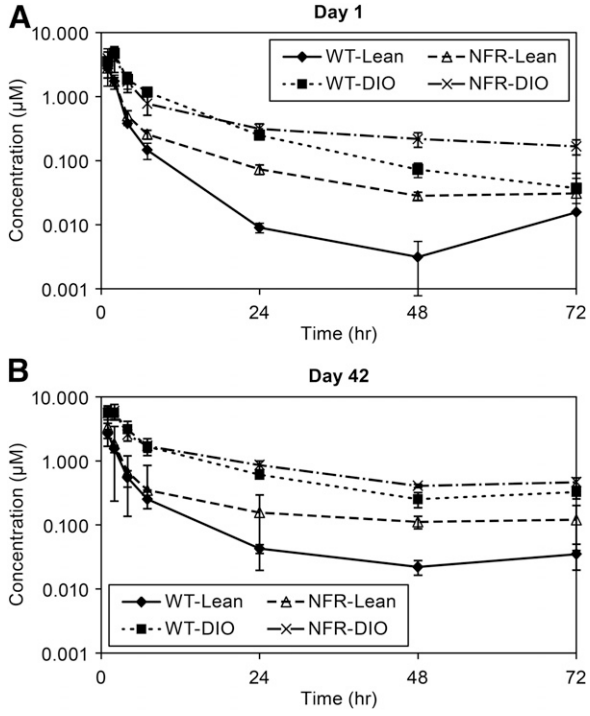

Fig. 3. Blood concentration-time profiles of anacetrapib in mice on day 1 and day 42 during daily dosing at 10 mg/kg. Anacetrapib was dosed once daily to WT-lean, WT-DIO, NFR-lean, and NFR-DIO mice at 10 mg/kg for 42 days. After dosing on days 1 and 42, n = 5 mice per subgroup underwent serial blood sampling for profiling of full concentration-time course (1–72 hours) as described in *Methods*. No additional doses of anacetrapib were given to these animals during the time course. Blood concentrations of anacetrapib were measured by LC-MS/MS as described in *Methods*. The data points represent mean and S.E. (n = 5).

TABLE 1

Anacetrapib blood pharmacokinetic parameters on days 1 and 42 of dosing

Anacetrapib was dosed orally once daily to WT-lean, WT-DIO, NFR-lean, and NFR-DIO mice at 10 mg/kg for 42 days. On days 1 and 42, five animals from each subgroup underwent serial blood sampling for a full blood pharmacokinetic profile. Blood concentrations were measured as described in *Methods*. Pharmacokinetic parameters were calculated using WinNonlin 5.1 software. Numbers represent mean and S.E. from n = 5 mice.

	WT Lean	WT DIO	NFR Lean	NFR DIO
Day 1				
$AUC_{0-\infty}$ (μM*h)	8.3 ± 2.1	35 ± 4.0	13 ± 1.3	51 ± 11
AUC_{0-24h} (μM*h)	7.8 ± 1.7	29 ± 3.8	9.8 ± 0.8	25 ± 6.2
C_{max} (μM)	2.7 ± 0.8	5.2 ± 1.5	2.8 ± 0.8	5.0 ± 1.3
T 1/2 (h)	14 ± 4.6	17 ± 3.3	23 ± 6.1	60 ± 25
Day 42				
$AUC_{0-\infty}$ (μM*h)	14 ± 1.2	93 ± 28	48 ± 26	118 ± 33
AUC_{0-24h} (μM*h)	9.2 ± 0.8	44 ± 4.5	13 ± 1.3	45 ± 8.5
C_{max} (μM)	2.7 ± 0.4	6.1 ± 1.3	3.4 ± 0.9	6.8 ± 1.3
T 1/2 (h)	74 ± 31	63 ± 36	152 ± 127[a]	66 ± 34[a]

[a]Some uncertainty in $t_{½}$ calculation, due to secondary peaks in blood concentration time profile.

the end of the 35-week post-dose period were below the quantitation limit for WT lean mice, and ranged from 0.002 to 0.02 μM in the other subgroups of mice.

