Clinical Safety, Pharmacokinetics, and Pharmacodynamics of the 11β-Hydroxysteroid Dehydrogenase Type 1 Inhibitor ABT-384 in Healthy Volunteers and Elderly Adults

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Clinical Safety, Pharmacokinetics, and Pharmacodynamics of the 11β-Hydroxysteroid Dehydrogenase Type 1 Inhibitor ABT-384 in Healthy Volunteers and Elderly Adults

Wei Liu, David A. Katz, Charles Locke, Dan Daszkowski, Yi Wang, Matthew J. Rieser, Walid Awni, Gerard J. Marek, and Sandeep Dutta

Abstract

ABT-384 is a potent and selective inhibitor of 11β-hydroxysteroid dehydrogenase type 1 (HSD-1), the enzyme that regenerates cortisol in several tissues. Two clinical studies of ABT-384 were undertaken to assess its safety, pharmacokinetics, target engagement, and pharmacologic effects in healthy subjects. Single doses from 1 to 240 mg, and multiple doses from 1 to 100 mg once daily for 7–14 days, were administered to healthy adults. Multiple doses from 10 to 100 mg once daily for 21 days were administered to elderly subjects. A total of 103 subjects received at least 1 dose of ABT-384. A maximum-tolerated dose was not defined in either study. The pharmacokinetic profiles of ABT-384 and its active metabolite support once daily dosing. Analysis of urine cortisol metabolites demonstrated full hepatic HSD-1 inhibition with regimens from 1 mg daily, and confirmed in vitro target selectivity. Pharmacologic effects included increases of adrenocorticotrophic hormone levels, cortisol production and androgen and estradiol levels. ABT-384 has a wide therapeutic index relative to full hepatic target engagement which is relevant for indications such as diabetes and metabolic syndrome. Its therapeutic index for other potential indications such as Alzheimer's disease remains to be established.

Keywords

hydroxysteroid dehydrogenase, enzyme inhibitor, clinical trial, pharmacodynamics, pharmacokinetics

11β-hydroxysteroid dehydrogenase type 1 (HSD-1) converts inactive cortisone to active cortisol in glucocorticoid (GC)-dependent tissues including liver, fat, brain, bone and eye.[1] 11β-hydroxysteroid dehydrogenase type 2 (HSD-2) serves a protective function in mineralocorticoid-sensitive tissues such as kidney by converting cortisol to cortisone.[2] ABT-384 (Figure 1) is a potent and selective inhibitor of HSD-1 and exhibits high affinity (Ki 0.1–2.7 nM) against rodent, monkey, and human HSD-1. ABT-384 is over 1000-fold selective in man for HSD-1 compared to HSD-2. A major pathway of ABT-384 catabolism identified preclinically is hydrolysis of the adamantane-1-carboxylic acid amide to form a carboxylic acid metabolite. The acid metabolite is approximately 5-fold less potent compared to ABT-384, and is over 100-fold selective in man for HSD-1 compared to HSD-2.

A range of medical conditions including Cushing's syndrome, obesity, insulin resistance, hypertension, dyslipidemia, osteoporosis, glaucoma, major depressive disorder, and Alzheimer's disease (AD) have been associated with excess levels of the stress hormone cortisol. An HSD-1 inhibitor, by blocking local tissue regeneration of cortisol, may have therapeutic potential for any of these diseases.[1] The initial intended therapeutic indication for ABT-384 is AD. There is a strong scientific rationale for the hypothesis that central nervous system (CNS) cortisol reduction, via HSD-1 inhibition, can have both cognitive benefits and disease modification potential in AD.

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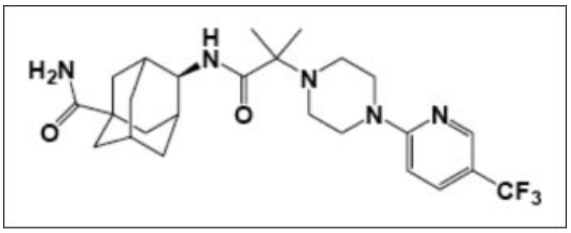

Figure 1. Structure of ABT-384 (1-Piperazineacetamide, *N*-[5-(aminocarbonyl) tricyclo[3.3.1.1^{3,7}]dec-2-yl]-α,α-dimethyl-4-[5-(trifluoromethyl)-2-pyridinyl]-, stereoisomer).

Increased basal plasma GC levels,[3,4] lack of cortisol release suppression by the synthetic GC dexamethasone,[5] and elevated cerebrospinal fluid GC levels[6,7] have been frequently described in AD. Higher mean 24-hour plasma GC levels have been associated with increased cognitive decline in AD.[8] Acute cortisol treatment decreases memory performance even in healthy humans.[9] Aged (12 months or more) HSD-1 knockout mice have enhanced spatial learning and memory compared to congenic controls,[10–12] implying that increased GC contributes to the normal loss of these functions during aging. Selective and potent HSD-1 inhibitors have improved cognitive performance in the mouse inhibitory avoidance model.[13]

In addition, multiple effects of GC in the brain are plausible AD pathologies as demonstrated in animal models. GC elevation leads to aggregation of Aβ oligomers in amyloid plaques (possibly via expression regulation of IDE, an enzyme involved in Aβ clearance[14]) and accumulated tau protein in neurofibrillary tangles,[15] both of which are hallmarks of AD. Excess GCs have multiple pathological effects in the hippocampus, a key region for learning and memory, including neurogenesis suppression,[16] long-term potentiation impairment,[17] altered dendritic morphology,[18] decreased expression of the brain-derived neurotrophic factor (BDNF),[19] and increased levels of excitotoxic amino acids.[20] Some of these pathological effects of elevated GCs have also been observed in the amygdala and prefrontal cortex.[21–24] GC-induced dysfunction of the hippocampus, amygdala and prefrontal cortex can lead to deficits of hypothalamic–pituitary–adrenal (HPA) axis negative feedback regulation, in turn resulting in persistent elevations of GCs.

Interrupting this destructive cycle by reducing levels of active GCs in the brain is expected to have beneficial effect in AD. Selective and potent HSD-1 inhibitor reduced basal tau phosphorylation in the hippocampus of normal mice, and significantly reduced β-amyloid levels in the hippocampus and cortex and accumulation of β-amyloid plaques in a transgenic mouse model of AD (AbbVie, data on file). The anatomical distribution of HSD-1 in the brain appears optimal to modulate the pathophysiology of AD. High HSD-1 expression is found in the hippocampus, prefrontal cortex, and parietal cortex.[25] AD plaques and tangles are generally first observed in these regions, which form a default mode network demarcating high basal metabolic activity in the brain while in a quiet resting state.[26,27] The regional expression of HSD-1 in the default mode network that first displays the pathological sequelae of AD, the critical role played by a key node in this network (medial temporal lobe including the hippocampus) in controlling the HPA axis, and the known deleterious effects of cortisol on cognitive function and apparent progression of AD make HSD-1 inhibition an attractive target for developing innovative AD therapeutics with potential for both symptomatic and disease modifying efficacy.

Several clinical trials of HSD-1 inhibitors have been completed, including positive proof-of concept studies in type 2 diabetes and metabolic syndrome. INCB13739 enhanced hepatic insulin sensitivity, and reduced glycosylated hemoglobin, total cholesterol, LDL, and blood pressure, in subjects with type 2 diabetes.[28] MK-0916 showed no significant effect on fasting plasma glucose or 2-hour post-prandial glucose and increased LDL-C relative to placebo, but led to reduction of glycosylated hemoglobin, blood pressure, and body weight in subjects with type 2 diabetes and at least 2 other National Cholesterol Education Program Adult Treatment Panel III criteria for metabolic syndrome.[29] The non-selective HSD inhibitor carbenoxolone has been reported to enhance hepatic insulin sensitivity, and improve verbal fluency in healthy elderly men and verbal memory in subjects with type 2 diabetes.[30] These clinical trial results demonstrate that HSD-1 inhibitors have therapeutic potential in metabolic diseases for which hepatic enzyme inhibition is a relevant pharmacologic effect.

Two Phase 1 clinical studies were undertaken to assess the safety, pharmacokinetics, target engagement, and pharmacologic effects of ABT-384 in healthy adults and elderly subjects.

Subjects and Methods

For each clinical study, Institutional Review Board (IRB) approval of the protocol, informed consent and subject information and advertising was obtained prior to the authorization of drug shipment to the study site. Any amendments to the protocol were approved by the IRB prior to implementation of any changes to the study design. Prior to the initiation of any screening or study-specific procedures, the investigator or his representative explained the nature of the study to the subject and answered all questions regarding this study. Each informed consent was reviewed, signed, and dated by the subject and the person who administered the informed consent.

Healthy Adults Study Design

Adult male or female subjects between 18 and 55 years old, in general good health (on the basis of medical history, physical examination, brief neurological examination, 12-lead electrocardiogram, vital signs, clinical chemistry tests, hematology tests, urinalysis, hepatitis/HIV tests, drug/alcohol screen, and [in females] pregnancy tests), and not using any medication (including over-the-counter, vitamins, and herbal supplements) were eligible for the study. History of diabetes, cancer (except basal cell carcinoma of the skin), or any clinically significant cardiovascular, respiratory (except mild asthma), renal, hepatic, gastrointestinal, hematologic, endocrinologic, neurologic, or psychiatric disease or disorder was exclusionary. History of gastric surgery, cholecystectomy, vagotomy, bowel resection, or any surgical procedure that might interfere with gastrointestinal motility, pH, or absorption was exclusionary. Safety and tolerability of the ABT-384 doses were assessed throughout the study by adverse event collection, physical examination, brief neurological examination, 12-lead electrocardiogram, vital signs including orthostatic blood pressure and pulse and clinical chemistry tests, hematology tests, urinalysis, drug/alcohol screen, and (in females) pregnancy tests. Adverse events were recorded until 30 days after the last dose of study drug. A study schematic is shown in Figure 2.

