

ARTICLE

Fatty Acid Amide Hydrolase Inhibition by JNJ-42165279: A Multiple-Ascending Dose and a Positron Emission Tomography Study in Healthy Volunteers

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Inhibition of fatty acid amide hydrolase (FAAH) potentiates endocannabinoid activity and is hypothesized to have therapeutic potential for mood and anxiety disorders and pain. The clinical profile of JNJ-42165279, an oral selective FAAH inhibitor, was assessed by investigating the pharmacokinetics, pharmacodynamics, safety, and binding to FAAH in the brain of healthy human volunteers. Concentrations of JNJ-42165279 (plasma, cerebrospinal fluid (CSF), urine) and fatty acid amides (FAA; plasma, CSF), and FAAH activity in leukocytes was determined in a phase I multiple ascending dose study. A positron emission tomography study with the FAAH tracer [¹¹C]MK3168 was conducted to determine brain FAAH occupancy after single and multiple doses of JNJ-42165279. JNJ-42165279 administration resulted in an increase in plasma and CSF FAA. Significant blocking of brain FAAH binding of [¹¹C]MK3168 was observed after pretreatment with JNJ-42165279. JNJ-42165279 produces potent central and peripheral FAAH inhibition. Saturation of brain FAAH occupancy occurred with doses ≥ 10 mg of JNJ-42165279. No safety concerns were identified.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ FAAH inhibition has been investigated as a candidate mechanism of action for CNS conditions for several years, notably for pain.

WHAT QUESTION DID THIS STUDY ADDRESS?

✓ We wanted to determine the dose-dependent effects on central FAA turnover in CSF and blockade of FAAH in brain, and determine the clinical doses that would give us confidence we were testing the hypothesis in phase II trials while affording the best safety margin.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

✓ This is the first report of the dose-dependent effects of FAAH inhibition on FAA turnover in CSF and occupancy of brain FAAH by an FAAH inhibitor in human volunteers.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

✓ Not many mechanisms are blessed with the range of biomarkers as FAAH inhibition we had when entering phase I; however, the value of target-specific biomarkers illustrated by this study may spur early investment in developing assays and methods for novel mechanisms that can be included in the first phase I trial.

The endocannabinoid system is abundantly present in tissues and organs throughout the body, especially in the brain. Activation of cannabinoid receptors by endogenous lipids (endocannabinoids) in the central nervous system (CNS) is hypothesized to provide homeostatic regulation through negative feedback inhibition of excitatory pathways.¹ Endocannabinoid activity can be enhanced by inhibition of fatty acid amide hydrolase (FAAH), the enzyme primarily responsi-

ble for degrading a variety of fatty acid amides (FAAs).² FAAs are a group of signaling lipids that participate in the control of many physiological processes including pain, cognition, mood regulation, sleep, energy metabolism, and inflammation. Consequently, FAAH inhibition has been proposed as a novel therapeutic approach for the treatment of mood and anxiety disorders, posttraumatic stress disorder, and pain. Selective FAAH inhibitors have been identified and several

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have been advanced for clinical development. In a proof-of-concept study with the FAAH inhibitor PF-04457845 in patients with osteoarthritis, peripheral inhibition of FAAH activity was observed; however, no effect on pain perception was reported.³ Whether FAAH inhibition can be useful in other conditions remains to be evaluated.

JNJ-42165279 is a potent, selective, and orally bioavailable inhibitor of FAAH.⁴ The compound is a substrate of the enzyme and inhibits its activity by covalent binding to the catalytic site. Enzyme activity is restored through slow hydrolysis of the drug fragment from the active site and regeneration of FAAH.⁴ The compound was recently tested in a phase I study.⁵

To confirm FAAH inhibition, determine the relationship between exposure and pharmacodynamic effect, guide dose selection, and identify the acceptable safety margin for phase II clinical studies, we evaluated the effect of different doses of JNJ-42165279 on FAAH activity and FAA turnover in plasma and cerebrospinal fluid (CSF) and on the occupancy of FAAH in brain with positron emission tomography (PET) using [¹¹C]MK3168, a radiolabeled reversible FAAH inhibitor developed for quantification of competition for binding at the catalytic site by other FAAH inhibitors.⁶

METHODS

The multiple ascending dose (MAD) study (clinicaltrials.gov identifier: NCT01964651) was conducted at the Janssen Clinical Pharmacology Unit, Merksem, Belgium from 21 October 2013 to 22 October 2014 and the PET occupancy study (clinicaltrials.gov identifier: NCT02169973) at the University Hospitals Leuven, campus Gasthuisberg, Belgium from 6 March to 15 July 2014.

