Safety, Tolerability, and Pharmacokinetics of FAAH Inhibitor BIA 10-2474: A Double-Blind, Randomized, Placebo-Controlled Study in Healthy Volunteers

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This study evaluated the safety, tolerability, pharmacokinetics, and pharmacodynamics of BIA 10-2474, a fatty acid amide hydrolase (FAAH) inhibitor, after first administration to healthy male and female participants. Participants (*n* = 116) were recruited into this phase I, double-blind, randomized, placebo-controlled, single ascending dose and multiple ascending dose (10-day) study. The primary outcome was the safety and tolerability of BIA 10-2474. Secondary outcomes were pharmacokinetics of BIA 10-2474 and pharmacodynamics, considering plasma concentrations of anandamide and three other fatty acid amides (FAAs) and leukocyte FAAH activity. Single oral doses of 0.25–100 mg and repeated oral doses of 2.5–50 mg were evaluated. BIA 10-2474 was well tolerated up to 100 mg as a single dose and up to 20 mg once daily for 10 days. In the cohort receiving repeated administrations of 50 mg, there were central nervous system adverse events in five of six participants, one with fatal outcome, which led to early termination of the study. BIA 10-2474 showed a linear relationship between dose and area under plasma concentration-time curve (AUC) across the entire dose range and reached steady state within 5–6 days of administration, with an accumulation ratio, based on AUC_{0-24h}, of <2 on Day 10. BIA 10-2474 was rapidly absorbed with a mean terminal elimination half-life of 8–10 hours (Day 10). BIA 10-2474 caused reversible, dose-related increases in plasma FAAs. In conclusion, we propose that these data, as well as the additional data generated since the clinical trial was stopped, do not provide a complete mechanistic explanation for the tragic fatality.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ The first-in-human trial with the fatty acid amide hydrolase (FAAH) inhibitor BIA 10-2474 resulted in a death in the 50-mg repeat dose cohort after 5 days. Published clinical data have only been from that cohort.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ By publishing all clinical data on side effects and pharmacokinetics from the single and multiple ascending dose phases, this study provides additional data and addresses some of the speculations on the mechanism of the clinical toxicity of BIA 10-2474.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ These data indicate that the serious toxicity observed after repeat administration of 50 mg BIA 10-2474 could not have been anticipated from the previous dose cohorts and that the pharmacokinetic data indicate that neither nonlinearity nor accumulation of BIA 10-2474 was likely to have contributed to the accident.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Although no mechanism for the toxicity has so far been identified, the additional information presented here may help the scientific community and regulatory bodies to elaborate on more comprehensive and powerful predictive toxicology, encompassing some specific genomic fingerprinting of the test organism/participant.

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The endocannabinoid system has been implicated in a growing number of physiological functions, and its modulation holds therapeutic promise in a variety of disparate diseases and pathological conditions.[1–6] The enzyme fatty acid amide hydrolase (FAAH) is primarily responsible for hydrolyzing the endocannabinoid anandamide (AEA) and related signaling lipids.[7] FAAH inhibitors may be efficacious in management of diseases in which elevated endocannabinoid tone might be beneficial, without the side effects associated with direct-acting cannabinoid agonists.[8,9] As a result, FAAH inhibitors are being considered as potential adjunctive therapies for pain, glaucoma, and post-traumatic stress disorder.[9–13]

This manuscript details the first-in-human clinical study of BIA 10-2474, a potent, time-dependent, orally available FAAH inhibitor, consisting of a double-blind, randomized, placebo-controlled, single ascending dose (SAD) and 10-day once-daily multiple ascending dose (MAD) study, including a food interaction part (FI). In the cohort receiving repeated administrations of 50 mg BIA 10-2474, there were central nervous system (CNS) adverse events in five of six participants, one with fatal outcome, which led to study termination.[14,15] The phenomenology of the acute clinical course leading to the tragic adverse events have been described elsewhere.[16] The nonclinical data on BIA 10-2474 available before the clinical trial (CT) and used to support the CT application are available in a series of publications.[17–25] In addition, several studies have explored possible off-target activities of BIA 10-2474 to better understand possible mechanisms responsible for the toxicity seen in the clinic.[17,26] A comprehensive disclosure of relevant nonclinical data is provided as supplementary material (**BIA 10-2474 nonclinical studies**), reporting the nonpublished and published studies in laboratory animals as part of the development program of BIA 10-2474 contained in the Investigator's Brochure, as submitted to the French National Agency for the Safety of Medicines and Health Products (ANSM) back in 2015.

METHODS

Study design

This was a single-center, phase I, double-blind, randomized, placebo-controlled trial (EudraCT No.: 2015-001799-24) including SAD and MAD parts, and an FI part (open-label design). The initial plan included a separate pharmacodynamic (PD) part, but this was not carried out. Instead, the PD component was limited to measuring FAAH activity and plasma fatty acid amide (FAA) concentrations. The trial was conducted at Biotrial, Rennes, France between July 2015 and March 2016, when it was terminated during the MAD phase. The SAD consisted of groups of eight healthy young male and female participants, each receiving a single oral dose of BIA 10-2474 or placebo (six verum and two placebo). In the first group, two participants (one verum and one placebo) were dosed 24 hours before the remaining six participants (five verum and one placebo). Remaining participants' dosing was to be staggered if there were safety concerns.

The FI part consisted of 12 healthy young male and female participants, each receiving 40 mg of BIA 10-2474 in the fed and fasting state in an open-label, two-way crossover design separated by at least 14 days. The MAD also consisted of groups of eight healthy young male and female participants, each receiving an oral dose of BIA 10-2474 or placebo (six verum and two placebo) once-daily for 10 days. At all stages, the safety data from the previous dose cohorts and pharmacokinetic (PK) data from at least the next-to-last dose cohorts were reviewed in a blinded manner before recommendations for the next dose level were made.

The study was approved by the national Independent Ethics Committee and Competent Authorities and was conducted according to the Helsinki Declaration, and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice recommendations and applicable local regulations. Written informed consent was obtained for each study participant.

Population

Participants were screened for eligibility within 28 and 7 days of admission to the first treatment period. Screening consisted of a discussion of informed consent, medical history, physical examination, vital signs, neurological examination, 12-lead electrocardiography (ECG), clinical laboratory tests (hematology, plasma biochemistry, coagulation, urinalysis, viral serology, alcohol and drugs of abuse screen, and a urine pregnancy test in women of childbearing potential), and review of the selection criteria. Participants were aged 18–55 years, with a body mass index of 19–30 kg/m^2 and were nonsmokers or ex-smokers for at least 3 months; women were either not of childbearing potential because of surgery or used double barrier or intrauterine device pregnancy protection. No medication other than the study drugs or that necessary for the treatment of adverse events (AEs) was allowed from screening until final discharge. A summary of participant demographics is provided in **Table S1**.