Part I of the study was an evaluation of escalating single doses of ABT-384 in a randomized, escalating-dose, double-blind, placebo-controlled study design. Each group consisted of 8 subjects who were randomized in a 3:1 ratio to receive ABT-384 or placebo. Subjects were confined to the study site and supervised for approximately 6 days. Confinement began on Day − 1 (1 day prior to the dosing day) and ended after completion of scheduled study procedures on Day 5. Subjects received a single dose of ABT-384 or placebo per os under fasting conditions on Day 1. ABT-384 single doses for sequential groups were 1, 8, 20, 50, 120, and 240 mg. Dosing of a higher dose did not proceed until the safety, tolerability and available pharmacokinetic data from prior groups had been analyzed. Dose escalation was limited by exposure limits based on the no adverse effect levels in preclinical toxicity studies of ABT-384. Serial blood samples for pharmacokinetic analysis were collected within 10 minutes prior to dosing (0 hour)

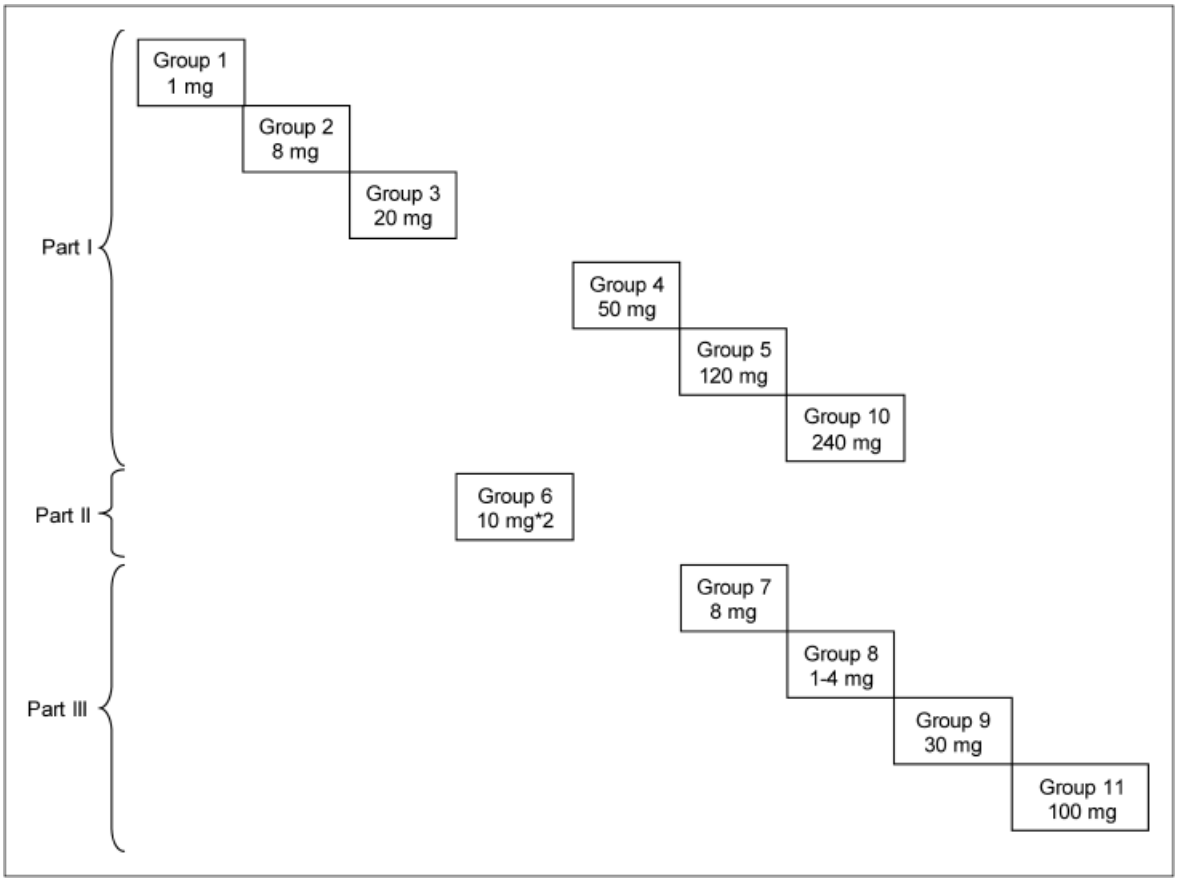

Figure 2. Healthy volunteers study schematic. Group numbers and doses are shown. Part I was a single ascending dose tolerability study. Part II was a food effect study. Part III was a multiple ascending dose tolerability study.

and at 0.5, 1, 1.5, 2, 3, 4, 6, 9,12, 16, 24, 36, 48, 72, and 96 hours after dosing.

Part II of the study was an open-label, two-period, fasting and non-fasting, randomized, crossover study designed to assess the effect of food on the pharmacokinetics of a single dose of ABT-384. Each of 12 subjects received a single dose of 10 mg ABT-384 under fasting and non-fasting conditions (high-fat breakfast) in randomized order. The washout interval between administrations of the 2 doses of ABT-384 was 14 days. Subjects in Part II were confined to the study site and supervised for approximately 6 days in each study period. Confinement began on Day − 1 (1 day prior to the dosing day) and ended after completion of scheduled study procedures on Day 5. In each period, serial blood samples for pharmacokinetic analysis were collected within 10 minutes prior to dosing (0 hour) and at 0.5, 1, 1.5, 2, 3, 4, 6, 9,12, 16, 24, 36, 48, 72, and 96 hours after dosing.

Part III of the study was a randomized, escalating-dose, double-blind, placebo-controlled, serial-group study designed to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of escalating multiple oral doses of ABT-384. Each group was to consist of 12 subjects who were randomized in a 3:1 ratio to receive ABT-384 or placebo. However, only 10 subjects were recruited for the fourth dose group, of whom 7 received ABT-384 and 3 received placebo.

For the first 3 groups of Part III, subjects were confined to the study site and supervised for approximately 13 days. Confinement began on Day − 2 (2 days prior to the dosing day) and ended after completion of scheduled study procedures on Day 11. Subjects received a once daily dose of ABT-384 or placebo per os 30 minutes after breakfast on Days 1 through 7. Subjects in 1 group were randomized in equal numbers to receive 3 different doses of ABT-384 to characterize the pharmacokinetics of ABT-384 at the lower dose range. ABT-384 doses were 1 (N = 3), 2 (N = 3), 4 (N = 3), 8 (N = 9), and 30 (N = 9) mg. Serial blood samples for pharmacokinetic analysis were collected as follows.

- Prior to dosing (0 hour) and at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 16, and 24 hours after dosing on Study Day 1.
- Prior to dosing (0 hour) on Study Days 5 and 6.
- Prior to dosing (0 hour) and at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 16, 24, 36, 48, 72, and 96 hours after dosing on Study Day 7.

In the fourth group of Part III, subjects were confined to the study site and supervised for approximately 20 days. Confinement began on Day − 2 (2 days prior to the dosing day) and ended after completion of scheduled study procedures on Day 18. Subjects received a once daily dose of 100 mg ABT-384 or placebo per os on Days 1 through 14. In addition to the above-described samples for Days 1, 5, and 6, serial blood samples for pharmacokinetic analysis were collected as follows.

- Prior to dosing (0 hour) and at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 16, and 24 hours after dosing on Study Day 7.
- Prior to dosing (0 hour) on Study Days 12 and 13.
- Prior to dosing (0 hour) and at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 16, 24, 36, 48, 72, and 96 hours after dosing on Study Day 14.

Urine samples for pharmacokinetic analysis were collected for 24 hours on Day 7, and also on Day 14 for the fourth group. Serial blood samples for measurement of adrenocorticotrophic hormone (ACTH) and cortisol were collected on Days − 1 (prior to dosing), 1 and 7. Urine samples for measurement of cortisol and its metabolites were collected for 24 hours on Day − 1 (prior to dosing) and 7. Dosing of a higher dose did not proceed until the safety, tolerability, and available pharmacokinetic data from prior groups had been analyzed. Dose escalation was limited by exposure limits based on the no adverse effect levels in preclinical toxicity studies of ABT-384 and the exposures achieved in Part I of this study.

Elderly Subjects Study Design

Adult male or female subjects age 65 or greater, in general good health (on the basis of medical history, physical examination, brief neurological examination, 12-lead electrocardiogram, vital signs and clinical chemistry tests, hematology tests, urinalysis, hepatitis/HIV tests, drug/alcohol screen and [in females] pregnancy tests), and not using any protocol-excluded medication were eligible for the study. History of dementia or any other clinically significant neurological or cardiovascular disease, any malignancy (except indolent tumors of the skin), or any clinically significant uncontrolled medical or psychiatric illness were exclusionary. History of gastric surgery, cholecystectomy, vagotomy, bowel resection, or any surgical procedure that might interfere with gastrointestinal motility, pH, or absorption was exclusionary. Clinically significant low creatinine clearance was exclusionary. Safety and tolerability of the ABT-384 doses were assessed throughout the study by adverse event collection, physical examination, brief neurological examination, 12-lead electrocardiogram, vital signs including orthostatic blood pressure and pulse and clinical chemistry tests, hematology tests, urinalysis, drug/alcohol screen, and (in females) pregnancy tests. Adverse events were recorded until 30 days after the last dose of study drug.