Design

The MAD study was a double-blind, randomized, placebo-controlled study of JNJ-42165279 in healthy volunteers conducted in two parts. The objective of the MAD Part 1 was the assessment of exposure to JNJ-42165279, FAAH activity in leukocyte, and FAA turnover in plasma and CSF. The key objectives of the MAD Part 2 were to determine safety, pharmacokinetics (PK), and peripheral pharmacodynamics (PD) of JNJ-42165279 in women and older volunteers. Volunteers were divided into five cohorts of eight volunteers each and were administered different doses of JNJ-42165279 ($n = 6$) (cohort A, 25 mg, young men; cohort B, 75 mg, young men; cohort C, 100 mg young women; cohort D, 100 mg, elderly men and women; cohort E, 10 mg, young men) or placebo ($n = 2$) once-daily for 10 consecutive days. The 10 mg cohort was added after the other cohorts had completed, specifically to assess the effects on CSF FAA turnover, based on the results of the PET study that suggested the potential for full occupancy in the brain with a 10 mg dose.

The PET study was an open-label study and was conducted in three parts. Part 1 determined the regional brain kinetics and binding properties of [¹¹C]MK3168(6). Dose-dependent blocking of [¹¹C]MK3168 retention by JNJ-42165279 was measured in Part 2, and accumulation of JNJ-42165279 on the FAAH enzyme after 1 week of dosing was explored in Part 3 (**Supplemental Figure S1**).

For further details on methods please refer to the **Supplementary Material**.

Study population

Healthy nonsmokers (men and women, 18–85 years) were enrolled in the MAD study. Volunteers with abnormal physical examination findings, ECG findings, clinical laboratory results, history of drug or alcohol use disorder within 6 months of screening were excluded from this study. JNJ-42165279 is a substrate for CYP3A and because of the potential for drug–drug interactions, use of CYP3A inhibitors or inducers were not allowed during the study.

The inclusion and exclusion criteria for the PET occupancy study were similar to the MAD study, except that only men (18–55 years) were enrolled, and volunteers exposed to ionizing radiation during the previous year in excess of 1 mSv or having clinically significant magnetic resonance imaging (MRI) abnormalities of the CNS at screening were excluded.

The studies were performed in accordance with Good Clinical Practice and were approved by the Ethics Committee of the University Hospital Antwerp, Belgium (MAD study) and University Hospitals Leuven, Belgium (PET occupancy study). All volunteers signed a written informed consent prior to study inclusion.

Assessments

Pharmacokinetics and pharmacodynamics

The concentration of JNJ-42165279 was quantified in plasma and CSF samples using a specific, validated, and sensitive liquid chromatography / tandem mass spectrometry (LC/MS/MS) method. The FAAH activity was measured in leukocytes and concentrations of FAAs N-arachidonoyl ethanolamine (AEA), N-oleoylethanolamine (OEA), and N-palmitoylethanolamide (PEA) were determined in plasma⁷; arachidonic acid (AA), AEA, and OEA were measured in CSF (for assay methods, see **Supplementary Material**). Mean and individual plasma concentration vs. time after JNJ-42165279 administration were plotted for each cohort. Plasma, urine, and CSF concentration data were determined at each timepoint. All PK parameters in plasma and urine for JNJ-42165279, calculated using noncompartmental methods, were summarized.

Behavioral evaluations were performed during the MAD study using the Addiction Research Center Inventory (ARCI-53) questionnaire,⁸ a computerized cognitive test battery from the Cognitive Drug Research System (CDR System™), and Bond and Lader Visual Analogue Scales (VAS) questionnaire⁸ for all cohorts except for cohort E. The cognitive battery and ARCI-53 were completed predose and 4 h after dosing on days 1 (baseline), 6, and 10.

During PET Part 2, PK samples for JNJ-42165279 were collected at baseline and at the beginning, middle, and end of the scan. The FAAH activity in leukocytes was tested at the midpoint of the scan (see **Supplementary Material**). Blood samples for pharmacogenomics were obtained at baseline.

PET study

The PET occupancy study was undertaken to confirm target engagement by JNJ-42165279 in the brain, and identify doses that could test the mechanism of action while having

acceptable safety margins. [^{11}C]MK3168, a highly selective reversible radiolabeled FAAH inhibitor with high affinity for human FAAH ($\text{IC}_{50} = 1.0 \pm 0.6 \text{ nM}$), was selected as a suitable candidate.⁶

To qualify the ligand for the occupancy study, an *in vivo* blocking study was conducted in rhesus macaque that demonstrated blocking of retention of [^{11}C]MK3168 by JNJ-42165279. Binding of [^{11}C]MK3168 to human leukocytes *ex vivo* could also be blocked by JNJ-42165279, supporting competition of the two inhibitors at the FAAH catalytic site (see **Supplementary Material**). Following qualification for use, a clinical PET study was conducted consisting of three parts.