Terminal $t_{½}$ of Anacetrapib upon Multiple Dosing. Since the elimination rate of anacetrapib from white adipose tissue was extremely slow, the sampling duration of 35 weeks was not sufficient to capture the true terminal phase of anacetrapib elimination. Therefore, the estimates of $t_{½}$ based on observed elimination may not be entirely accurate; however, they represent a semiquantitative assessment of elimination kinetics of anacetrapib in each tissue.

The estimated $t_{½}$ values of anacetrapib in mouse blood and tissues were generally very long, in the range of 40–300 days (Table 2). In blood, anacetrapib exhibited a 3-fold to 7-fold longer $t_{½}$ in WT DIO, NFR lean, and NFR DIO mice compared with WT lean mice, suggesting that both adipose partitioning and binding to CETP have a potential role in determining the blood $t_{½}$. The $t_{½}$ of anacetrapib in brown adipose tissue appeared to be similar across mouse strains (~100–150 days). In white adipose tissue, the $t_{½}$ was estimated to be 171 days and 277 days in WT DIO and NFR DIO mice, respectively, whereas no reliable $t_{½}$ estimate could be obtained from WT lean and NFR lean mice owing to the absence of a terminal decay phase. In liver, the $t_{½}$ was estimated to be 115–230 days. In brains, the $t_{½}$ was highly variable but shorter than in the other tissues (26–130 days).

Because of the terminal sampling mode (and hence the lack of individual time profiles) in the postdose phase of the study, we relied on the mean concentration-time curves to obtain estimates of the terminal $t_{½}$ in blood and tissues of the respective groups of mice. It was not possible to directly assess the intersubject variability and S.E. or $t_{½}$ could not be calculated.

Discussion

This study provides evidence in a preclinical species that disposition of anacetrapib in adipose tissue plays a key role in the long-term accumulation and retention of the drug and ultimately contributes to the observed long terminal $t_{½}$ in systemic circulation. Of note, we measured the pharmacokinetic profile from mouse blood rather than plasma as measurement of the full concentration-time profile (0–72 hours on treatment days 1, 15, 28, and 42) required serial sampling from individual animals, which was possible only by sampling blood, owing to limitations in the total blood volume available from mice.

Whereas preclinical studies in rats and monkeys have shown that anacetrapib is characterized by low clearance, a high degree of protein binding, and a high volume of distribution (Tan et al., 2010), the data from these single-dose pharmacokinetic studies were unable to predict the tissue accumulation properties and the long $t_{½}$ observed in this study. In a previous mouse pharmacokinetic study where anacetrapib was given i.v. as a single dose at 0.5 mg/kg, the estimated terminal $t_{½}$ values were 11 and 23 hours for WT lean and DIO mice, respectively (unpublished data). These results were consistent with the day 1 observed $t_{½}$ in the present study but much shorter than the $t_{½}$ after the 6-week multiple dose regimen (Table 2). This phenomenon was also observed in humans, where single-dose studies underpredicted the $t_{½}$ after longer-term treatment (Krishna et al., 2008; Gutstein et al., 2012). A potential explanation is that the mechanistic causes of long $t_{½}$ are linked to the drug's tissue accumulation and retention characteristics. Further insight could be gained from understanding the processes that govern the entry of drug into, and release of drug out of, adipose and other tissues.