This was a randomized, escalating-dose, double-blind, placebo-controlled, serial-group study designed to assess the safety, tolerability, pharmacokinetics, and

pharmacodynamics of escalating multiple oral doses of ABT-384. The first group consisted of 12 subjects who were randomized in a 3:1 ratio to receive ABT-384 or placebo. The second and third groups each consisted of 8 subjects who were randomized in a 3:1 ratio to receive ABT-384 or placebo. Subjects were confined to the study site and supervised for approximately 25 days. Confinement began on Day − 2 (2 days prior to the dosing day) and ended after completion of scheduled study procedures on Day 23. Subjects returned to the study site on Days 25 and 27 for blood sample collection and adverse event assessment. Subjects received a once daily dose of ABT-384 or placebo per os 30 minutes after breakfast on Days 1 through 21. ABT-384 doses for sequential groups were 10, 50, and 100 mg. Serial blood samples for pharmacokinetic analysis were collected as follows.

- Prior to dosing (0 hour) and at 0.5, 1, 2, 3, 4, 6, 9, 14, and 24 hours after dosing on Day 1.
- Prior to dosing (0 hour) and 4 hours after dosing on Day 14.
- Prior to dosing (0 hour) on Days 19 and 20.
- Prior to dosing (0 hour) and at 0.5, 1, 2, 3, 4, 6, 9,14, 24, 36, 48, 96, and 144 hours after dosing on Day 21.

Urine samples for pharmacokinetic analysis were collected for 24 hours on Day 21. Serial blood samples for measurement of ACTH, cortisol, cortisol binding globulin, testosterone, estradiol, dehydroepiandrosterone, dehydroepiandrosterone sulfate, and androstendione were collected on Days − 1 (prior to dosing), 7, and 14. Urine samples for measurement of cortisol and its metabolites were collected for 24 hours on Days − 1 (prior to dosing), 7, 14, and 21. Dosing of a higher dose did not proceed until the safety, tolerability, and available pharmacokinetic data from prior groups had been analyzed. Dose escalation was limited by exposure limits based on the no adverse effect levels in preclinical toxicity studies of ABT-384 and the exposures achieved in the healthy adults study.

The clinicaltrials.gov identifier is NCT00968422.

Subject Demographics and Disposition

Subject demographics are presented in Table 1. One hundred one subjects completed the healthy adults study. One subject was lost to follow-up after the confinement period, 3 subjects withdrew consent for reasons not related to safety, and 1 subject (who received placebo) withdrew consent because he experienced an adverse event. All 28 subjects completed the elderly subjects study.

Table 1. Subject Demographics

Parameter	Mean (SD)	Min–Max
Healthy Adults Part I—Single Ascending Dose (N = 48)		
Age (years)	33.4 (10.5)	19–52
Weight (kg)	72.1 (11.1)	49–91
Height (cm)	169.8 (9.0)	150–189
Sex	34 Males (71%), 14 Females (29%)	
Race	43 White (90%), 4 Black (8%), 1 Asian (2%)	
Healthy Adults Part II—Food Effect (N = 12)		
Age (years)	30.0 (9.5)	19–50
Weight (kg)	76.2 (12.6)	60–96
Height (cm)	174.3 (11.8)	151–191
Sex	9 Males (75%), 3 Females (25%)	
Race	12 White (100%)	
Healthy Adults Part III—Multiple Ascending Dose (N = 46)		
Age (years)	33.3 (11.0)	19–55
Weight (kg)	76.9 (11.0)	57–101
Height (cm)	173.5 (10.3)	145–206
Sex	38 Males (83%), 8 Females (17%)	
Race	35 White (76%), 9 Black (20%), 1 Native American (2%), 1 Mixed (2%)	
Elderly Subjects—Multiple Ascending Dose (N = 28)		
Age (years)	69.6 (3.5)	65–76
Weight (kg)	78.9 (12.1)	51–105
Height (cm)	169.1 (8.7)	151–185
Sex	13 Males (46%), 15 Females (54%)	
Race	27 White (96%), 1 Mixed (4%)	

Analytical Methods

The plasma and urine analytical methods for ABT-384 and its acid metabolite utilized on line solid phase extraction (SPE) with HPLC separation and tandem mass spectrometric (MS/MS) detection. Stable label D_6 internal standards (IS) for both ABT-384 and the metabolite were used. Both IS were labeled via perdeuterated substitutions on the α,α-dimethyl moiety. Sample preparation involved supplementation of an aliquot of the plasma or urine samples with the IS solution in acetonitrile. Sample matrix cleanup was done with a Thermo Fisher Scientific TurboFlow HTLC Cyclone P, 1.0 mm × 50 mm on line SPE column (San Jose, CA). The HPLC separation was performed on a Waters Xbridge C18, 3.5 mm, 2.1 mm × 100 mm analytical column (Milford, MA), with a mobile phase of 70:30 (v:v) acetonitrile:water with 0.5% acetic acid and 2 mM ammonium acetate. The isocratic flow rate was 0.2 mL/min. Detection of the analytes was achieved with the Thermo Fisher Scientific TSQ Quantum Ultra mass spectrometer (San Jose, CA). Ionization was by positive ion electrospray with a spray voltage of 4500 and source collisionally induced dissociation of 12 eV. The MS/MS analysis was operated in the selected reaction monitoring (SRM) mode with a peak width of 0.2 amu in the first quadrupole and 0.7 amu in the third quadrupole. The collision energy for fragmentation was set at 33 eV. The nominal SRM transitions were m/z 494 → 272, 500 → 278, 495 → 272, and 501 → 278 for ABT-384, ABT-384 IS, acid metabolite, and acid metabolite IS, respectively. Reference standards for ABT-384, the acid metabolite, and both IS were synthesized by AbbVie.

The plasma method had intra-run accuracy between −4.0% and 3.7% bias with precision ≤11.1% CV for both analytes. The inter-run accuracy was between −1.2% and 2.4% bias and precision was ≤9.1% CV. The lower limit of quantitation in plasma was approximately 0.2 ng/mL for ABT-384 and the metabolite. The upper limit of quantitation in plasma was approximately 200 ng/mL for both analytes. Stability of ABT-384 and the metabolite in the plasma matrix was demonstrated with no appreciable degradation after 9 hours room temperature exposure, 5 freeze–thaw cycles, and 598 days frozen storage at approximately −20°C. The urine method had intra-run accuracy between −7.8% and 13.2% bias with precision ≤13.2% CV for both analytes. The inter-run accuracy was between −1.5% and 6.9% bias and precision was ≤10.0% CV. The lower limit of quantitation in urine was approximately 1.3 ng/mL for ABT-384 and the metabolite. The upper limit of quantitation in urine was approximately 1150 ng/mL for both analytes. The analytes were stable in urine with no appreciable degradation after 8 hours room temperature exposure, 4 freeze–thaw cycles, and 329 days frozen storage at approximately −20°C.

Urine assays for cortisol, cortisone, 5-α-tetrahydrocortisol, 5-β-tetrahydrocortisol and tetrahydrocortisone, and serum assay for cortisol, were developed and performed at PPD (Richmond, VA).

The urine assay for cortisol and cortisone involved isolating analytes through liquid–liquid extraction. Final extracts were analyzed via HPLC separation and tandem mass spectrometric (MS/MS) detection. To counteract loss to container surfaces, isopropanol was added to human urine samples at a 1:10 volume ratio. Calibration standards and quality controls were prepared in Dulbecco's phosphate-buffered saline containing isopropanol (1:10 isopropanol/PBS, v/v). Study samples were treated with isopropanol in a similar manner at the collection site. Stable label IS were used for both analytes. Sample preparation involved fortifying an aliquot of the human urine samples with the IS solution in methanol. Sample matrix cleanup was done via LLE; the organic layer was separated and then evaporated under a nitrogen stream at 45°C, and the remaining residue was reconstituted with 400 μL of 50:50 methanol/water, v/v. The HPLC separation was performed on an Acquity BEH C8, 2.1 mm × 50 mm, 1.7 μm (Waters, Milford, MA) using 40:60:0.1 methanol/water/acetic acid, v/v/v (Mobile Phase A) and methanol (Mobile Phase B) and a gradient pump program. Detection of the analytes was achieved with a API 4000 triple quadrupole mass spectrometer (Sciex, Framingham, MA). Ionization was by negative ion electrospray with a spray voltage of −4200 V and source collisionally induced dissociation solenoid setting of 4. The MS/MS analysis was operated in multiple reaction monitoring (MRM) mode with unit resolution in the first quadrupole and unit resolution in the third quadrupole. The collision energy for fragmentation was set at −24 eV. The nominal MRM transitions were m/z 419.3 → 329.2, 426.3 → 336.3, 421.3 → 331.3, and 425.3 → 335.3 for cortisone, cortisone-d_7, cortisol, and cortisol-d4. Reference standards were obtained from various sources as follows: cortisone from Sigma (St. Louis, MO), cortisol from USP, cortisone-d_7 from IsoSciences (King of Prussia, PA), and cortisol-d_4 from C/D/N Isotopes (Pointe-Claire, QU). The human urine method had intra-run accuracy between 0.692% and 3.56% bias with precision ≤−1.09% CV. The inter-run accuracy was between 1.48% and 7.17% bias and precision was ≤1.32% CV. The lower limit of quantitation in human urine was approximately 1.00 ng/mL for each analyte.