For Part 1 of the PET study, five volunteers underwent dynamic PET scans following intravenous injection of [^{11}C]MK3168 alone, to develop a suitable kinetic model for determination of the total distribution volume (V_T) of the tracer in the brain.

Part 2 investigated FAAH occupancy as a function of a single oral dose of JNJ-42165279. Doses were selected from a possible range of 2.5–100 mg. The choice of dose was adaptive, and was adjusted based on information as it accrued over the study. Six volunteers participated in Part 2; each volunteer underwent three PET scans: a baseline scan and a PET scan after two different single doses. PK samples for JNJ-42165279 were taken at baseline, at the time of tracer injection, and at 45 min and 90 min posttracer injection, and samples were taken for leukocyte FAAH activity at the midpoint (~4 h and 45 min postdose of JNJ-42165279) of the PET scan. The baseline scan and first post treatment scan were both obtained on the first dosing day, ~4 h apart.

The PET tracer was injected 1 h after administration of JNJ-42165279 (i.e., the estimated T_{max} of JNJ-42165279). The second and third (treatment) scans were separated by at least 1 week to allow normalization of FAAH enzyme activity after the first dose of JNJ-42165279. Part 3 evaluated the potential for accumulation of JNJ-42165279 at the FAAH enzyme during chronic dosing in four volunteers. Volunteers underwent three PET scans: a baseline scan, a scan 24 h after a single dose, and the third scan 24 h after the last dose of 1 week of daily dosing to measure occupancy level at trough and test whether accumulation at the FAAH enzyme in brain would occur. Doses were chosen that were predicted not to saturate FAAH binding over a 24-h dosing interval based on the results of Part 2.

PET and MRI and input function determination

Arterial blood sampling for measurement of parent tracer and radio metabolite analysis was conducted for each PET scan. The plasma PK of [^{11}C]MK3168 and radio metabolites was derived from discrete arterial samples (analysis described in **Supplementary Material**) as an input function. The tissue response function in brain was measured from the PET images. PET scans were acquired on a HiRez Biograph 16 slice PET/CT camera (Siemens, Erlangen, Germany) with [^{11}C]MK3168 tracer produced as previously described.⁶ A slow bolus injection ($356 \pm 57 \text{ MBq}$; molar activity $111 \pm 67 \text{ GBq}/\mu\text{mol}$) of [^{11}C]MK3168 was synchronized with the start of a 90-min list-mode acquisition. A low dose computed tomography (CT) scan was acquired

immediately prior to tracer injection for attenuation correction. Acquired data were reconstructed using ordered subset expectation-maximization algorithm (five iterations / eight subsets, 27 frames) with corrections applied for dead time, scatter, randoms, decay, and tissue attenuation.

MR image volumes were obtained as anatomical reference for coregistration of PET images using 3D T1-MPRAGE (magnetization-prepared rapid gradient-echo sequence, repetition time (TR) = 9.6 ms; echo time (TE) = 4.6 ms; flip angle, 8° ; voxel size: $0.98 \times 0.98 \times 1.2 \text{ mm}$). MRI was performed on a 3T Philips Ingenia system (Philips Healthcare, Best, The Netherlands).

Kinetic modeling

Kinetic modeling for determining enzyme occupancy was based on the analysis of time activity curves (TACs) at baseline and during treatment scans from each participant. Generation of TACs and kinetic modeling was performed with PMod v. 3.4 software (Zurich, Switzerland). Automated delineation of 83 volumes of interest (VOIs) according to the Hammers brain template⁹ was performed using the PNeuro tool in PMod. For data reduction and to obtain a better signal-to-noise ratio, smaller regions were united into composite VOIs to delineate the frontal, temporal, parietal and occipital lobes, striatum, thalamus, and cerebellum. A two-tissue compartment reversible model was used for quantification and nondisplaceable binding potential (BP_{ND}) and total distribution volume (V_T) estimations.¹⁰

Safety assessments

For both of the studies (MAD and PET occupancy), safety assessments included incidence or type of treatment-emergent adverse events (TEAEs), physical examinations, laboratory assessments, 12-lead ECG, and vital signs. TEAEs following enrollment were evaluated separately for each part of the PET study.

RESULTS

Study population

Of the total of 55 enrolled volunteers, 54 volunteers completed the study (MAD study, 39 of 40; PET study, 15 of 15). All volunteers in the MAD study were included in the PK, biomarker, and PD analysis set. Most of the volunteers enrolled in these studies were identified as “white” or “Caucasian” (MAD, $n = 36$; PET, $n = 15$) and a total of four volunteers reported protocol deviations (MAD study, $n = 3$; PET study, $n = 1$). Baseline demographics are shown in **Supplemental Table S1**.