A comparison of the anacetrapib concentration-time profiles in brown versus white adipose tissue suggests that brown adipose might constitute a "transient depot" for the drug, whereas white adipose is likely to be a major long-term depot for the drug in vivo. Brown adipose achieved the highest concentrations during the treatment period but rapidly declined after cessation of treatment. In contrast, decline of drug levels from white adipose was much slower. It is also evident that adiposity enhances the extent of drug distribution into the adipose, as obese animals had adipose concentrations three to four times greater than lean animals. Overall, the observed high affinity of anacetrapib to

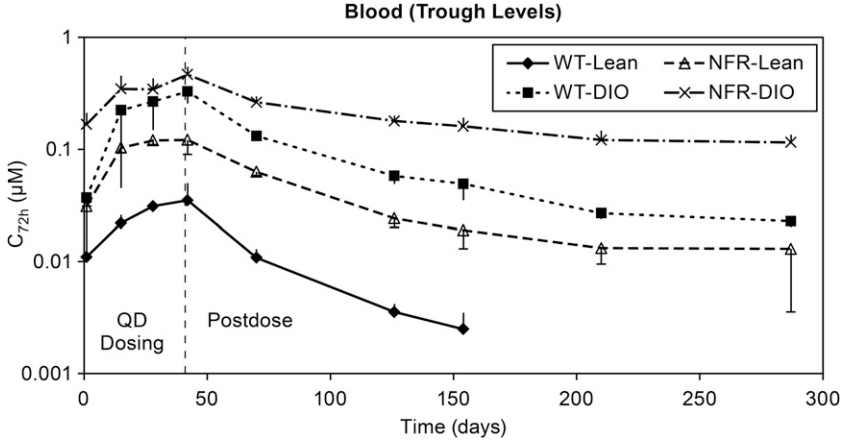

Fig. 4. Mean anacetrapib blood concentration-time profiles (trough levels) in mice during 6-week dosing (10 mg/kg daily) and to 35 weeks postdose. Anacetrapib was dosed orally at 10 mg/kg daily for 6 weeks in WT-lean, WT-DIO, NFR-lean, and NFR-DIO mice. Blood samples were collected at 72 hours (trough levels) on days 1, 15, 28, and 42 during daily dosing and then 4, 12, 16, 24, and 35 weeks postdose (= days 70, 126, 154, 210, 287). Drug levels were measured by LC-MS/MS as described in *Methods*. Data points indicate mean and S.E. from $n = 5$ mice of the respective treatment groups. Absence of error bars indicates that the error bars are smaller than the size of the symbols. For the WT-lean group, blood concentrations in most animals were below the limit of quantitation at the time points beyond 154 days.