The urine assay for 5α-tetrahydrocortisol, 5β-tetrahydrocortisol and tetrahydrocortisone involved subjecting analytes to enzyme hydrolysis and then isolating them through liquid–liquid extraction. Final extracts were analyzed via HPLC separation and tandem mass spectrometric (MS/MS) detection. To counteract loss to container surfaces, isopropanol was added to urine samples at a 1:10 volume ratio. A stable

label cortisol-d_4 IS was used for all 3 analytes. Sample preparation involved fortifying an aliquot of the human urine samples with the IS solution in methanol. Sample matrix cleanup was done via LLE; the organic layer was separated and then evaporated under a nitrogen stream at 45°C, and the remaining residue was reconstituted with 5.00 mL of 50:50 methanol/water, v/v. The HPLC separation was performed on an Acquity BEH C8, 2.1 mm × 50 mm, 1.7 μm analytical column (Waters) using 40:60:0.1 methanol/water/acetic acid, v/v/v (Mobile Phase A) and methanol (Mobile Phase B) and a gradient pump program. Detection of the analytes was achieved with a API 4000 triple quadrupole mass spectrometer (Sciex, Framingham, MA). Ionization was by negative ion electrospray with a spray voltage of −4200 V and source collisionally induced dissociation solenoid setting of 4. The MS/MS analysis was operated in MRM mode with unit resolution in the first quadrupole and unit resolution in the third quadrupole. The collision energy for fragmentation was set at −24 eV. The nominal MRM transitions were m/z 423 → 333 for tetrahydrocortisone and 425 → 335 for 5α-tetrahyrdocortisol, 5β-tetrahydrocortisol, and IS. Reference standards were obtained from Research Plus, Inc. (Barrington, IL) and IS was from CDN Isotopes. The method had intra-run accuracy between −0.959% and 7.17% bias with precision ≤6.81% CV. The inter-run accuracy was between −6.57% and 2.23% bias and precision was ≤10.1% CV. The lower limit of quantitation in human urine was approximately 20.0 ng/mL for each analyte.

The serum assay for cortisol involved isolating cortisol through protein precipitation. Final extracts were analyzed via HPLC separation and tandem mass spectrometric (MS/MS) detection. Calibration standards and quality controls were prepared in Dulbecco's phosphate-buffered saline with 5% albumin. Stable label IS were used for both analytes. Sample preparation involved fortifying an aliquot of the human serum samples with the IS solution in methanol. Samples were prepared through protein precipitation with 150 μL of acetonitrile; a 175-μL portion of the supernatant was removed and diluted with 200 μL of water. The HPLC separation was performed on a VanGuard BEH C18, 2.1 mm × 5 mm, 1.7-μm loading column (Waters) and a BEH C18, 2.1 mm × 50 mm, 1.7-μm analytical column (Waters) using 40:60:0.1 methanol/water/acetic acid, v/v/v (Mobile Phase A) and methanol (Mobile Phase B) and a gradient pump program. Detection of the analytes was achieved with a API 5000 triple quadrupole mass spectrometer (Sciex, Framingham MA). Ionization was by negative ion electrospray with a spray voltage of −4200 V and source collisionally induced dissociation solenoid setting of 4. The MS/MS analysis was operated in MRM mode with unit resolution in the first quadrupole and unit resolution in the third quadrupole. The collision energy for fragmentation was set at −24 eV. The nominal MRM transitions were m/z 421 → 331 and 425 → 335 for cortisol and cortisol-d4. Reference standards were obtained from various sources as follows: cortisol from USP, and cortisol-d_4 from CDN Isotopes. The human serum method had intra-run accuracy between 0.428% and 10.2% bias with precision ≤6.32% CV for all analytes. The inter-run accuracy was between 0.783% and 5.94% bias and precision was ≤3.93% CV. The lower limit of quantitation in human serum was approximately 1.00 ng/mL for cortisol.

Plasma ACTH was measured by PPD Global Central Labs (Highland Heights KY) using an Immulite-2000 solid phase 2-site sequential immunoassay according to instructions from the manufacturer. Serum CBG was measured by Quest Diagnostics Clinical Trials (Valencia CA) using a Genesys 6000 competitive radioimmunoassay according to instructions from the manufacturer. Dehydroepiandrosterone sulfate (DHEA-S) was measured by Mayo Medical Laboratories (Rochester, MN) using an Immulite-2000 immunoassay according to instructions from the manufacturer.

Androstendione, DHEA, estradiol, and testosterone were measured by Mayo Medical Laboratories using published methods.[31–35]

Statistical Methods

Pharmacokinetic parameters were calculated based on noncompartmental methods using WinNonlin-Professional™, Version 5.2 (Pharsight Corporation, Mountain View, CA) and summarized by descriptive statistics. Plasma concentration data from all subjects who received ABT-384 were included in the pharmacokinetic analyses. Plasma concentrations below lower limit of quantitation were assigned as zero during the analyses. Dose proportionality and pharmacokinetic linearity was investigated using one-way analysis of covariance (ANCOVA) for dose-normalized C_{max}, dose-normalized AUC, T_{max}, and β of ABT-384 and of the acid metabolite. Repeated measures analysis was performed to address the attainment of steady state. The model had effects for dose level, day, and the interaction of dose level and day as factors. To assess food effect on pharmacokinetics, an analysis of variance (ANOVA) was performed for T_{max}, β, and the natural logarithms of C_{max}, AUC_t, and AUC_∞ of ABT-384 and of the acid metabolite. The model included effects for sequence, subject nested within sequence, period, and regimen. From the analyses of the natural logarithms of C_{max} and AUC, 90% confidence intervals were provided for the bioavailability under non-fasting conditions relative to that under fasting conditions. For target engagement and selectivity, and pharmacologic effect parameters, linear mixed effect models were used. Adverse events were coded by Medical Dictionary for

Regulatory Affairs. The number and percentage of subjects reporting treatment emergent adverse events were tabulated by preferred term and system organ class with a breakdown by regimen. Laboratory test values, vital signs measurements and ECG parameters that were potentially clinically significant, according to predefined criteria, were identified. One-way ANCOVAs were performed on the clinical laboratory variable data. Linear mixed effect models were performed on vital signs and ECG parameter data. No subjects or data were excluded from safety analyses.

Results

Safety

Healthy Adults. No deaths, other serious adverse events, or discontinuations due to adverse events occurred in ABT-384 subjects. In Parts I and II, 8 (8/48, 17%) subjects who received single doses of ABT-384 and 2 (2/12, 17%) subjects who received single doses of placebo reported at least 1 treatment-emergent adverse event (Table 2). In Part III, 8 (8/34, 24%) subjects who received multiple doses of ABT-384 and 3 (3/12, 25%) subjects who received multiple doses of placebo reported at least 1 treatment-emergent adverse event (Table 2). The adverse events reported by more than 2 subjects who received ABT-384 (single or multiple doses) were headache (N = 4) and dizziness (N = 3). All adverse events were mild in severity, except for 1 event of dizziness considered moderate in severity. The only adverse event considered possibly or probably related to ABT-384 by the investigator was a macular rash. ABT-384 at a single dose of 240 mg was associated with a mean increase in diastolic blood pressure of 7.4 mmHg compared with placebo at 2 hours after dosing (near fasting T_{max}). ABT-384 at daily doses of 100 mg was associated with a mean increase in the QTcF of 5.1 mseconds compared with placebo at 4 hours after dosing (near non-fasting T_{max}) when data from multiple days were analyzed jointly. ABT-384 at daily doses of 100 mg was also associated with a mean increase in the QRS interval of 1.5 msecond compared with placebo when data from several times of measurement on Day 7 were analyzed jointly. There were no other potentially clinically meaningful individual or mean changes of clinical laboratory tests, vital signs or ECG parameters including QTcF. A maximum-tolerated dose was not defined.

Elderly Subjects. No deaths, other serious adverse events or discontinuations due to adverse events occurred. Twelve (12/21, 57%) subjects who received ABT-384 and 4 (4/7, 57%) subjects who received placebo reported at least 1 treatment-emergent adverse event. All adverse events were mild or moderate in severity. The adverse events reported by 2 or more subjects who received ABT-384 were diarrhoea (N = 4), headache (N = 4), dyspepsia (N = 2), and somnolence (N = 2). The majority of adverse event were considered possibly or probably related to ABT-384 by the investigator. Mean values of hepatic enzymes (alanine aminotransferase, aspartate aminotransferase, gamma-glutaminyl transferase, alkaline phosphatase) showed monotonic increases with both ABT-384 dose and treatment duration (Table 3). These changes were considered to be potential indications of hepatocellular injury associated with increasing ABT-384 exposure. Subsequent review of the healthy volunteer study results revealed similar but smaller hepatic enzyme increases (data not shown). Decreases in lymphocytes were considered potentially clinically meaningful (Table 3). None of the individual changes on hepatic enzymes or lymphocytes were considered to be clinically meaningful. ABT-384 was associated with heart rate increases of 3.6 bpm (10 mg QD), 4.3 bpm (50 mg QD), and 5.0 bpm (100 mg QD) as well as similar increases of pulse rate. There were no other potentially clinically meaningful individual or mean changes of clinical laboratory tests, vital signs, or ECG parameters. A maximum-tolerated dose was not defined.

Pharmacokinetics

Healthy Adults—Single Dose Administration. Following single-dose administration of ABT-384, only 2 subjects had ABT-384 plasma concentration levels higher than lower limit of quantitation following the 1 mg dose. C_{max} increased linearly over the 8–120 mg dose range, but was less than dose proportional at the 240 mg dose ($P = .032$). AUC was dose proportional over the 8–240 mg range. For the acid metabolite, no subject had detectable levels following the 1 mg dose. The acid metabolite C_{max} increased dose proportionally over the 8–240 mg dose range while AUC increased more than proportionally with ABT-384 dose ($P \leq .014$). The mean elimination half-lives ranged from 10 to 15 hours for ABT-384 and 31 to 44 hours for the acid metabolite (Table 4).

Healthy Adults—Food Effect. Food had limited impact on the bioavailability (C_{max} and AUC) of ABT-384 and its acid metabolite (Table 5). For both analytes, C_{max} under non-fasting conditions was equivalent to that under fasting conditions. Administration of ABT-384 under non-fasting conditions resulted in a 20% increase in ABT-384 AUC central value and 12% increase in acid metabolite AUC central value relative to the fasting condition.