On the dosing day of each part and/or treatment period, participants were fasting from food for a minimum of 8 hours before dosing and remained fasted for 1 hour post dosing. Water was allowed except for 1 hour before and after each dosing. From 24 hours before admission until discharge of each treatment period, participants were requested to abstain from consuming alcohol. Participants were requested to abstain from consumption of xanthine-containing beverages during the treatment periods. BIA 10-2474 was a light-blue hard gelatin capsule (0.25, 2.5, and 10 mg). The placebo was identical but without the active ingredient.

Dose selection

Selection of the single doses was based on extrapolation of *in vivo* data from nonclinical pharmacologic models, and safety data using safety margins derived from the no-observed-adverse-effect-level in the most sensitive species (details are given in Supplementary Information). That was 10 mg/kg/day in the rat, and this dose gave a human equivalent dose of 100 mg. To afford the maximal safety margin possible, the starting dose chosen in the SAD part was 0.25 mg with staggered dosing, in that only one participant received this dose level at first, with the remaining participants dosed 24 hours later. The dose levels of the following groups were increased until the dose reached 100 mg (1.25, 2.5, 5, 10, 20, and 40 mg). The doses selected for the remainder of the study (40 mg for the FI part; 2.5, 5, 10, 20, and 50 mg for MAD) were chosen on the basis of (blinded) emerging safety and PK to end with a detailed/broadened characterization of safety and pharmacokinetics of BIA 10-2474 and to determine a safe and tolerable dose of BIA 10-2474, maximum inhibition of FAAH, or significant increases in plasma endocannabinoid concentration (anandamide). The decision to progress to the next higher dose was made jointly by the Biotrial Investigator and medical monitor of BIAL. Nevertheless, the final decision rested with the Investigator.

Safety assessments

Safety and tolerability assessments included laboratory tests (blood chemistry, hematological profile, coagulation, and urinalysis), physical examination, ECG, and vital signs. Any undesirable sign, symptom, or

medical condition occurring after starting the study, whether reported spontaneously or when prompted, was recorded regardless of suspected relation to the study medication. AEs were coded according to the Medical Dictionary for Regulatory Activities (MedDRA, version 18.1). For the laboratory safety data, clinically significantly abnormal values were considered as AEs.

If the investigators identified any safety concerns, the dosing of participants for the following dose level was to be staggered with up to four participants initially dosed and a 24-hour follow-up before the remaining participants were dosed. In the absence of any safety concerns, dosing was not staggered for the following dose.

Blood sampling and analysis

Details of blood sampling for PK and pharmacodynamic analysis are given in Supplementary Information.

RESULTS

Safety

In the SAD part, AEs were mild (except one moderate back pain in SAD 100 mg 2 days after dosing), unspecific, with no clear dose relationship (**Table 1**). Tolerability was comparable to placebo even at higher doses. In the FI part, AEs were also mild (except for moderate orthostatic hypotension occurring more than 2 weeks after dosing) and transient. BIA 10-2474 was well tolerated at fasting or fed state.

In the MAD part (cohorts 1 to 4, up to 20 mg/day), AEs were mild (except moderate nasopharyngitis in 20 mg/day) and transient (**Table 1**). Although more participants experienced AEs with 10 and 20 mg/day BIA 10-2474, no AE was dose-dependent and causal relationship between BIA 10-2474 and these events was unlikely.

Possible CNS-related events included two participants (2.3%) with blurred vision (10 mg/day) and three (3.6%) with a headache (single doses of 40 mg, and 10 and 20 mg/day for 10 days). The blurred vision was mild and brief (<2.5 hours), without associated symptoms and not observed in any other cohort, particularly not at 20 mg/day. Of the three headaches, one was considered unlikely to be related to study medication (onset 16 days after a single dose of 40 mg). The other two were mild and started 24 hours post last dose. One resolved spontaneously and the other with 1 g of paracetamol. No participant showed any clinically relevant change in laboratory safety parameters or any clinically relevant abnormality on ECGs. Five participants were reported with transitory symptomatic orthostatic hypotension, but distribution was similar between BIA 10-2474 ($n = 3$) and placebo ($n = 2$).

During the 50-mg MAD part (up to day 5), five of six participants reported unexpected CNS serious adverse events (SAEs), for which causality cannot be excluded (**Table 1**). One male participant showed serious and sudden deterioration of clinical status with fatal outcome. No formal magnetic resonance imaging (MRI) diagnostic or autopsy report is currently available, although MRI imaging for three participants has been presented by Kerbrat *et al.*[16] One participant on BIA 10-2474 and two on placebo remained asymptomatic. All 84 participants previously treated with BIA 10-2474 were re-evaluated, and no neurological symptoms or MRI abnormalities were observed, except one case of vascular cerebellar lesion with an unknown exact date of occurrence.

Pharmacokinetics and pharmacodynamics

Following a single dose (**Figure 1a,b**, **Table 2**), BIA 10-2474 was rapidly absorbed with dose-related increases in maximum observed plasma concentration (C_{max}) and a linear decline in plasma concentrations with mean terminal elimination half-life between 4.51 and 9.28 hours. Noncompartmental PK parameters are summarized in **Table 2**. There was a clear dose proportionality for both C_{max} and AUC after single doses of BIA 10-2474 using the power model (**Table 3** and **Figure S1**). Of note, individual assessments were made during the analysis and no individual profile was found to be an outlier or to deviate from expected values.

Following repeated doses (**Figure 1c,d**), plasma profiles at Days 1 and 10 were similar to the SAD part, with concentrations peaking quickly and decreasing thereafter. Noncompartmental PK on both Days 1 and 10 are summarized in **Table 4**. There was less than a twofold accumulation of BIA 10-2474 after 10 days (**Table 4**). Dose proportionality was demonstrated for C_{max} and AUC to either Day 1 (all doses) or Day 10 (excluding 50 mg/day), using the power model (**Table 3** and **Figure S1**). From visual inspection, steady-state pre-dose concentration (C_{trough}) values appeared to be reached within 5–6 days post dose. Individual plasma profiles at Day 1 (**Figure 1e,f**) of the 50 mg/day BIA 10-2474 were generally similar, and no outliers or deviation from expected values was apparent.

Exposure of BIA 10-2474 decreased ~15% following single oral administration of 40 mg BIA 10-2474 in the presence of food (**Figure S2**).

Metabolites identified from *in vitro* studies (BIA 10-2445, BIA 10-2583, BIA 10-2631, and BIA 10-2639) had low plasma abundance following a single dose, being undetectable at dose levels below 40 mg (**Table 2**), or following repeated ascending doses (**Table 4**), being undetectable at dose levels below 20 mg for 10 days. The calculated kinetic parameters revealed that their contribution for overall kinetic evaluation was very low (both C_{max} and $AUC_{0\text{-}\tau}$) because the sum of the four metabolites was less than 8.0% of the parent BIA 10-2474 (**Tables 2** and **4**).