Pharmacokinetic and pharmacodynamics

Following oral administration, JNJ-42165279 was rapidly absorbed. The plasma concentration increased in a dose-dependent manner, and exhibited a multiexponential decline (**Figure 1**). Mean plasma half-life ranged from 8.14–14.1 h, resulting in higher exposures on day 10 than day 1. The estimated accumulation ratios for C_{max} ranged from ~98–156% and for AUC ranged from ~144–337% for JNJ-42165279 doses (10–100 mg, **Supplemental Table S2**).

Mean concentrations of JNJ-42165279 in CSF after 7 days were 2.67 ng/mL (cohort E), 8.95 ng/mL (cohort A), and

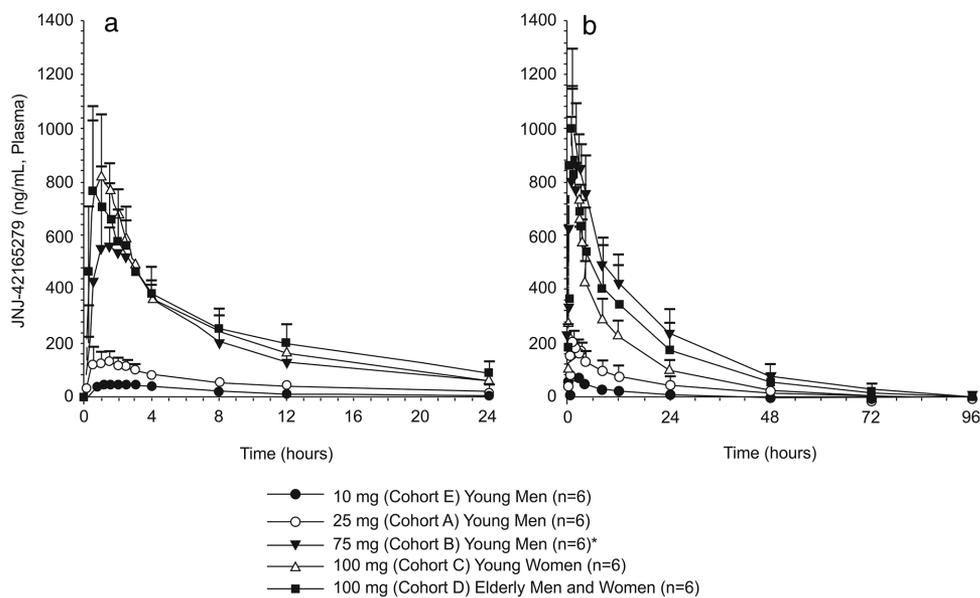


Figure 1 Arithmetic mean (+SD) JNJ-42165279 plasma concentration–time profiles **a**: Day 1, 0–24-h postdose; **b**: Day 10, 0–96 h postdose. * $n = 5$ for Day 1.

31.8 ng/mL (cohort D, men only), which were slightly over 3% of plasma values at C_{max} on day 10 (**Figure 1**). This was consistent with the high plasma protein binding of the compound across species, with the unbound fraction in humans estimated to be 6.4–7.5%.

FAAH activity in leukocytes

Across the 10–100 mg JNJ-42165279 dose range, FAAH activity remaining in leukocytes attained a mean trough of 7.85–10.4% (relative to predose values) after a single dose and a mean trough of 0.58–10.5% after once-daily (QD) dosing for 10 days (**Figure 2**).

FAAs in plasma

Single doses of JNJ-42165279 in the range of 10–100 mg produced mean peak concentrations of AEA in plasma that were 5.5–10-fold higher than mean peak placebo values, whereas mean peak OEA and PEA concentrations were 4.3–5.6-fold higher than mean peak placebo. Similar changes in mean FAA concentrations were observed after daily administration of 25–100 mg for 10 days (**Figure 3**).

FAAs in CSF

Daily administration of JNJ-42165279 for 7 days increased the mean CSF AEA concentrations by ~45-fold (10 mg), ~41-fold (25 mg), and ~77-fold (75 mg) and mean OEA concentrations by ~6.6-fold (10 mg), ~5.8-fold (25 mg), and ~7.4-fold (75 mg), relative to predose values. The concentrations of the product of FAA hydrolysis (arachidonic acid: AA) in CSF were similar on day 6 compared with baseline after administration of 25 mg or 75 mg JNJ-42165279 and placebo (**Table 1**).

Behavioral evaluations. No treatment effects were observed on the Bond and Lader VAS. Women of nonchild-bearing potential receiving 100 mg of JNJ-42165279

reported a slight similarity to sedatives and dissimilarity to stimulants on the ARCI-53; no groups reported experiences similar to marijuana or hallucinogens. Analysis of cognitive performance testing indicated a significant negative treatment effect ($P \leq 0.05$), as well as treatment-by-visit ($P \leq 0.01$) effect, on quality of working memory (QWM) in elderly volunteers receiving 100 mg of JNJ-42165279 ($P \leq 0.01$ vs. placebo, and 25 and 100 mg of JNJ-42165279 in young volunteers). This was driven by slight impairment relative to baseline, occurring 4 h postdose on days 1, 6, and 10. Significant treatment effects on quality of episodic secondary memory performance were also observed, driven by improved performance by the cohort of women of nonchild-bearing potential given 100 mg, at all-timepoints compared with baseline. No treatment effects were observed on tapping, tracking, and postural stability.