Healthy Adults—Multiple-Dose Administration. The mean + SD plasma concentration-time profiles of ABT-384 are presented in Figure 3. The mean (SD) pharmacokinetic parameters of ABT-384 and the acid metabolite on Day 1 and last day of dosing are presented in Table 6.

Upon multiple dosing of 1–100 mg ABT-384 to healthy volunteers, dose-normalized ABT-384 exposure

Table 2. Adverse Events Reported During Healthy Adults and Elderly Subjects Studies

MedDRA Preferred Term	Placebo (N = 12)	ABT-384 (N = 36)
Healthy adults, Part I (single ascending dose)		
Nausea	0	1
Headache	0	1
Dermatitis	0	1
Musculoskeletal pain	1	0
Dizziness	1	0

MedDRA Preferred Term	ABT-384 (N = 12)
Healthy adults, Part II (food effect)	
Vessel puncture site pain	1
Viral infection	1
Musculoskeletal chest pain	1
Dizziness	1
Headache	1
Oropharyngeal pain	1
Dermatitis contact	1

MedDRA Preferred Term	Placebo (N = 12)	ABT-384 (N = 34)
Healthy adults, Part III (multiple ascending dose)		
Dizziness	1	2
Headache	1	2
Ear pain	0	1
Abdominal pain	0	1
Diarrhoea	0	1
Nausea	0	1
Toothache	0	1
Feeling cold	0	1
Feeling hot	0	1
Viral infection	1	1
Laceration	0	1
Elevated mood	0	1
Insomnia	0	1
Cough	0	1
Nasal congestion	0	1
Oropharyngeal pain	0	1
Rash macular	0	1

MedDRA Preferred Term	Placebo (N = 7)	ABT-384 (N = 21)
Elderly subjects		
Diarrhoea	1	4
Headache	1	4
Dyspepsia	0	2
Somnolence	1	2
Lethargy	2	1

on Day 1 following 1, 2, and 4 mg dose were statistically significantly lower than those at 8 mg or higher doses, consistent with the observations in single dose. However, at steady state, the C_{max} and AUC of ABT-384 increased less than proportionally with ABT-384 dose ($P \leq .015$). Steady state was achieved for ABT-384 by Day 6 of QD dosing. The accumulation ratios of ABT-384 at steady state were 1.4–2.3 across the 8–100 mg dose range. The mean elimination half-lives of ABT-384 ranged from 12 to 16 hours. The mean percentages of the ABT-384 doses

Table 3. Mean Changes and Potentially Clinically Significant (PCS) Individual Values of Clinical Laboratory Test Values in Elderly Subjects in General Good Health

Laboratory Variable [PCS Criterion]	Regimen	Last Day LSM	Difference (SE) vs. Placebo	PCS Values
SGPT/ALT (U/L) [>3 × ULN]	Placebo QD	15.36		None
	10 mg ABT-384 QD	17.77	2.41 (8.94)	None
	50 mg ABT-384 QD	27.49	12.13 (9.72)	None
	100 mg ABT-384 QD	45.43	30.07 (10.09)**	None
SGOT/AST (U/L) [>3 × ULN]	Placebo QD	18.86		None
	10 mg ABT-384 QD	19.67	0.81 (4.32)	None
	50 mg ABT-384 QD	23.66	4.80 (4.83)	None
	100 mg ABT-384 QD	35.17	16.32 (4.83)**	None
GGT (U/L)	Placebo QD	18.07		[a]
	10 mg ABT-384 QD	18.39	0.32 (8.71)	[a]
	50 mg ABT-384 QD	23.74	5.67 (9.63)	[a]
	100 mg ABT-384 QD	46.94	28.86 (10.05)**	[a]
Alkaline Phosphatase (U/L) [>3 × ULN]	Placebo QD	64.33		None
	10 mg ABT-384 QD	64.66	0.33 (7.14)	None
	50 mg ABT-384 QD	69.98	5.65 (7.72)	None
	100 mg ABT-384 QD	84.82	20.49 (7.72)*	None
Lymphocytes ($\times 10^9$/L)	Placebo QD	1.77		[a]
	10 mg ABT-384 QD	1.80	0.02 (0.13)	[a]
	50 mg ABT-384 QD	1.57	−0.21 (0.14)	[a]
	100 mg ABT-384 QD	1.29	−0.48 (0.14)**	[a]

LSM, least squares mean.
[a]There was no predefined PCS criterion.
*$P < 0.05$.
**$P < 0.01$.

excreted as unchanged drug in urine were negligible (≤0.04%). Exposure (C_{max} and AUC) of the acid metabolite increased more than proportionally with ABT-384 dose ($P \leq .011$). Steady state was achieved for the acid metabolite by Day 12 of ABT-384 QD dosing. The accumulation ratios of the acid metabolite ranged from 4.9 to 7.6 across the 8–100 mg dose range. The mean half-lives of the acid metabolite ranged from 31 to

Table 4. Mean (SD) Pharmacokinetic Parameters of ABT 384 and Its Acid Metabolite in Healthy Volunteers Following Single Dose Administration

Pharmacokinetic Parameters (Units)	Group 1, 1 mg ABT-384 (N = 6)[a]	Group 2, 8 mg ABT-384 (N = 6)	Group 3, 20 mg ABT-384 (N = 6)	Group 4, 50 mg ABT-384 (N = 6)	Group 5, 120 mg ABT-384 (N = 6)	Group 10, 240 mg ABT-384 (N = 6)
ABT-384						
C_{max} (ng/mL)	0.293, 0.245	38.2 (13.3)	109 (20.4)	309 (114)	570 (150)	842 (353)
T_{max} (h)	0.5, 1.0	2.2 (0.7)	2.3 (1.0)	1.9 (0.6)	1.8 (0.7)	3.3 (1.6)
$t_{1/2}$ (h)	—	10.4 (1.59)	15.1 (3.19)	15.2 (3.11)	14.8 (4.67)	13.3 (0.70)
AUC_∞ (ng h/mL)	—	409 (138)	1050 (296)	2700 (903)	5430 (1880)	12300 (4990)
CL/F (L/h)	—	22.0 (9.54)	20.5 (6.50)	19.9 (5.21)	24.4 (8.48)	22.7 (10.4)
Acid Metabolite of ABT-384						
C_{max} (ng/mL)	—	0.673 (0.216)	2.18 (0.373)	7.05 (2.69)	14.7 (6.80)	35.6 (20.0)
T_{max} (h)	—	14.5 (6.3)	9.5 (1.2)	20.2 (6.3)	20.2 (6.3)	20.2 (15.9)
$t_{1/2}$ (h)	—	39.7 (13.9)	32.5 (7.09)	32.2 (3.70)	45.5 (21.2)	53.2 (27.3)
AUC_∞ (ng h/mL)	—	45.8 (14.1)	141 (32.4)	484 (205)	1230 (557)	4280 (3910)

SD, standard deviation; C_{max}, maximum post-dose plasma concentration; T_{max}, time to C_{max}; $t_{1/2}$, terminal elimination half-life; AUC_∞, area under the plasma time-concentration curve from time 0 to infinite time; CL/F, oral clearance.
[a]Only 2 subjects had plasma concentration levels > LLOQ; individual values are presented.

Table 5. Food Effect on ABT-384 and Its Acid Metabolite Exposure

	Central Value[a]		Relative Bioavailability	
Pharmacokinetic Parameter	Non-Fasting (N = 12)	Fasting (N = 12)	Point Estimate[b]	90% Confidence Interval
ABT-384				
C_{max}	54.12	58.22	0.930	0.841–1.028
AUC_t	626.51	522.50	1.199	1.080–1.331
AUC_∞	636.26	530.65	1.199	1.082–1.329
Acid Metabolite of ABT-384				
C_{max}	1.24	1.14	1.086	0.976–1.208
AUC_t	64.04	57.10	1.122	0.930–1.353
AUC_∞	80.43	72.52	1.109	0.981–1.254

[a]Antilogarithm of the least squares means for logarithms.
[b]Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

83 hours. The acid metabolite was not detectable in urine. The metabolite/parent ratio appeared to increase with dose.

Elderly Subjects—Multiple Dose Administration. The mean (SD) pharmacokinetic parameters of ABT-384 and the acid metabolite on Day 1 and Day 21 are presented in Table 7.

Following multiple dosing of 10–100 mg ABT-384 in elderly subjects, the ABT-384 C_{max} increased less than proportionally with dose ($P \leq .013$); AUC increased dose proportionally. Steady state was achieved for ABT-384 by Day 14 of QD dosing. The median AUC_{0-24} accumulation ratios of ABT-384 at steady state were 1.4–1.7. The mean elimination half-lives of ABT-384 ranged from 14 to 16 hours. Exposure (C_{max} and AUC) of the acid metabolite increased more than proportionally with ABT-384 dose ($P \leq .009$). The median AUC_{0-24} accumulation ratios of acid metabolite ranged from 6.3 to 7.8. The mean elimination half-lives of acid metabolite ranged from 35 to 45 hours. Steady state was achieved for