BIA 10-2474 caused dose-related increases in plasma AEA (12.5-fold AUC at 100 mg BIA 10-2474 compared with placebo), with measurable changes after the 1.25-mg dose (**Table 5** and **Figure 2a**). AEA plasma concentrations increased in a dose-dependent manner (**Table 5** and **Figure S3**). This increase followed the plasma BIA 10-2474 concentration with a time lag of about 4 to 10 hours between maximum PK and PD responses and a return to baseline >72 hours post dose; AEA levels remained elevated at 72 hours post dose after 10, 20, 40, and 100 mg BIA 10-2474. BIA 10-2474 also caused dose-dependent increases in at least the extent of plasma systemic exposure of N-oleoyl ethanolamide, N-palmitoyl ethanolamide, and N-linoleoyl ethanolamide concentrations (**Tables S2** and **S3**). The data available following BIA 10-2474 single doses suggest that leukocyte FAAH inhibition occurred sooner than the increases of AEA concentrations (**Figure 2b**). From visual inspection, BIA 10-2474 (0.25 mg), a dose with no effect in any plasma FAA concentrations, presented more than 50% FAAH inhibition, and an apparent maximum FAAH inhibition (> 97%) was noted after administration of 5 mg BIA 10-2474 and above.

Table 1 Summary of adverse events following doses of BIA 10-2474

	Single Ascending Dose (SAD) and Food Interaction (FI)								
		BIA 10-2474							
	Placebo	0.25 mg	1.25 mg	2.5 mg	5 mg	10 mg	20 mg	40 mg*	100 mg
	N = 16	*N* = 6	*N* = 6	*N* = 6	*N* = 6	*N* = 6	*N* = 6	*N* = 18	*N* = 6
Any AE, n (%)	*6 (37.5%)*	*0*	*0*	*1 (16.7%)*	*0*	*1 (16.7%)*	*0*	*5 (27.8%)*	*1 (16.7%)*
Dizziness								1	
Headache								1	
Soft feces	**1**								
Diarrhea	**1**								
Nausea	1							1	
Back pain								1	1[#]
Pain in extremity	**1**								
Orthostatic hypotension	**2**			**1**		**1**		1[#]	

	Multiple Ascending Dose (MAD) up to 20 mg				
		BIA 10-2474			
	Placebo	2.5 mg	5 mg	10 mg	20 mg
	N = 10	*N* = 6	*N* = 6	*N* = 6	*N* = 6
Any AE, n (%)	*0*	*1 (16.7%)*	*1 (16.7%)*	*3 (50%)*	*4 (66.7%)*
Abdominal pain		**1**			**1**
Blurred vision				**2**	
Nasopharyngitis					1 + 1[#]
Postural dizziness				**1**	**1**
Headache				**1**	1
Sciatica			1		
Diarrhea		**1**			
Presyncope				1	

	Multiple Ascending Dose (MAD) at 50 mg	
Primary SOC	**Event**	***No.* events**
Nervous system disorders	Transient global amnesia	1[#§]
	Headache	2 + 3[#]
	Hemiparesis	1[£§]
	Dizziness	2
	Nervous system disorder	1[£§H]
	Blurred vision	1
	Diplopia	1[#§]
	Dysarthria	1[#§&]
Gastrointestinal disorders	Diarrhea	2
	Abdominal pain	1
	Nausea	1
	Soft feces	1[#]
Surgical and medical procedures	Hot Flush	1[#]
Vascular disorders		
General disorders and administration site conditions	Asthenia	1

(Continued)

Table 1 (Continued)

	Multiple Ascending Dose (MAD) at 50 mg	
Primary SOC	**Event**	***No.* events**
	Feeling drunk	1[#§&]
Eye disorders	Vitreous floaters	1[§*]

Summary of adverse events following single oral doses up to 100 mg BIA 10-2474 (AEs, Safety Set, SAD and FI parts) and summary of adverse events following multiple oral doses up to 50 mg BIA 10-2474 (AEs, Safety Set, MAD part).
AE, adverse event; SOC, standard of care; bold font, the cases considered by investigator as treatment related; *, SAD and FI combined; #, moderate in severity; £, severe in severity; §, serious adverse event; H, required in-patient hospitalization; &, present in the participant with fatal outcome.

In the MAD part, BIA 10-2474 caused dose-related increases in plasma AEA concentrations on Days 1 and 10 (**Table 5**, **Figure 2c** and **Figure S3**). Plasma changes in AEA followed BIA 10-2474 exposure, with a similar time lag between maximum PK and PD and AEA levels, and remained elevated at 72 hours post dose. BIA 10-2474 caused dose-dependent increases in plasma N-oleoyl ethanolamide, N-palmitoyl ethanolamide, and N-linoleoyl ethanolamide concentrations (**Table S3**).

DISCUSSION

In presenting the data from the BIA 10-2474 phase I study, our objective was to make all data currently in our possession concerning this study available for review and evaluation by third parties, although it should be noted that some data and clinical material (blood samples, imaging, autopsy, etc.) are still *sub judice* and not available for publication. Equally important is the invitation to critically and imaginatively explore the data for possible and hitherto overlooked warning signs of the serious toxicology that was observed in the 50-mg MAD cohort.

There has been considerable discussion in the literature on the design of this trial and the choice of BIA 10-2474 as a candidate.[27–31] Some of the issues raised, such as potency and selectivity of BIA 10-2474, have been addressed[17] and will not be discussed in any detail. It should also be stressed that the design received all necessary regulatory authorization.[32,33]

Following single oral doses of 0.25–100 mg and repeated oral doses of 2.5–20 mg, BIA 10-2474 was safe and well tolerated. It was rapidly absorbed with dose proportionality to C_{max} and AUC after both single and once-daily repeated doses. BIA 10-2474 reached steady state within 5–6 days of administration, with an accumulation ratio observed on Day 10 of less than twofold. Human metabolites identified *in vitro* (BIA 10-2445, BIA 10-2583, BIA 10-2631, and BIA 10-2639) had low plasma abundance, and their calculated kinetic parameters revealed a negligible contribution to the overall exposure. The design was a standard combination of eight single ascending dose (SAD) cohorts, food interaction studies, and four multiple ascending dose (MAD) cohorts receiving the compound for 10 days.[31] The starting dose in the SAD phase was based on the no-observed-adverse-effect-level of 10 mg/kg/day in the rat, the most sensitive species,[20,21] giving a human equivalent dose of 100 mg. At the starting dose of 0.25 mg and up to the highest dose tested in the SAD phase of 100 mg, treatment-emergent adverse effects were reported by 15 participants receiving any dose of BIA 10-2474 (17.9% of participants receiving drug) and 5 participants receiving placebo (20.8%). None of these adverse effects were reported by more than three participants, none were considered serious, and none resulted in the withdrawal of participants from the study.