[¹¹C]MK3168 radiometabolite quantification

Radiometabolite analysis in baseline scans demonstrated rapid metabolism of [¹¹C]MK3168 with 30–40% of parent tracer remaining 10 min postinjection (p.i.), and almost complete elimination of [¹¹C]MK3168 from plasma at 60 min p.i. Pretreatment with JNJ-42165279 significantly slowed the rate of metabolism of the tracer, and the extent of slowing was dose-dependent (**Supplemental Figure S2**). The final measured FAAH occupancy in brain was highly correlated with the fraction of parent tracer in plasma measured at 60 min p.i. (**Supplemental Figure S3**), suggesting that estimation of brain occupancy could be predicted by the intact tracer ratio.

Kinetic modeling

Uptake of the intact tracer [¹¹C]MK3168 was high and uniform over all gray matter regions with a standardized uptake value (SUV) of ~3.0 at 20 min and ~2.0 at 90 min (~15

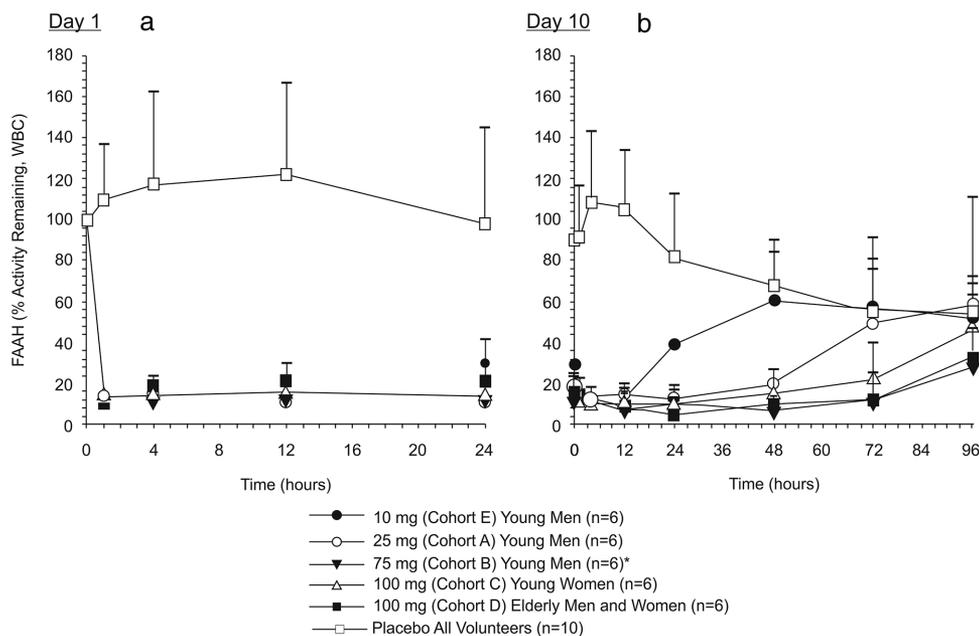


Figure 2 Mean (+SD) leukocyte fatty acid amide hydrolase (FAAH) activity-time profiles after once daily dosing for 10 days. **a:** Day 1; 024-h postdose; **b:** Day 10; 096 h postdose. * $n = 5$ for Day 1. FAAH, fatty acid amide hydrolase.

and 10 kBq/cc, respectively) (**Figure 4, Supplemental Figure S7**).

Single doses of 10, 25, and 50 mg of JNJ-42165279 resulted in 96–98% occupancy (**Supplemental Figure S8**), indicating saturation of brain FAAH at estimated C_{max} . Lower doses of 2.5 and 5 mg showed lesser, although appreciable, occupancy. Based on these results, 2.5 and 10 mg of JNJ-42165279 were selected for testing at trough after single and repeated doses. FAAH occupancy at trough after the first dose of 10 mg was >80% and >50% for 2.5 mg. Concentrations increased with repeated dosing, consistent with accumulation ratio, although this did not translate to increased occupancy with repeated dosing (**Supplemental Table S3**).

Safety assessments

No deaths, serious, or severe treatment emergent adverse event (TEAEs) or discontinuations due to TEAEs were observed in either of the two studies. The overall number of volunteers with TEAEs were similar between all the cohorts in the MAD study. A total of three volunteers experienced TEAEs during Part C of the PET study (**Supplemental Table S4**).