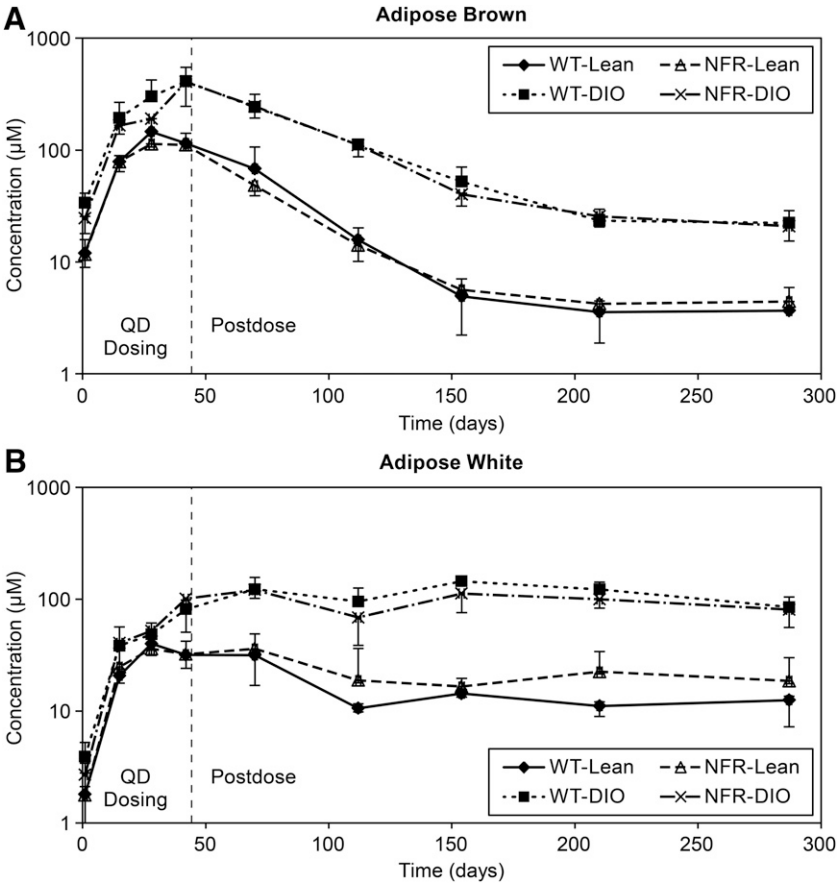

Fig. 5. Mean anacetrapib brown and white adipose concentration-time profiles in mice during 6-week dosing (10 mg/kg daily) and to 35 weeks postdose anacetrapib was dosed orally at 10 mg/kg daily for 6 weeks in WT-lean, WT-DIO, NFR-lean, and NFR-DIO mice. Brown adipose and epididymal white adipose tissues were collected at 72 hours (trough levels) on days 1, 15, 28, and 42 during daily dosing and then 4, 10, 16, 24, 35 weeks postdose (= days 70, 112, 154, 210, 287). Drug levels were measured by LC-MS/MS as described in *Methods*. Data points indicate mean drug levels from $n = 5$ mice of the respective groups.

adipose tissue can be explained by the lipophilicity (the reported LogD is 7.1; Merck unpublished data) and the neutral properties of the molecule.

There was an apparent slight rise in anacetrapib levels in white adipose tissue of DIO mice after cessation of dosing, which could be due to interanimal variability, potential redistribution from other tissues, or a combination of both. It is important to note that in the present study, epididymal white adipose tissue was sampled and used as a surrogate for the totality of white adipose tissue. We cannot preclude the possibility that different adipose depots of the body (e.g., subcutaneous, retroperitoneal, others) might exhibit differences in terms of the disposition kinetics of anacetrapib. A redistribution of drug from one depot to another is a possibility, although the mechanistic details of this process (if it does occur) are currently unknown.

It is known that white and brown adipose tissues are different with respect to their physiologic functions (i.e., white adipose stores excess energy as triglycerides, whereas brown adipose is specialized in the dissipation of energy through the production of heat). Also, brown adipose tissue has been shown to be metabolically active (for review, see Saely et al., 2012). It is likely that anacetrapib, because of its lipophilicity, has a high affinity to lipid stores in adipocytes, which could help to explain the longer retention of the drug in white versus brown adipose tissue. Whether metabolism plays a role in removal of anacetrapib from brown adipose tissue is currently not known.