At least three other FAAH inhibitors have been evaluated in phase I studies, including the reversible inhibitor V158866 and the irreversible inhibitors PF-04457845 and JNJ-42165279, with no identified toxicity associated with FAAH inhibition.[9,12,13] In the case of the irreversible FAAH inhibitors, this was despite them being tested at doses considerably higher than required for complete FAAH inhibition, as measured in leukocytes. In the case of JNJ-42165279, at least 10 times the dose required for complete FAAH inhibition was tested,[13] and for PF-04457845, 133 times the dose was used.[12] Complete FAAH inhibition for long periods was not associated with any specific safety concerns even when doses exceeded a maximal pharmacological dose by 1 or 2 orders of magnitude. In the present study, approximately maximal FAAH inhibition was observed from 5 mg, making the maximal dose tested, 100 mg (20-fold higher), within the range of previously tested multiples for FAAH inhibition. The clinical data with PF-04457845 were available prior this clinical trial and did not suggest any prior reasons to consider a CT with BIA 10-2474, an irreversible inhibitor like PF-04457845, as having a high risk resulting from the primary pharmacological activity.

Regulatory toxicology studies with BIA 10-2474 had similarly not given cause to consider this phase I study to be high risk.[18,19,21–25] This risk assessment was shared by ANSM and the ethics committee overseeing study approval.[32] Consequently, there was no requirement to comply with the EMA guidance for high-risk compounds. Eddleston *et al.*[29] agree with this perspective despite being otherwise critical, largely as a result of much of the relevant data, now published, being unavailable. Although recent guidelines indicate that maximal exposure in phase I trials should not exceed the pharmacologically active range (e.g., ref. 34), previous guidelines, applicable when this trial was designed and approved, are less clear-cut.[35]

The PK data from the SAD phase demonstrate that over the tested dose-range (0.25–100 mg) exposure to BIA 10-2474 increased in a dose-proportional manner. This was true of the MAD phase after both the initial dose (up to 50 mg) and after 10 daily administrations (up to 20 mg). Both C_{max} and AUC increased between the first and 10th administration by an average factor of 1.24 and 1.44, respectively. This predicts a steady-state level in the 50 mg cohort of around two-thirds that achieved in the 100 mg SAD cohort.

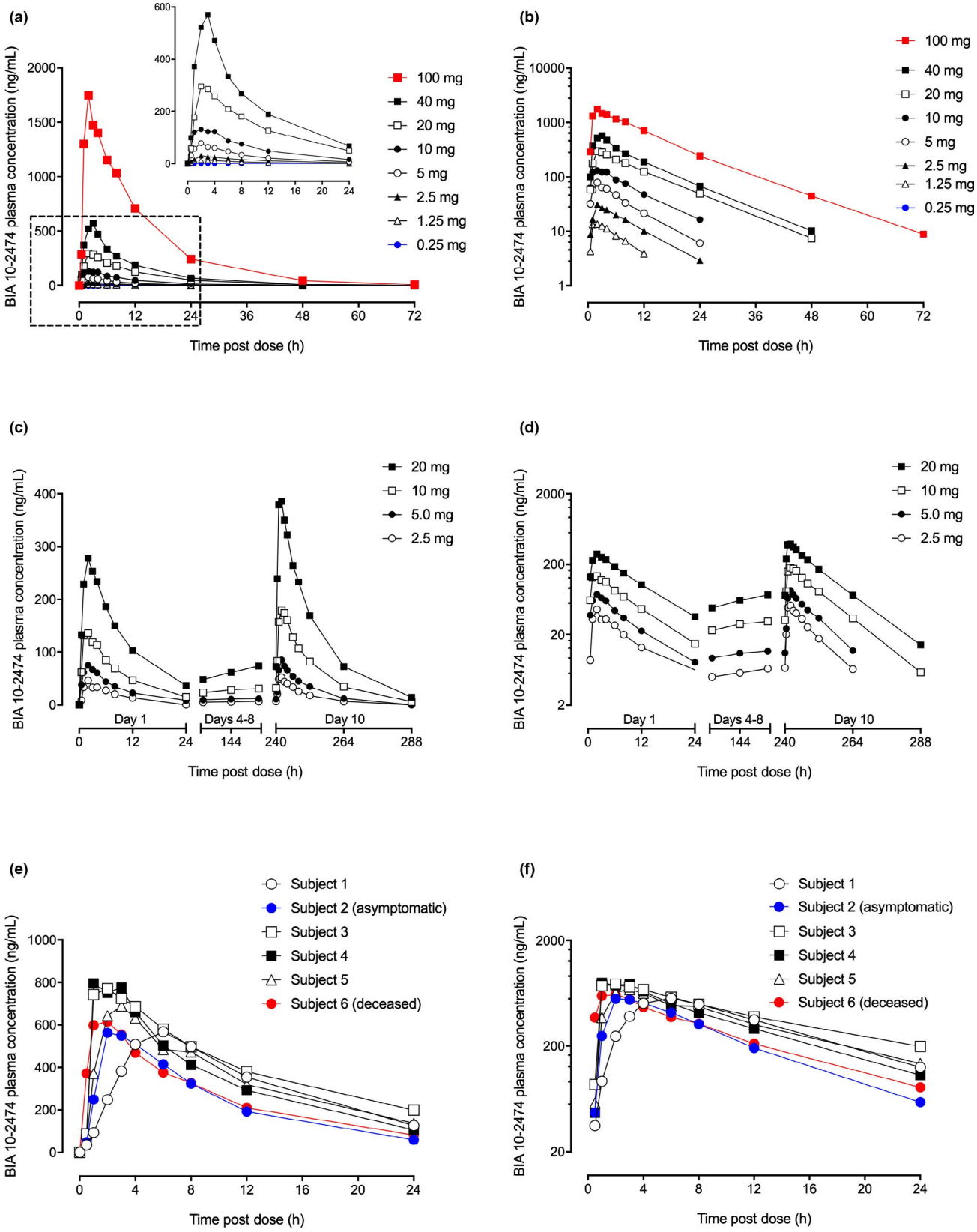

Figure 1 BIA 10-2474 mean plasma concentrations after (**a** and **b**) single and (**c** and **d**) multiple oral dose administration. The inset represents concentrations up to 24 hours and dose levels from 0.25 to 40 mg. Panels (**e**) and (**f**) depict BIA 10-2474 plasma concentrations after single oral dose administration of 50 mg BIA 10-2474 in six participants during Day 1 of the multiple administration period. Plasma concentrations from Days 4 to 8 are predose (C_{trough}) estimates. Symbols represent the means of six determinations per group. C_{trough}, lowest concentration.