The most commonly (≥ 2 TEAEs in any dose) reported TEAEs during the MAD study were headache ($n = 13$), back pain ($n = 10$), and fatigue ($n = 7$) and in the PET study were headache ($n = 2$) and ecchymosis at the arterial line puncture site ($n = 2$). One to two volunteers in the pooled placebo group, 10 mg and 100 mg, cohorts (each) were observed to have elevations in liver transaminases. The elevations were in the range of 1–2 times the upper limit of normal and returned to pretreatment levels after dose discontinuation (**Supplemental Table S4**).

There were no TEAEs related to any other clinical laboratory values and no clinically significant treatment effects

were observed for any vital signs, ECG parameters including change from baseline of QTc intervals, and physical examination. All TEAEs were either mild (MAD and PET study) or moderate (MAD study) in severity.

DISCUSSION

Fatty acid amides participate in the control of a number of diverse physiological processes such as pain, cognition, and mood regulation.¹¹ Modulation of FAA turnover by selective inhibition of FAAH has been suggested as a treatment for a range of disorders. To assess the suitability of FAAH inhibition for the treatment of mood and anxiety disorders, we evaluated the extent of central and peripheral FAAH inhibition and the minimum dose necessary to sustain inhibition throughout a dosing interval. High levels of central and peripheral FAAH inhibition and near complete occupancy of brain FAAH were observed after doses of 10 mg of JNJ-42165279. The changes in plasma and CSF AEA turnover and brain FAAH occupancy were dose-dependent over the tested range.

Inhibition of FAAH activity in leukocytes was rapid and plasma AEA levels increased ≥ 10 -fold at the lowest dose tested in the MAD study (10 mg). Dose-dependent effects were more pronounced for rate of recovery of FAAH activity in leukocytes and decline in FAA concentrations relative to the observed maximum effects on FAAH activity and FAA concentrations. The relative effect on central turnover of AEA was greater: concentrations of AEA in CSF increased 40–70-fold after 7 days of dosing. The relative effect of JNJ-42165279 on turnover of AEA was also more pronounced than on turnover of OEA and PEA in the periphery, and on OEA concentrations in CSF. This likely reflects AEA being the preferred substrate for FAAH. These data differ somewhat from observations in rat, and may be related to the much