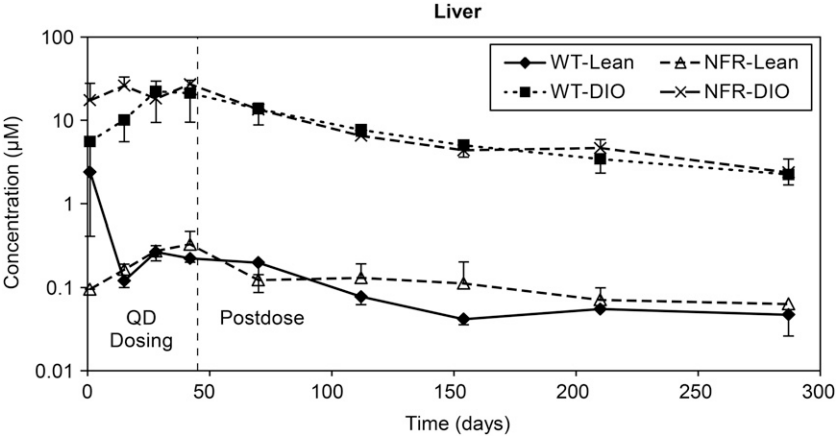

Fig. 6. Mean anacetrapib liver concentration-time profiles in mice during 6-week dosing (10 mg/kg daily) and to 35 weeks postdose. Anacetrapib was dosed orally at 10 mg/kg daily for 6 weeks in WT-lean, WT-DIO, NFR-lean, and NFR-DIO mice. Livers were collected at 72 hours (trough levels) on days 1, 15, 28, and 42 during daily dosing and then 4, 10, 16, 24, and 35 weeks postdose (= days 70, 112, 154, 210, 287). Drug levels were measured by LC-MS/MS as described in *Methods*. Data points indicate mean drug levels from $n = 5$ mice of the respective groups.

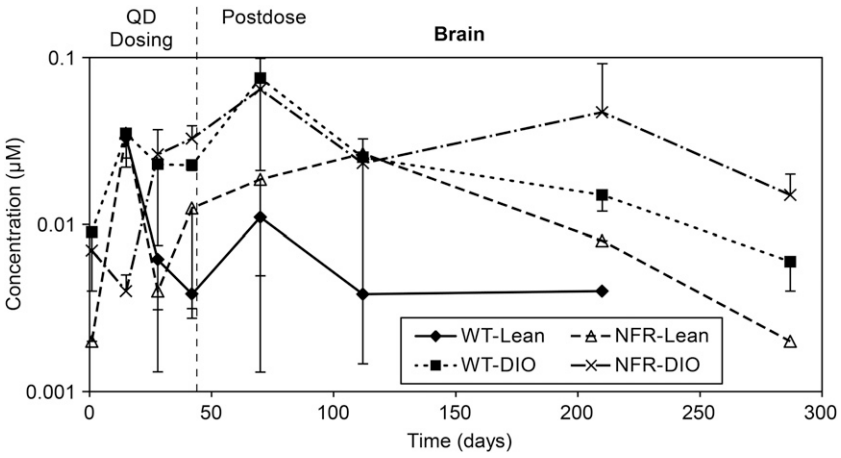

Fig. 7. Mean anacetrapib brain concentration-time profiles in mice during 6-week dosing (10 mg/kg daily) and to 35 weeks postdose. Anacetrapib was dosed orally at 10 mg/kg daily for 6 weeks in WT-lean, WT-DIO, NFR-lean, and NFR-DIO mice. Brain samples were collected at 72 hours (trough levels) on days 1, 15, 28, and 42 during daily dosing, and then 4, 10, 24, and 35 weeks post-dose (= day 70, 112, 210, 287). Drug levels were measured by LC-MS/MS as described in *Methods*. Data points indicate mean drug levels from n = 5 mice of the respective groups.

Our results revealed nearly 100-fold higher day 42 levels of anacetrapib in the livers of DIO versus lean mice. This is not entirely surprising, as the liver is a major site of lipoprotein turnover (uptake, excretion, metabolism), and anacetrapib has been shown to be associated with lipoproteins (Roddy et al., 2014). As obesity induced by dietary fat likely increases the deposition of lipoproteins into the liver, this could cause higher hepatic drug accumulation in DIO mice. Differences in hepatic drug metabolism between lean and DIO mice appear less plausible since anacetrapib is a low-clearance compound, and to date there has been no evidence that adiposity would impact the extent of anacetrapib metabolism.

Despite its lipophilicity, anacetrapib did not exhibit any appreciable extent of accumulation in the mouse brain. In fact, in-house data from a brain penetration study conducted in P-glycoprotein (P-gp) *mdr1a* knockout mice indicate that anacetrapib is a P-gp substrate, as the measured brain-to-plasma AUC ratios in WT and P-gp *mdr1a* knockout mice were 0.015 and 0.55, respectively (unpublished). Thus, the presence of P-gp at the blood-brain barrier likely limits the accumulation of free drug in the brain.