Table 2 Pharmacokinetic parameters of BIA 10-2474 and metabolites following single ascending doses (SAD)

BIA 10-2474							
Dose (mg)	C_{max} (ng/mL)	t_{max} (hour)	AUC_{0-t} (ng·hour/mL)	$AUC_{0-\infty}$ (ng·hour/mL)	$t_{1/2}$ (hour)	V/F (L)	CL (L/hour)
0.25	3.1 (9.09)	2.0 (1.0–3.0)	NA	NC	NC	NC	NC
1.25	16.2 (17.1)	1.5 (1.0–3.0)	98.0 (29.0)	142 (23.9)	6.54 (14.4)	87.8 (30.4)	9.28 (25.2)
2.5	31.2 (22.6)	1.5 (0.50–2.0)	289 (33.3)	338 (25.1)	7.04 (13.3)	79.0 (29.0)	7.85 (30.9)
5	85.4 (21.0)	2.0 (0.50–2.0)	693 (20.9)	752 (19.9)	6.57 (18.6)	64.6 (23.0)	6.84 (18.4)
10	146 (35.4)	3.0 (1.0–4.0)	1,441 (30.6)	1,614 (31.7)	7.01 (17.1)	66.9 (29.8)	6.74 (31.3)
20	308 (17.6)	2.0 (1.0–4.0)	4,073 (12.8)	4,168 (12.7)	8.70 (8.67)	61.3 (19.0)	4.87 (14.4)
40	790* (42.9)	2.0 (1.0–3.0)	8,751* (48.5)	8,829* (48.1)	7.84 (24.8)	73.2 (28.3)	6.98 (50.6)
100	1,772 (12.5)	2.0 (1.0–3.0)	22,991 (23.5)	23,166 (23.7)	9.13 (26.3)	57.0 (16.3)	4.51 (22.4)
BIA 10-2474 metabolites							
Metabolite	C_{max} (ng/mL)	t_{max} (hour)	AUC_{0-t} (ng hour/mL)	$AUC_{0-\infty}$ (ng hour/mL)	$t_{1/2}$ (hour)	V/F (L)	CL (L/hour)
40 mg BIA 10-2474							
BIA 10-2445	4.66 (44.7)	12.0 (3.0–24.0)	51.3 (71.4)	NC	NC	NC	NC
BIA 10-2583	NA	NA	NC	NC	NC	NC	NC
BIA 10-2631	NA	NA	NC	NC	NC	NC	NC
BIA 10-2639	8.09 (30.7)	2.0 (1.0–3.0)	59.2 (30.6)	11 (34.1)	9.78 (67.1)	NC	NC
100 mg BIA 10-2474							
BIA 10-2445	11.4 (27.1)	12.0 (3.0–24.0)	303 (68.3)	NC	NC	NC	NC
BIA 10-2583	NA	NA	NC	NC	NC	NC	NC
BIA 10-2631#	7.1 (30.9)	18.0 (2.0–24.0)	359 (36.3)	NC	NC	NC	NC
BIA 10-2639	19.6 (29.1)	2.0 (1.0–3.0)	206 (40.7)	271 (35.6)	9.20 (15.2)	NC	NC

Pharmacokinetics parameters of BIA 10-2474 and metabolites BIA 10-2445, BIA 10-2583, BIA 10-2631, and BIA 10-2639 after the administration of single ascending doses of BIA 10-2474 (SAD part). Values are presented as mean (coefficient of variation, CV%); t_{max}, values are median with range in parenthesis. Plasma levels of BIA 10-2445, BIA 10-2583, BIA 10-2631 and BIA 10-2639 were not detected at dose levels below 40 mg BIA 10-2474.
AUC, area under plasma concentration-time curve; CL/F, apparent total body clearance; C_{max}, maximum observed plasma concentration; NA, not available; NC, not calculated; $t_{1/2}$, apparent terminal half-life; t_{max}, time of occurrence of C_{max}; V/F, apparent volume of distribution; *, combines data from both SAD and food interaction under fasting conditions; #, N = 4.

Though a direct evaluation of FAAH activity in circulating leukocytes was not possible due to accuracy failure (see Supplementary Pharmacokinetic and Pharmacodynamic Analysis), BIA 10-2474 appeared to cause maximal inhibition of FAAH activity, as evidenced by the dose-dependent increases in plasma FAAs. Similar problems in FAAH activity evaluation were reported by Pawsey *et al.*[9] with the reversible inhibitor V158866. There was a dose-dependent relationship between BIA 10-2474 exposure and changes in plasma FAAs with relevant AEA increases at a BIA 10-2474 plasma exposure of around 1,500 ng·h/mL, which was achieved with 10 mg/day.

One consequence of mechanism-based irreversible enzyme inhibitors is that functional loss of enzyme activity can last substantially longer than exposure to the drug. In the case of BIA 10-2474, the drug half-life in plasma is between 6 and 10 hours, whereas FAAH activity in plasma appears to remain inhibited by more than 90% for at least 72 hours at doses above 2.5 mg. These data are comparable to other irreversible inhibitors such as PF-04457845, which has a half-life between 11 and 23 hours but which can inhibit FAAH by greater than 90% for 6 days or more,[12] and JNJ-42165279 which has a half-life of 8–14 hours but

Table 3 Relationship between main PK parameters and dose of BIA 10-2474

Single Ascending Dose (SAD)					
Dose (mg)	Fold increase in dose[#]	C_{max} (ng/mL)	Fold increase in C_{max}[#]	AUC_{0-t} (ng·hour/mL)	Fold increase in AUC_{0-t}[#]
0.25	—	—	—	—	—
1.25	—	16.2	—	98.0	—
2.5	2.0	31.2	1.9	289	—
5	2.0	85.4	2.7	693	2.4
10	2.0	146	1.7	1,441	2.1
20	2.0	308	2.1	4,073	2.8
40	2.0	790	2.6	8,751	2.2
100	2.5/-	1,772	2.2	22,991	2.6
Overall[*]	20		14.5		19.6
DPF[+]	1.0		0.8		0.9
Exponent[†]		0.93 (0.81; 1.05)		1.05 (0.88; 1.21)	