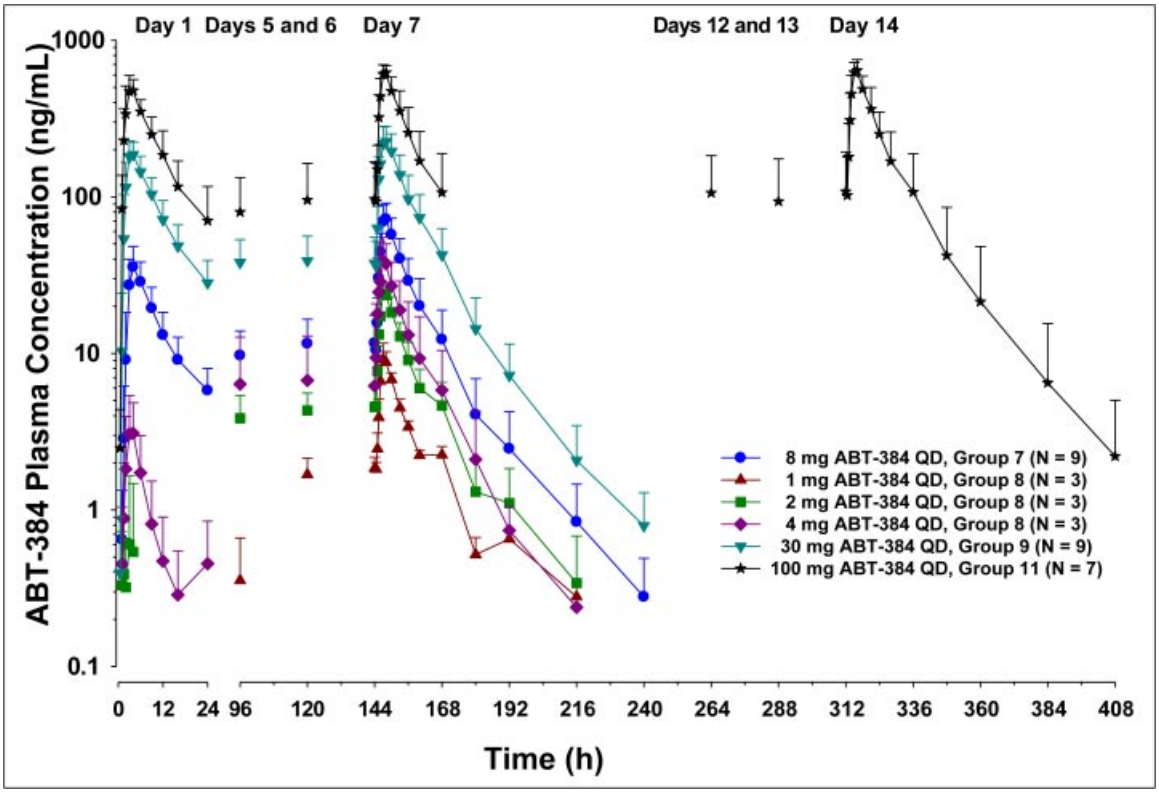

Figure 3. Mean ± SD plasma concentration-time profiles for ABT-384 are shown on a log-linear scale.

Table 6. Mean (SD)[a] Pharmacokinetic Parameters of ABT-384 and Its Acid Metabolite in Healthy Volunteers Following Once-Daily Administration

Parameter (Units)	1 mg QD	2 mg QD	4 mg QD	8 mg QD	30 mg QD	100 mg QD
	Day 1					
N	3	3	3	9	9	7
ABT-384						
C_{max} (ng/mL)	ND	1.81, 0.285	3.54 (2.25)	35.9 (12.5)	198 (44.1)	518 (84.6)
T_{max} (h)	ND	3.0, 2.0	3.7 (0.6)	4.1 (0.8)	3.3 (0.5)	3.6 (1.3)
AUC_{0-24} (ng h/mL)	ND	6.93, 0.45	21.7 (15.3)	343 (124)	1900 (463)	4850 (1230)
Acid metabolite of ABT-384						
C_{max} (ng/mL)	ND	ND	ND	0.679 (0.223)	4.92 (1.75)	16.6 (6.93)
T_{max} (h)	ND	ND	ND	18.7 (5.3)	19.0 (7.5)	15.1 (6.5)
AUC_{0-24} (ng h/mL)	ND	ND	ND	12.2 (3.91)	86.4 (26.9)	307 (110)

Parameter (Units)	1 mg QD	2 mg QD	4 mg QD	8 mg QD	30 mg QD	100 mg QD	
	Day 7						Day 14
ABT-384							
C_{max} (ng/mL)	9.41 (2.01)	25.2 (3.4)	40.1 (15.6)	72.6 (19.1)	234 (61)	647 (68)	655 (91)
T_{max} (h)	3.3 (0.6)	3.3 (0.6)	3.3 (0.6)	3.6 (0.5)	4.1 (1.2)	3.6 (0.5)	3.7 (0.5)
C_{min} (ng/mL)	1.74 (0.28)	4.39 (1.72)	5.55 (4.81)	10.5 (5.5)	35.7 (16.0)	92.1 (69.9)	98.1 (78.2)
$t_{1/2}$ (h)	14.3 (1.1)	14.5 (6.3)	16.9 (7.8)	16.3 (3.4)	14.5 (1.2)	NC	14.8 (2.2)
AUC_{0-24} (ng h/mL)	96.6 (12.6)	257 (41)	393 (231)	778 (269)	2650 (840)	6760 (2020)	6890 (2130)
CL/F (L/h)	10.5 (1.4)	7.91 (1.17)	12.8 (7.0)	11.3 (3.4)	12.3 (3.8)	NC	15.5 (3.8)
f_e (%)	0	0	0.03	0.03	0.04	0.03	0.03
Acid metabolite of ABT-384							
C_{max} (ng/mL)	0.306 (0.055)	1.24 (0.25)	2.04 (1.25)	4.30 (1.41)	20.4 (7.6)	75.4 (36.4)	103 (58)
T_{max} (h)	6.3 (2.5)	7.3 (2.9)	8.0 (6.9)	7.9 (4.3)	11.1 (5.7)	6.7 (2.3)	5.6 (2.5)
C_{min} (ng/mL)	0.069 (0.120)	0.977 (0.185)	1.55 (0.96)	3.38 (1.12)	15.5 (6.1)	59.3 (30.5)	77.9 (44.9)
$t_{1/2}$ (h)	82.8 (3.9)	31.6 (5.7)	32.6 (8.9)	39.2 (6.9)	40.7 (6.0)	NC	51.2 (18.4)
AUC_{0-24} (ng h/mL)	6.04 (1.40)	26.8 (4.7)	44.0 (28.8)	95.5 (31.5)	445 (164)	1640 (820)	2140 (1250)

[a]Median and range for R_{ac}; C_{min}, minimum post-dose plasma concentration during dosing interval; AUC_{0-24}, area under the plasma time-concentration curve, 24 hours; f_e, fraction excreted in urine; NC, not calculated; ND, not detected.

acid metabolite by Day 14 of ABT-384 QD dosing. The metabolite/parent exposure ratio appeared to be dose dependent.

Target Engagement and Selectivity

In the liver, cortisol, and cortisone are converted to their tetrahydro metabolites which are rapidly excreted in the urine. Thus, the ratio of cortisol metabolites (5-α-tetrahydrocortisol + 5-β-tetrahydrocortisol) to cortisone metabolite (tetrahydrocortisone) in urine is a proxy for ratio of hepatic levels of HSD-1 product (cortisol) to substrate (cortisone).[1] So that the measurement is independent of interindividual variation of urination patterns or diurnal cortisol rhythms, data are typically reported from a 24-hour urine collection. The mean of this ratio prior to study drug administration, representing uninhibited HSD-1 activity, was 1.02. ABT-384 regimens of 1–100 mg QD for 7 days in healthy volunteers (Figure 4, left panel) and of 10–100 mg QD for 21 days in elderly subjects (data not shown) reduced this HSD-1 ratio by 87–97%.

Small amounts of cortisol and cortisone are excreted in the urine rather than reabsorbed into circulation from the kidney. The ratio of cortisone to cortisol in urine represents renal levels of HSD-2 product (cortisone) to substrate (cortisol).[1] The mean of this ratio prior to study drug administration, representing uninhibited HSD-2 activity, was 2.95. ABT-384 regimens of 1–100 mg QD for 7 days in healthy volunteers (Figure 4, right panel) and of 10–100 mg QD for 21 days in elderly subjects in general good health (data not shown) did not affect this HSD-2 ratio.

Pharmacologic Effects

Following a single dose of ABT-384, increased plasma ACTH levels were observed in healthy volunteers with increasing dose ($P = .015$, Figure 5, upper left panel). No substantial ACTH increase was observed only 2 hours

Table 7. Mean (SD) Pharmacokinetic Parameters of ABT-384 in Elderly Subjects Following Once-Daily Administration

Pharmacokinetic Parameters (Units)	Group 1, 10 mg ABT-384 QD (N = 9)	Group 2, 50 mg ABT-384 QD (N = 6)	Group 3, 100 mg ABT-384 QD (N = 6)
Day 1			
ABT-384			
C_{max} (ng/mL)	66.4 (19.3)	303 (84.3)	527 (107)
T_{max} (h)	3.2 (0.8)	3.8 (2.0)	4.0 (1.3)
AUC_{0-24} (ng h/mL)	629 (227)	2570 (571)	5590 (1380)
Acid metabolite of ABT-384			
C_{max} (ng/mL)	1.36 (0.641)	7.90 (1.56)	23.0 (6.45)
T_{max} (h)	19.6 (6.8)	14.0 (7.8)	21.5 (6.1)
AUC_{0-24} (ng h/mL)	24.5 (10.3)	150 (30.0)	395 (121)
Day 21			
ABT-384			
C_{max} (ng/mL)	101 (25.1)	358 (63.2)	667 (141)
T_{max} (h)	3.2 (0.8)	3.8 (1.3)	3.8 (1.6)
C_{min} (ng/mL)	14.2 (6.07)	44.8 (22.4)	105 (32.6)
$t_{1/2}$ (h)	15.5 (3.94)	15.2 (3.38)	16.6 (2.42)
AUC_{0-24} (ng h/mL)	1040 (318)	3540 (817)	7690 (1780)
CL/F (L/h)	10.6 (3.72)	14.6 (2.66)	13.7 (3.58)
f_e (%)	0.01	0.04	0.03
Acid Metabolite of ABT-384			
C_{max} (ng/mL)	8.00 (3.53)	47.7 (20.0)	148 (56.3)
T_{max} (h)	7.8 (2.4)	6.3 (2.9)	13.2 (8.6)
C_{min} (ng/mL)	5.96 (2.61)	32.8 (16.9)	99.2 (28.0)
$t_{1/2}$ (h)	37.1 (9.77)	47.3 (13.9)	46.5 (8.88)
AUC_{0-24} (ng h/mL)	170 (73.6)	984 (450)	3020 (986)