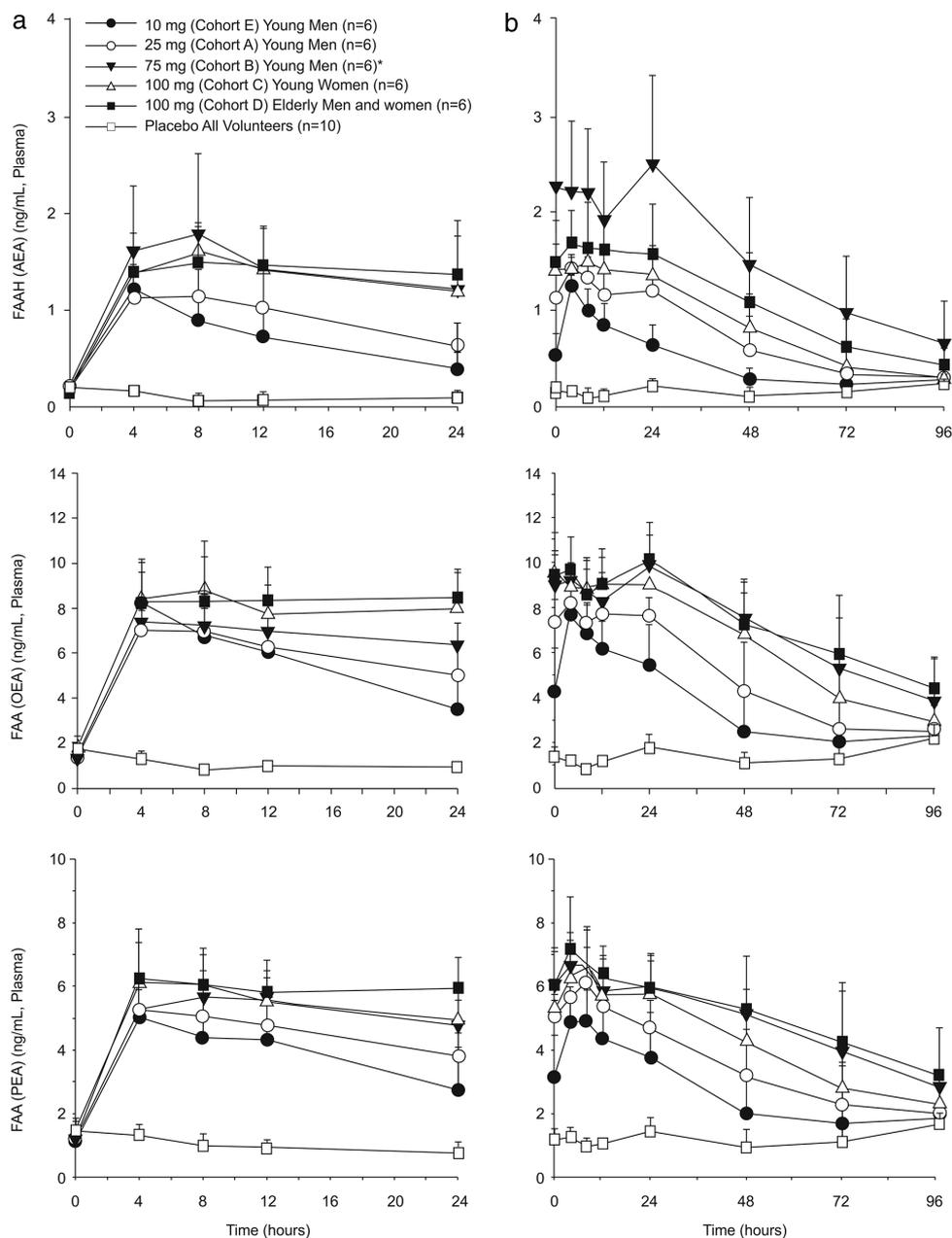


Figure 3 Mean (standard deviation) plasma fatty acid amide (FAA) concentration–time profiles **a**: Day 1, 0–24-h postdose; **b**: Day 10, 0–96 h postdose. **n* = 5 for Day 1. FAAH, fatty acid amide hydrolase; AEA, N-arachidonylethanolamine (anandamide); OEA, oleoylethanolamide; PEA, N-palmitoylethanolamide.

higher affinity of JNJ-42165279 for human over rat FAAH.⁴ Moreover, inactivation of OEA and PEA may be mediated by other mechanisms in addition to FAAH.^{12–14} Effects on FAA turnover and leukocyte FAAH activity consistent with this report have been observed previously with PF-04457845.³

The PK properties of JNJ-42165279 support once-daily dosing. Inhibition of FAAH in the periphery can be readily monitored by measuring substrate levels in plasma, and the terminal half-life of 8–14 h for JNJ-42165279 and the prolonged inhibition of FAAH activity (due to slowly reversible covalent binding of JNJ-42165279 to the FAAH enzyme)⁴

indicate that inhibition can be sustained throughout the dosing interval. Occupancy of brain FAAH approaching saturation could be demonstrated for all doses ≥ 10 mg at the estimated C_{max} , declining to 80–85% occupancy 24 h after dosing with 10 mg. The brain occupancy estimates were supported by the correlation with peripheral inhibition of leukocyte FAAH enzyme activity (**Supplemental Figure S4**). While the expected accumulation in plasma concentrations occurred with repeated dosing of 2.5 or 10 mg, increases in occupancy at trough were not observed beyond a slight numerical increase in occupancy at 10 mg. Rapid recovery

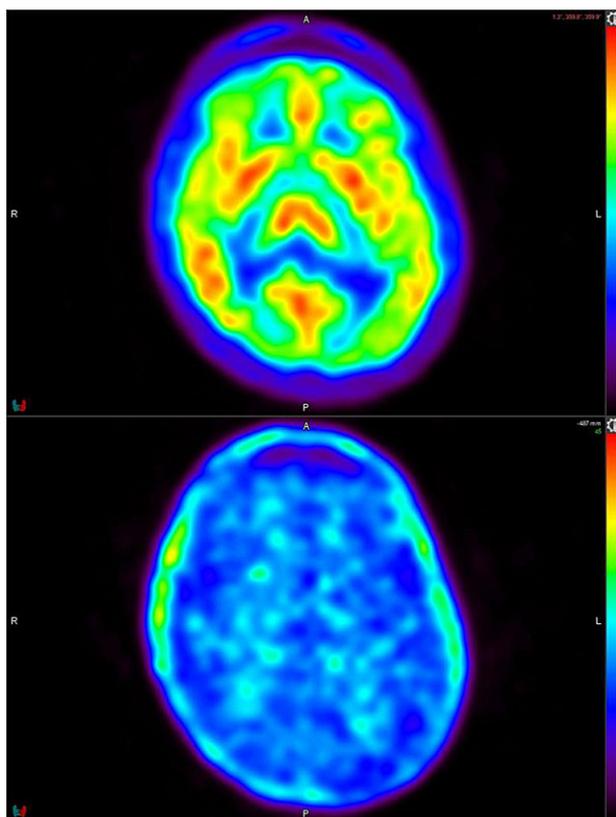
Table 1 Arithmetic mean (standard deviation) of fatty acid amide (FAA) concentrations in cerebrospinal fluid of young male volunteers