Multiple Ascending Dose (MAD)										
	Day 1					Day 10				
Dose (mg)	Fold increase in dose[#]	C_{max} (ng/mL)	Fold increase in C_{max}[#]	$AUC_{0-\tau}$ (ng·h/mL)	Fold increase in $AUC_{0-\tau}$[#]	Fold increase in dose[#]	C_{max} (ng/mL)	Fold increase in C_{max}[#]	$AUC_{0-\tau}$ (ng·h/mL)	Fold increase in $AUC_{0-\tau}$[#]
2.5	—	46.0	—	397	—	—	52.6	—	526	—
5	2.0	76.2	1.7	708	1.8	2.0	85.7	1.6	909	1.7
10	2.0	143	1.9	1393	2.0	2.0	190	2.2	2198	2.4
20	2.0	290	2.0	2983	2.1	2.0	396	2.1	4651	2.1
50	2.5	667	2.3	7768	2.6					
Overall[*]	20		14.5		19.6	8		7.53		8.84
DPF[+]	1.0		0.8		0.9	1.0		0.9		1.1
Exponent[†]		0.93 (0.81; 1.05)		1.05 (0.88; 1.21)			1.06 (0.88; 1.24)		1.11 (0.93; 1.29)	

Relationship between main pharmacokinetic parameters and dose of BIA 10-2474 following single oral doses (SAD part) and multiple (MAD part) oral doses. AUC, area under plasma concentration-time curve; C_{max}, maximum observed plasma concentration; PK, pharmacokinetic; [#], fold increase in dosage or parameters between adjacent dosages (start at 1.25 mg for $^{1}C_{max}$ and at 2.5 mg for $^{2}AUC_{0-t}$); *, fold increase in dosage or parameter over the dosage range 0.25 to 100 mg BIA 10-2474 (range 1.25 to 100 mg for $^{1}C_{max}$ and 2.5 to 100 mg for $^{2}AUC_{0-t}$); [+], DPF, dose proportionality factor, ratio of fold increase in parameter divided by fold increase in dosage; [†], exponent of the power model (95% confidence interval); —, data not calculated or available.

inhibits FAAH beyond 24 hours.[13] As those substances were safe, neither this long-lasting inhibition of FAAH, nor the subsequent changes in FAAs, are likely to be the cause of the toxicity seen with BIA 10-2474.

Following the unfortunate death, several contributing factors have been suggested. Eddleston *et al.*[29] made several suggestions, as did the CSST (ANSM's specialised scientific committee: Comité Scientifique Spécialisé Temporaire).[32] These related specifically to the properties of BIA 10-2474 rather than the trial itself and will only be discussed briefly here. For example, the question of specificity has been addressed elsewhere,[17,26] and despite the identification of some off-target activity of BIA 10-2474, the relevance to the SAEs observed in the clinic has not yet been demonstrated.

As Eddleston *et al.*[29] suggest, staggered dosing of participants in a given cohort is recommended if late SAEs are anticipated. Staggered dosing was used in the present protocol at the beginning of the SAD part, and the protocol allowed for staggered dosing for all subsequent cohorts if concerns on safety and/or tolerability arose. As this was not the case prior to the 50-mg MAD cohort, staggered dosing was not used. According to the data in Kerbrat *et al.*,[16] the first symptoms in the 50-mg cohort occurred 5–7 days after the initial administration. Thus, even if the 24-hour delay that the protocol allowed had been used, the participants would still have been exposed. In addition, as the early appearance of symptoms reported by the surviving participants were not immediately considered as severe or serious AEs by experienced clinicians, these ill-defined prodromes might not be the most sensitive or reliable markers for halting a trial. Consideration should also be given to stopping criteria based on data that take account of moderate nonserious adverse reactions.

The CSST[32] suggested that the PK became nonlinear between 40 and 100 mg. As shown by the data presented, there is no evidence to support the nonlinearity of the PK parameters related to exposure, C_{max}, and AUC over the dose range tested in humans. Coupled to

Table 4 Pharmacokinetic parameters of BIA 10-2474 and metabolites after multiple ascending dose (MAD)

BIA 10-2474															
	Day 1							Day 10							
Dose (mg)	C_{max} (ng/mL)	t_{max} (hour)	AUC_{0-t} (ng·hour/mL)	$AUC_{0-\tau}$ (ng·hour/mL)	$t_{1/2}$ (hour)	V/F (L)	CL (L/h)	C_{max} (ng/mL)	t_{max} (h)	C_{min} (ng/mL)	$AUC_{0-\tau}$ (ng·hour/mL)	$t_{1/2}$ (hour)	V/F (L)	CL (L/hour)	Racc
2.5	46.0 (21.5)	2.0 (2.0–2.0)	384 (27.3)	397 (19.2)	6.73 (21.6)	56.1 (18.7)	5.90 (17.4)	52.6 (22.1)	2.0 (1.0–2.0)	6.24 (21.4)	526 (20.4)	8.04 (11.0)	57.0 (26.0)	4.89 (18.5)	1.15
5	76.2 (25.5)	2.0 (1.0–2.0)	695 (38.0)	708 (34.4)	6.40 (24.2)	63.2 (20.4)	7.48 (46.6)	85.7 (23.1)	2.0 (1.0–2.0)	10.8 (56.6)	909 (33.5)	7.87 (19.1)	65.3 (17.4)	6.06 (36.8)	1.31
10	143 (27.4)	2.0 (1.0–2.0)	1,393 (26.2)	1,393 (26.2)	7.12 (19.4)	69.0 (22.8)	6.94 (31.8)	190 (24.5)	2.0 (1.0–3.0)	31.9 (49.5)	2,198 (27.9)	9.09 (19.8)	61.4 (17.9)	4.82 (24.9)	1.59
20	290 (15.5)	1.5 (1.0–3.0)	2,983 (14.4)	2,983 (14.4)	7.74 (11.8)	66.3 (7.34)	6.02 (15.0)	396 (12.9)	2.0 (0.5–2.0)	69.2 (11.9)	4,651 (10.0)	10.2 (10.8)	64.1 (15.4)	4.33 (9.26)	1.57
50	667 (15.2)	2.0 (1.0–6.0)	7,768 (19.6)	7,768 (19.6)	8.67 (22.8)	67.9 (9.78)	5.68 (26.5)	NA	NA	NA	NC	NC	NC	NC	NC

BIA 10-2474 metabolites															
	Day 1							Day 10							
Metabolite	C_{max} (ng/mL)	t_{max} (hour)	AUC_{0-t} (ng·hour/mL)	$AUC_{0-\infty}$ (ng·hour/mL)	$t_{1/2}$ (h)	V/F (L)	CL (L/h)	C_{max} (ng/mL)	t_{max} (hour)	AUC_{0-t} (ng·hour/mL)	$AUC_{0-\infty}$ (ng·hour/mL)	$t_{1/2}$ (hour)	V/F (L)	CL (L/hour)	Racc
20 mg BIA 10-2474											**20 mg BIA 10-2474**				
BIA 10-2445	NA	NA	NA	NA	NC	NC	NC	8.27 (3.42)	4.0 (4.0–12.0)	105 (64.7)	NC	NC			
BIA 10-2583	NA	NA	NA	NA	NC	NC	NC	NA	NA	NA	NC	NC			
BIA 10-2631*	NA	NA	NA	NA	NC	NC	NC	7.41 (3.9)	4.0 (2.0–24.0)	203 (197)	NC	NC			
BIA 10-2639	4.1 (0.5)	1.0 (1.0–2.0)	21.8 (6.8)	26.6 (8.5)	9.94 (1.79)	NC	NC	5.8 (1.7)	1.0 (1.0–2.0)	47.3 (20.6)	122* (27.8)	13.4* (3.6)			