Although the impact of adiposity on anacetrapib's long $t_{½}$ is clear, the role of CETP binding in this phenomenon is more complex. CETP seems to have an impact on on-treatment blood concentrations, with higher concentrations observed in NFR mice compared with WT mice. During the washout phase, blood concentrations in most WT lean animals were below the limit of quantitation 24 weeks after cessation of treatment. Small but measurable amounts of drug were present in blood 35 weeks after cessation of treatment in the other three subgroups. Approximately 7%–11% of day 42 drug levels remained in the blood of WT DIO and NFR lean mice, whereas 25% of day 42 drug levels remained in NFR DIO mice. This numeric trend suggests that CETP probably has an impact on the rate of elimination of anacetrapib from blood.

On the other hand, on-treatment drug concentrations as well as elimination rates from adipose are similar for WT and NFR mice. Since CETP is present in a number of tissues, including adipose, one would reasonably expect that drug binding to CETP in adipose occurs, but the relative impact of it is probably small given the enormous capacity of adipose tissue for retaining the drug and the large pool of drug in adipose resulting from continued accumulation owing solely to its physical properties.

In CETP transgenic mice, CETP has also been shown to be expressed in tissues other than adipose, such as liver, kidney, small intestine, spleen, and heart (Jiang et al., 1992). Overall, it was found that the CETP expression levels in these mice are similar to levels in humans, and in the case of NFR mice the CETP mRNA abundance appears somewhat higher than in humans. Taken together, these findings support the idea that CETP is likely not a governing factor for disposition of anacetrapib into adipose tissue.

It remains to be determined whether the mechanisms contributing to the long $t_{½}$ of anacetrapib are similar to those governing the $t_{½}$ of other lipophilic drugs, such as amiodarone and probucol (Vadiei et al., 1997; Jeon et al., 2011). In the case of anacetrapib, to date, the long $t_{½}$ has not been associated with any adverse effect, and in chronic dosing, anacetrapib has been shown to exhibit a favorable safety profile (Gutstein et al., 2012; Gotto et al., 2014a, b). Several thousand patients, across a wide range of body weights, have been treated with anacetrapib for more than 3 years in completed or ongoing clinical trials. Anacetrapib has been generally safe and well tolerated in these patients. Adipose tissue retention and long $t_{½}$ have so far not been linked to any safety concerns.

In conclusion, the findings from this study clearly demonstrate that disposition into adipose tissue is a key factor governing the long-term accumulation and elimination kinetics of anacetrapib in vivo, and it is likely to contribute to the long $t_{½}$ of the drug. In contrast, the effect of binding to CETP on the kinetics of anacetrapib is less conspicuous and may be more difficult to determine. Efforts are currently under way to establish a preclinical pharmacokinetic model that is able to describe the disposition of anacetrapib in the different tissues. If successful, such a model could shed further light on the mechanisms of long $t_{½}$.

TABLE 2

Estimates of anacetrapib $t_{½}$ (days) in mouse blood and tissues after 6-week dosing and to 35 weeks postdosing

Anacetrapib was dosed orally once daily to WT-lean, WT-DIO, NFR-lean, and NFR-DIO mice at 10 mg/kg for 42 days. Blood and tissues were collected during dosing and at 4, 12, 16, 24, and 35 weeks postdose. Drug levels were measured by LC-MS/MS as described in *Methods*. The $t_{½}$ estimates were calculated from the last three data points of the mean concentration time curves from blood and tissues.

	WT Lean	WT DIO	NFR Lean	NFR DIO
Blood	39	125	256	292
Liver	230	115	125	143
Adipose brown	94	115	118	145
Adipose white	NC[a]	171	NC[a]	277
Brain	57	47	26	130

[a]Not calculated: A reliable $t_{½}$ estimate could not be obtained from these tissues, as the terminal phase could not be determined.

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Authorship Contributions

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