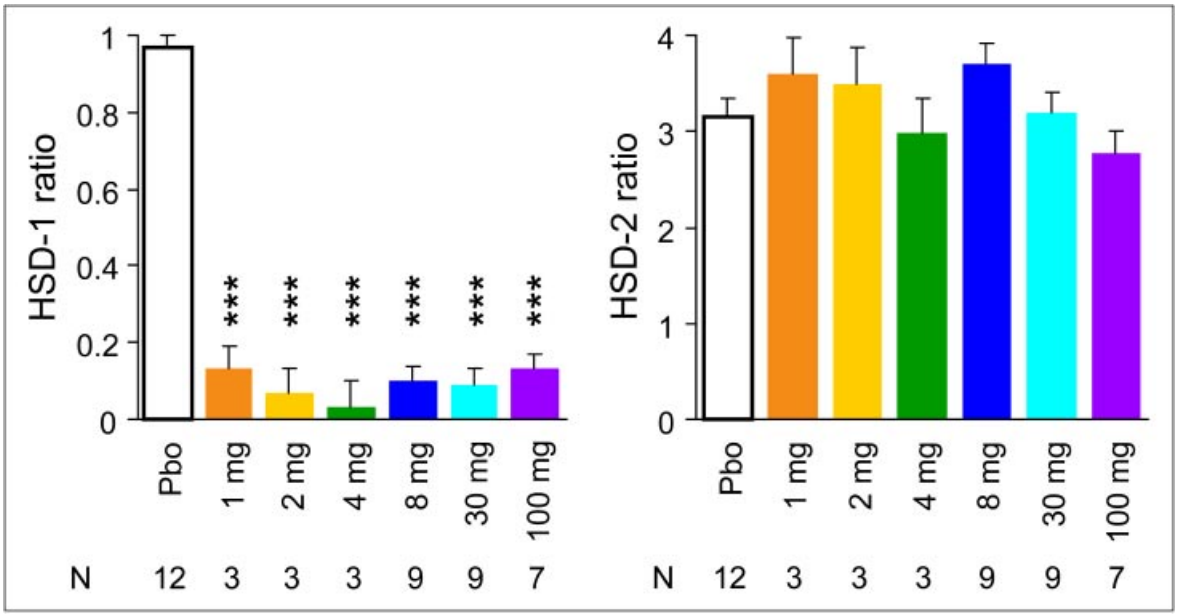

Figure 4. ABT-384 inhibits its target HSD-1 and is selective against the related enzyme HSD-2. Urine was collected for 24-hour periods on Day − 1 (prior to dosing) and Day 7. The HSD-1 ratio (left panel) is urine cortisol metabolites (5-α-tetrahydrocortisol + 5-β-tetrahydrocortisol) divided by urine cortisone metabolite (tetrahydrocortisone). The HSD-2 ratio (right panel) is urine cortisone divided by urine cortisol. Bars and whiskers represent Day 7 least squares means and standard errors. Pbo = placebo; ***$P < .001$ for an ABT-384 regimen compared to placebo.

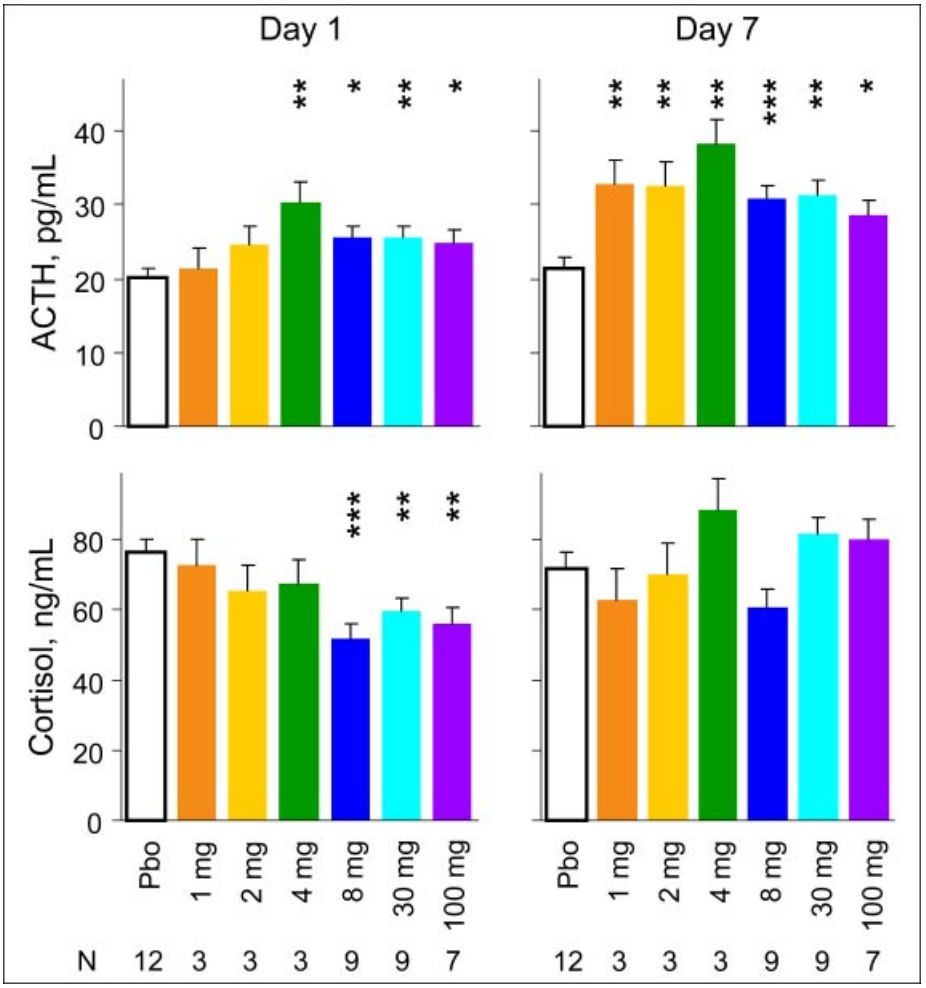

Figure 5. ABT-384 increases plasma ACTH levels, leading to a decrease of serum cortisol following a single dose but limited changes after 7 days of dosing. Plasma and serum samples were collected at 2, 4, 12, and 24 hours after dosing on Day − 1 (prior to dosing), Day 1 and Day 7. Data shown are plasma ACTH (upper panels) and serum cortisol (lower panels) averaged across the 4-time points following dosing on Day 1 (left panels) and Day 7 (right panels). Bars and whiskers represent least squares means and standard errors. Pbo = placebo; *$P < .05$, **$P < .01$, ***$P < .001$ for an ABT-384 regimen compared to placebo.

after the dose. The largest increases were observed at 4 and 24 hours after the dose. Little effect on ACTH was observed at 12 hours after the dose, during the nadir of its diurnal cycle when no or little effect was expected. Decreased serum cortisol levels were observed in healthy volunteers with increasing dose ($P = .001$, Figure 5, lower left panel). Decreases were observed 2, 12, and 24 hours after the dose, although not consistently across dose levels at each time point. Little effect on cortisol was observed at 4 hours after the dose. Data shown are averaged across time points, reflecting the statistically significant main effects of dose on ACTH and cortisol levels. Plasma ACTH and serum cortisol after a single dose were not measured in elderly subjects.

Following 7 daily doses of ABT-384, increased plasma ACTH levels were observed in healthy adults with increasing dose ($P < .001$, Figure 5, upper right panel). Substantial ACTH increases were observed 2, 4, and 24 hours after the dose. Little effect on ACTH was observed at 12 hours after the dose, during the nadir of its diurnal cycle. A trend for ACTH increases was observed with increasing dose in elderly subjects ($P = .085$, mean increases of 40–56% compared to placebo). A trend for increased serum cortisol levels was observed in healthy adults with increasing dose ($P = .052$, Figure 5, lower right panel). A dose × time interaction ($P < .001$) reflected serum cortisol increases at 2 hours after the dose among subjects who received 4, 30 or 100 mg ABT-384, and 24 hours after the dose among subjects who received 100 mg ABT-384. In elderly subjects, there was no trend for change of serum cortisol levels with increasing dose ($P = .6$). A dose × time interaction ($P = .044$) was mainly attributable to mean decreases of 25–37% compared to placebo just prior to dosing (i.e.,

24 hours after the prior dose) among subjects who received 50 or 100 mg ABT-384. No differences from placebo were observed at 14 hours after dosing, during the nadir of cortisol's diurnal cycle.

The main GCs excreted in urine are the tetrahydro metabolites of cortisol and cortisone. These generally constitute over 50% of total GCs in urine. The sum of cortisol, cortisone, and their tetrahydro metabolites is used as a proxy for total cortisol production.[1] So that the measurement is independent of interindividual variation of urination patterns or diurnal cortisol rhythms, data are typically reported from a 24-hour urine collection. ABT-384 regimens of 2–100 mg QD for 7 days in healthy volunteers (Figure 6, left panel) and of 10–100 mg QD for 21 days in elderly subjects (data not shown) increased total cortisol production by 115–180%. The increase associated with 1 mg QD for 7 days in healthy volunteers was somewhat smaller. In both studies, nearly all of the increase was in tetrahydrocortisone, consistent with the observed full blockade of conversion of cortisone to cortisol in the liver.