Pharmacokinetic parameters of BIA 10-2474 and metabolites BIA 10-2445, BIA 10-2583, BIA 10-2631, and BIA 10-2639 after the administration of multiple oral doses on day 1 (for all doses) and 10 (excluding 50 mg/day) (MAD part). Values are presented as mean (coefficient of variation, CV%); t_{max}, values are median with range in parenthesis. Plasma levels of BIA 10-2445, BIA 10-2583, BIA 10-2631 and BIA 10-2639 were not detected at dose levels below 20 mg BIA 10-2474.

AUC, area under plasma concentration-time curve; CL/F, apparent total body clearance; C_{max}, maximum observed plasma concentration; NA, not available; NC, not calculated; $t_{1/2}$, apparent terminal half-life; t_{max}, time of occurrence of C_{max}; V/F, apparent volume of distribution. *, $N = 5$.

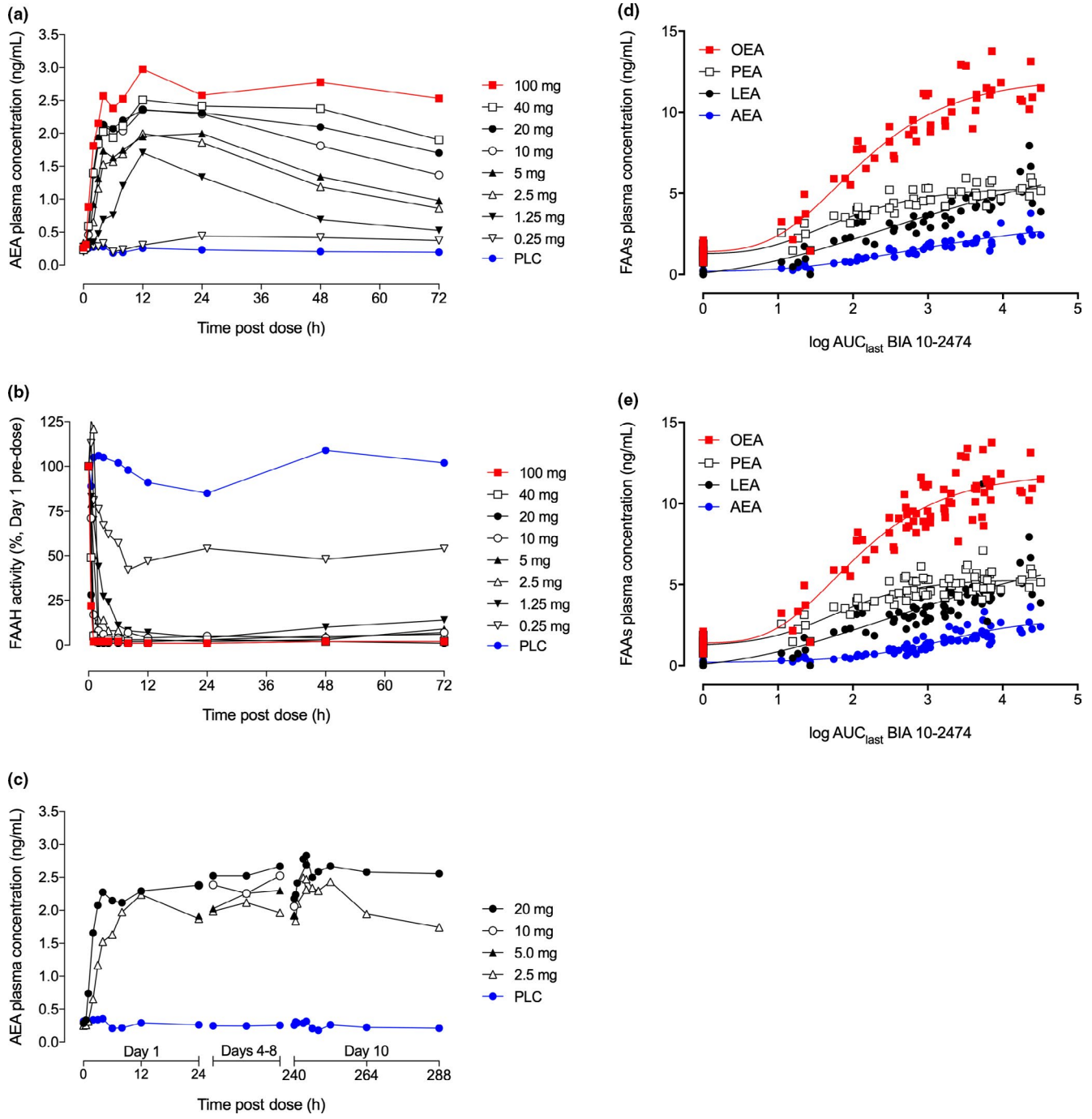

Figure 2 AEA mean plasma concentrations (**a**) and after FAAH activity (**b**) single oral dose administration (SAD part) and AEA mean plasma concentrations after (**c**) repeated oral dose administration (MAD part). Relationship between BIA 10-2474 dose and fatty acid amides (FAAs) concentrations: The sigmoidal line of best fit is shown for each FAA ((**d**), SAD data only; (**e**), SAD enriched with Day 1 MAD data). Symbols represent the means of six determinations per group. AEA, N-arachidonyl ethanolamide; AUC, area under plasma concentration-time curve; FAAH, Fatty acid amide hydrolase; LEA, N-linoleoyl ethanolamide; OEA, N-oleoyl ethanolamide; PEA, N-palmitoyl ethanolamide; PLC, placebo.

this is the observation that the PK data of the prior cohort (20-mg MAD) were not known before starting dosing of the 50-mg cohort. Firstly, the PK parameters following single doses up to 100 mg were already known. Secondly, no one has disputed the linearity of exposure up to 40 mg. Thirdly, the data from the previous MAD cohorts, up to 10 mg, showed that exposure remained linear after 10 daily administrations and that the increase in exposure remained substantially below the levels attained during the 100-mg single-dose administration. While we agree with suggestions that in the future these parameters should be known before proceeding to the next dose, these data do not indicate that unexpected changes to PK parameters were responsible for the SAEs experienced by the 50-mg cohort.