FAA		10 mg <i>n</i> = 6	25 mg <i>n</i> = 6	75 mg <i>n</i> = 6	Placebo <i>n</i> = 6
AA	Predose	—	1.85 (0.52)	2.04 (0.86)	2.31 (1.39) ^a
	Day 7	—	1.44 (0.52) ^b	1.81 (0.72) ^b	1.74 (0.78) ^a
AEA	Predose	0.28 (0.31)	0.52 (0.16)	0.38 (0.21)	0.41 (0.39)
	Day 7	12.6 (4.86)	21.24 (7.39) ^b	28.88 (9.52) ^b	0.57 (0.27)
OEA	Predose	21.2 (5.33)	20.15 (10.19)	20.21 (8.25)	16.2 (5.46)
	Day 7	139 (42.0)	116.81 (26.07) ^b	150.24 (34.64) ^b	22.8 (23.3)

^a*n* = 4.^b*n* = 5.

AA, arachidonic acid; AEA, N-arachidonylethanolamine (anandamide); OEA, oleoylethanolamide.

AA was not analyzed for the 10 mg cohort as no treatment effect had been seen with the higher doses.

**Figure 4** Distribution of [¹¹C]MK3168 in human brain under baseline and blocked conditions. Top image: [¹¹C]MK3168 PET scan at baseline; Bottom image: [¹¹C]MK3168 PET scan in the same volunteer after receiving 10 mg JNJ-42165279.

of FAAH enzyme activity is seen with lower exposures, as indicated by the recovery of WBC FAAH activity 24 h after repeat dosing with 10 mg (**Figure 2**).

Nervous system TEAEs (>40%) were the most common, which is not unexpected for a potentially neuromodulatory mechanism. These TEAEs were generally mild, and were more prevalent at doses >25 mg. Evidence of behavioral effects in healthy volunteers was very limited, with conflicting effects on derived memory parameters in the 100 mg cohorts, and weak endorsement of sedative-like effects by women of nonchild-bearing potential receiving 100 mg. Modulation of neuronal activity by the endocannabinoid system

may be strongly activity-dependent,¹⁵ and effects may differ in pathological states in which increased excitation of neuronal circuitry might occur. In contrast, single clinical doses of the cannabinoid agonist nabilone (which would be less dependent on activity state for effects) are associated with notable impairment on cognitive performance and strong endorsement of several of the ARCI-53 scales in healthy volunteers.¹⁶ Older volunteers and women of nonchild-bearing potential were included to provide information on safety and tolerability prior to conducting proof-of-concept studies. Most of the older volunteers were women and the average age of the women of nonchild-bearing potential was 49, which does not allow for meaningful testing of the independent effects of age and gender on the PK of JNJ-42165279 or PD effects. Reevaluation of the impact of age and gender will be undertaken with the availability of data from subsequent clinical studies.

The persistence of central effects of JNJ-42165279 on FAAH inhibition were greater than we anticipated, based on peripheral effects. The plasma AEA turnover was dose-dependent and a maximal effect was observed at doses ≥75 mg (**Figure 3**). However, ~24 h after stopping the 10 mg dosing, ≥80% brain FAAH occupancy was still present, while ~50% FAAH activity had recovered in leukocytes (**Figure 2**). An increase in CSF AEA was also dose-dependent, although the range of responses after a 25 mg dose overlapped significantly with those after 75 mg, suggesting a maximum limit in changes in FAAH turnover in these healthy young adult males. The results of the CSF and PET studies lowered our initial estimates of the minimum dose required for phase II studies targeting mood and anxiety disorders and, importantly, allowed a greater safety margin based on preclinical toxicology studies. The 25 mg dose was selected as the clinical dose for use in proof-of-concept studies based on model predictions from the occupancy study demonstrating that brain FAAH inhibition could be sustained over the dosing interval in the majority of volunteers and based on the peripheral inhibition of FAAH across volunteers, also sustained over the dosing interval, while affording a larger safety margin than higher doses. As clinical efficacy has not yet been investigated for this compound, we regard the PD biomarkers as confirmation of pharmacology and hypothesize this dose to be sufficient for testing for clinical effects in our proof-of-concept trials. Given the meaningful pharmacologic effects seen at 10 mg, the lower dose appears to be a strong candidate for future clinical studies if 25 mg is found to be

effective. A recent study using a 4 mg once-daily dose of the selective FAAH inhibitor PF-04457845 demonstrated clinical efficacy in patients suffering from cannabis use disorder (CUD) in terms of reduced cannabis withdrawal symptoms, cannabis use, and sleep disturbances.¹⁷ This dose had been previously shown to be more than sufficient to result in 97% inhibition of WBC FAAH and elevate plasma FAAs.¹⁸ Dose ranging was not done, so these clinical results can only suggest that complete inhibition of FAAH is necessary for efficacy, at least in CUD.

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