The increase from 20 to 50 mg has been suggested by the CSST[32] and General Inspectorate of Social Affairs (IGAS)[33] to

Table 5 Summary of AEA PK following placebo and BIA 10-2474 dosing

Single ascending dose (SAD)							
Dose (mg)	C_{max} (ng/mL)	t_{max} (hour)	AUC_{0-t} (ng·hour/mL)				
PLC	0.317 (22.0)	2.50 (0.0–24.0)	15.1 (29.7)				
0.25	0.469 (38.9)	36.0 (0.0–48.0)	27.5 (34.1)				
1.25	1.71 (12.7)	12.0 (12.0–24.0)	68.0 (13.1)				
2.5	2.08 (13.6)	12.0 (12.0–24.0)	101 (17.2)				
5	2.02 (8.27)	24.0 (6.0–24.0)	109 (11.2)				
10	2.49 (8.95)	12.0 (4.0–24.0)	137 (8.92)				
20	2.47 (9.64)	12.0 (8.0–24.0)	149 (6.20)				
40	2.57 (28.9)	18.0 (12.0–48.0)	160 (26.42)				
100	3.07 (20.7)	12.0 (4.0–48.0)	188 (22.4)				
Multiple Ascending Dose (MAD)							
	Day 1			Day 10			
Dose (mg)	C_{max} (ng/mL)	t_{max} (hour)	AUC_{0-24} (ng·hour/mL)	C_{max} (ng/mL)	t_{max} (hour)	$AUC_{0-\tau}$ (ng·hour/mL)	Ratio ($AUC_{D10/D1}$)
PLC	0.377 (11.7)	3.5 (0.5–4.0)	6.45 (12.2)	0.356 (14.7)	32.0 (0.5–72.1)	5.80 (15.8)	0.899
2.5 mg	2.24 (16.5)	12.0 (12.0–12.0)	42.8 (17.1)	2.62 (11.95)	3.5 (2.0–8.0)	54.2 (9.41)	1.27
5 mg	2.36 (25.3)	12.0 (12.0–12.0)	44.9 (23.4)	2.74 (21.4)	3.5 (2.0–8.0)	56.3 (17.7)	1.25
10 mg	2.62 (17.9)	12.0 (4.0–24.0)	52.2 (19.3)	2.86 (18.7)	2.5 (1.0–4.0)	60.8 (17.9)	1.16
20 mg	2.49 (20.1)	24.0 (4.0–24.0)	51.2 (19.6)	2.94 (23.6)	3.0 (2.0–24.0)	62.8 (21.7)	1.22

Summary of AEA pharmacokinetics (PK) following placebo and BIA 10-2474 oral doses (SAD part; MAD part, excluding 50 mg/day). Values are presented as mean (coefficient of variation, CV%); t_{max} values are median with range in parenthesis.
AEA, anandamide; AUC, area under plasma concentration-time curve; C_{max}, maximum observed plasma concentration; PLC, placebo; $t_{1/2}$, apparent terminal half-life; t_{max}, time of occurrence of C_{max}.

have been too large, but this was before much of the data were available. A similar increase was used in the SAD phase, from 40 to 100 mg, resulting in greater C_{max} and AUC values than occurred in the 50-mg MAD cohort, without any indication of adverse effects. Also, the PK in the 50-mg cohort was as predicted from the data of the SAD cohorts and the 10-mg and 20-mg MAD cohorts. No sudden jump to exposure levels any greater than those which had been shown to be safe occurred. Nevertheless, the amount of BIA 10-2474 administered to the 50-mg cohort did exceed the amount received by previous volunteers (i.e., 200 mg in the 20-mg MAD cohort) following the fifth administration. Prior to this, there were no SAEs observed or reported.

Some have speculated that the toxic effects of BIA 10-2474 represent a threshold effect once total exposure achieves a certain level, due to either an accumulation or a cumulative pharmacological effect that did not produce any physiological effects below a certain level. There is little evidence for the accumulation effect, either in humans or in animals.[17,21] The second hypothesis is more speculative, but, since the CT, several off-targets have been identified. Some of these interactions occur at exposures similar to or only slightly higher than those required to inhibit FAAH. These include ABHD6 and carboxyl esterases.[17,26,36] It seems unlikely that these targets are responsible for the toxicity seen in the clinic. In contrast, there are other interactions that occur at much higher concentrations that have been associated with threshold effects in humans, including PNPLA6.[17,26,36] The concentrations required for interaction with this enzyme are higher than those achieved in the clinic; doses used in toxicology studies which did result in inhibition of this enzyme were not associated with any signs suggesting neurotoxicity; and finally, the clinical signs of toxicity that occur following PNPLA6 inhibition are dissimilar to those reported for BIA 10-2474.[17]

One potential issue with irreversible inhibitors that bind covalently to their target enzyme is the possible haptenization of the enzyme-inhibitor combination,[37] with the subsequent risk of generating an autoimmune response. To date, there is no evidence to support this mechanism in the toxicity of BIA 10-2474,[38] and the clinical signs seen in the 50-mg cohort are not consistent with such an effect as indicated by CSST.[32] There were no changes to any leukocyte parameters in the toxicology studies[18,19,21–23,25] which might be expected if haptenization occurred.

Most have concentrated on the hypothesis that the toxicity of BIA 10-2474 is centrally mediated. However, other possibilities

deserve consideration. The symptoms described by the volunteers in the 50-mg cohort, such as headache, visual disturbances, ataxia, and cognitive changes correspond closely with those of central vascular disorders including primary angiitis of the CNS, reversible cerebral vasoconstriction syndrome, and posterior reversible encephalopathy syndrome (PRES). In most of these syndromes, the MRI abnormalities are somewhat different from those seen in this trial. However, PRES is an interesting possibility as the MRI lesions are subcortical and often symmetrical[39] and PRES is associated with inflammation, which was observed in the participant that died. However, transcranial doppler assessment did not indicate intracranial vasoconstriction in the present study and all these disorders can be induced by drugs.[40] In addition, in most cases they are reversible, and for the surviving volunteers, the symptoms appear to have regressed or are regressing. All 50-mg cohort participants showed radiologic imaging evidence of microhemorrhage in the pons, hippocampus, and amygdala,[16] suggesting that the vascular hypotheses should be pursued.

In conclusion, despite global scientific and medical scrutiny over the preceding 5 years since the CT, the adverse effects of BIA 10-2474 seen in the clinic remain fundamentally unexplained.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

J.-F.R., P.M., P.P., A.W.H., and P.S.-d.-S. wrote the manuscript. J.-F.R. and P.S.-d.-S. designed the research. J.-F.R. and A.S. performed the research. J.-F.R., P.M., H.G., A.F., and P.S.-d.-S. analyzed the data.

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