Urine cortisol output represents renal exposure to cortisol.[2] Increased urine cortisol output was observed in healthy volunteers with increasing dose ($P = .002$, Figure 6, right panel). Similarly increased urine cortisol output was observed in elderly subject ($P = .019$) with statistically significant increases compared to placebo among subjects who received 50 or 100 mg ABT-384.

In addition to cortisol, ACTH increases secretion of other adrenal steroids. Following observation of increased plasma ACTH levels with ABT-384 dosing in healthy volunteers, levels of several additional steroids were measured in elderly subjects. Increases of the androgens dehydroepiandrosterone (75–125%, $P < .001$), dehydroepiandrosterone sulfate (44–65%, $P < .001$), and androstendione (80–99%, $P = .033$) were observed with all ABT-384 doses. Estradiol increased among subjects who received 100 mg ABT-384 QD (81%, $P = .001$) but not among those who received 10 or 50 mg ABT-384 QD. Testosterone was not affected ($P = .9$).

ABT-384 did not affect cortisol-binding globulin levels in elderly subjects ($P > .1$ for each regimen). Cortisol-binding globulin was not measured in healthy volunteers.

Discussion

In these 2 clinical studies of the HSD-1 inhibitor ABT-384, single doses from 1 to 240 mg, and multiple doses from 1 to 100 mg for 7–21 days a maximum-tolerated dose was not defined. The only potentially clinically significant safety finding was an increase of mean hepatic enzyme values with both ABT-384 dose and treatment

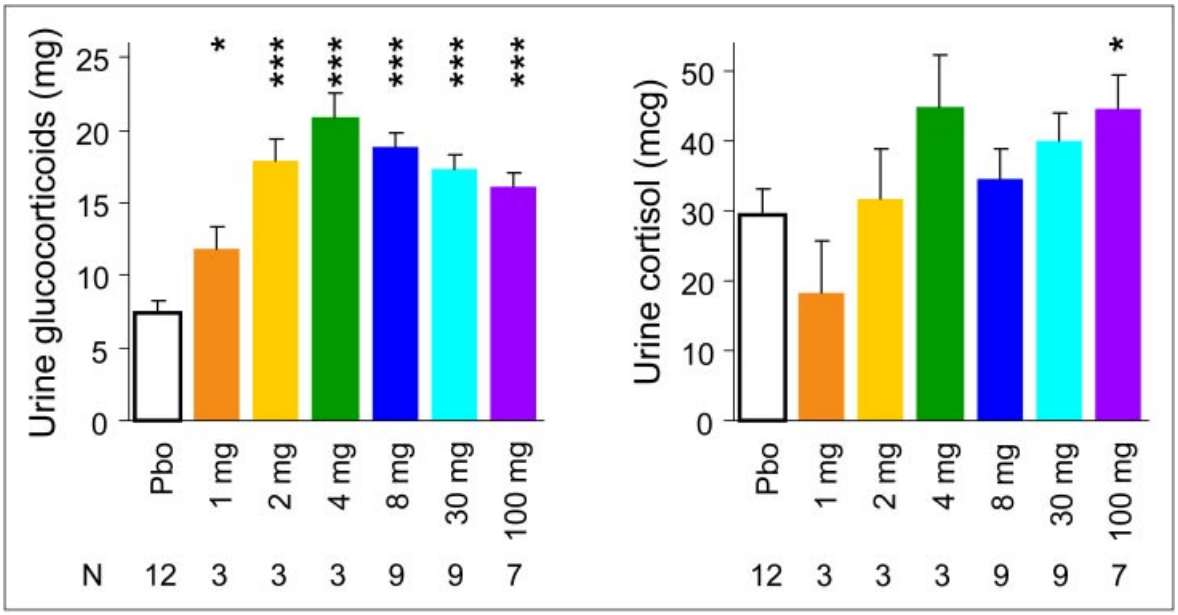

Figure 6. ABT-384 increases cortisol production and renal cortisol exposure after 7 days of dosing. Urine was collected for 24-hour periods on Day − 1 (prior to dosing) and Day 7. Cortisol production (left panel) is represented by the 24-hour sum of urine glucocorticoids (cortisol + cortisone + 5-α-tetrahydrocortisol + 5-β-tetrahydrocortisol + tetrahydrocortisone). The mean of this sum prior to study drug administration, representing normal cortisol production, was 7.75 mg. Renal cortisol exposure (right panel) is represented by 24-hour urine cortisol. Mean 24-hour urine cortisol, representing normal excretion, was 37 μg. Bars and whiskers represent Day 7 least squares means and standard errors. Pbo = placebo; *$P < .05$, ***$P < .001$ for an ABT-384 regimen compared to placebo.

duration in elderly subjects and to a lesser degree in healthy volunteers. Conclusions regarding ABT-384's safety are tempered by the limited number of healthy subjects in these studies. The 100 mg ABT-384 daily dose provides a wide safety margin above the 1 mg daily dose at which full inhibition of hepatic HSD-1 was observed (albeit only in 3 subjects, limiting the strength of a conclusion that a 1 mg daily dose will be fully efficacious), and also above the 2–10 mg daily doses at which non-human primate findings (AbbVie, data on file) suggest will achieve brain HSD-1 inhibition in man.

In vitro enzyme-binding results suggest that the acid metabolite is approximately one-fifth as potent, compared to ABT-384, as an inhibitor of human HSD-1. Both ABT-384 and the acid metabolite were moderately to highly bound to human plasma protein (97–98%). The exposure of acid metabolite increased more than dose proportionally from 11% to 23% following single doses and from 7% to 40% following multiple doses. These results suggest that acid metabolite could contribute to the pharmacological effect, particularly at higher doses. Additionally, the longer half-life of acid metabolite than ABT-384 suggests that acid metabolite may still contribute to the overall pharmacological effect even when ABT-384 concentrations are low. These characteristics support once-daily administration of ABT-384.

The amounts of ABT-384 and acid metabolite were negligible in urine, suggesting that renal excretion was not the major elimination pathway for ABT-384 and acid metabolite, consistent with the in vivo metabolism study in rat which showed ABT-384 was extensively metabolized and no ABT-384 level was detectable in rat urine or bile. In preclinical animal studies ABT-384 bioavailability was >40%. Although ABT-384's absolute bioavailability in human is not known, it is predicted to be >10% based on in vitro and in vivo data. As the observed apparent clearance for ABT-384 (~10–20 L/h) following single and multiple-dose studies was relatively low, it suggests that the elimination of ABT-384 is likely to be dependent on hepatic intrinsic clearance.

The pharmacokinetic results from the lower dose administrations presented a limited number of measurable ABT-384 plasma concentrations at the 1 and 2 mg single-dose levels, while the exposures were close to the linear range following multiple doses of ABT-384. The greater than dose-proportional increase in exposure following single doses of 1–8 mg ABT-384, as well as a dose-proportional increase in exposure (AUC_{0-24} and C_{min}) following multiple doses of 1–30 mg ABT-384, suggest a potential non-specific binding process involved in the distribution of ABT-384, which will become saturable at higher dose or repeated dosing. The higher dose-normalized AUC_{0-24} values following multiple doses and the dose-normalized AUC_{∞} values following single-doses support this hypothesis.

Exposures (C_{max} and AUC_{0-24}) of ABT-384 and the acid metabolite on Day 1 and at steady state (Day 21) in elderly subjects were compared to the exposures on Day 1 and at steady state (Day 14) in healthy adult subjects (Figure 7). Results of the statistical analyses showed similar central values on ABT-384 C_{max} and AUC_{0-24} between elderly and young subjects on both Day 1 and at steady state. Exposure (C_{max} and AUC_{0-24}) of the acid metabolite in elderly were approximately 50% higher than adult subjects on both Day 1 and at steady state, although no statistical significance was observed. The half-life for both ABT-384 and the acid metabolite in elderly subjects were similar to those in adult subjects. Other demographic variables (weight and sex) were also explored in the statistical analyses for their impact on pharmacokinetics of ABT-384 and the acid metabolite. The impact of these demographic variables on the various pharmacokinetic parameters was limited and not consistent between single and multiple doses.

Analysis of urine cortisol metabolites demonstrated full (at least ~90%) hepatic HSD-1 inhibition with regimens from 1 mg daily, and confirmed in vitro target selectivity. Little or no difference of hepatic target engagement was observed between daily regimens of 1–100 mg ABT-384. With full target engagement at 1 mg daily and no clear safety signal at 100 mg daily ABT-384 appears to have a wide therapeutic margin, at least for indications such as diabetes and metabolic syndrome for which hepatic enzyme inhibition is relevant.

The relative contribution to systemic cortisol levels of tissue cortisol regeneration catalyzed by HSD-1, compared to that of the HPA axis that leads to de novo cortisol synthesis, has not been extensively characterized. It has been estimated that as much as 50% of systemic cortisol may come from the HSD-1 pathway.[36] The pharmacologic effects of ABT-384 on HPA axis activity suggest that tissue regeneration is a major source of systemic cortisol. On the first day of dosing, ABT-384 doses of 4–100 mg were associated with increased plasma ACTH levels and doses of 8–100 mg were associated with decreased serum cortisol levels. After multiple days of dosing, all ABT-384 doses were associated with increased plasma ACTH levels but not substantial difference of serum cortisol levels. Moderate increases (in healthy volunteers) and decreases (in elderly subjects) of serum cortisol were observed in subjects who received 30–100 mg ABT-384. The increase of ACTH appears to be a compensatory response of the HPA axis to restore circulating cortisol homeostasis in the presence of an HSD-1 inhibitor.

Substantial changes of other steroid levels, secondary to increased ACTH, were associated with ABT-384 administration. Increased urine tetrahydrocortisone indicates elevation of hepatic cortisone. Increased circulating

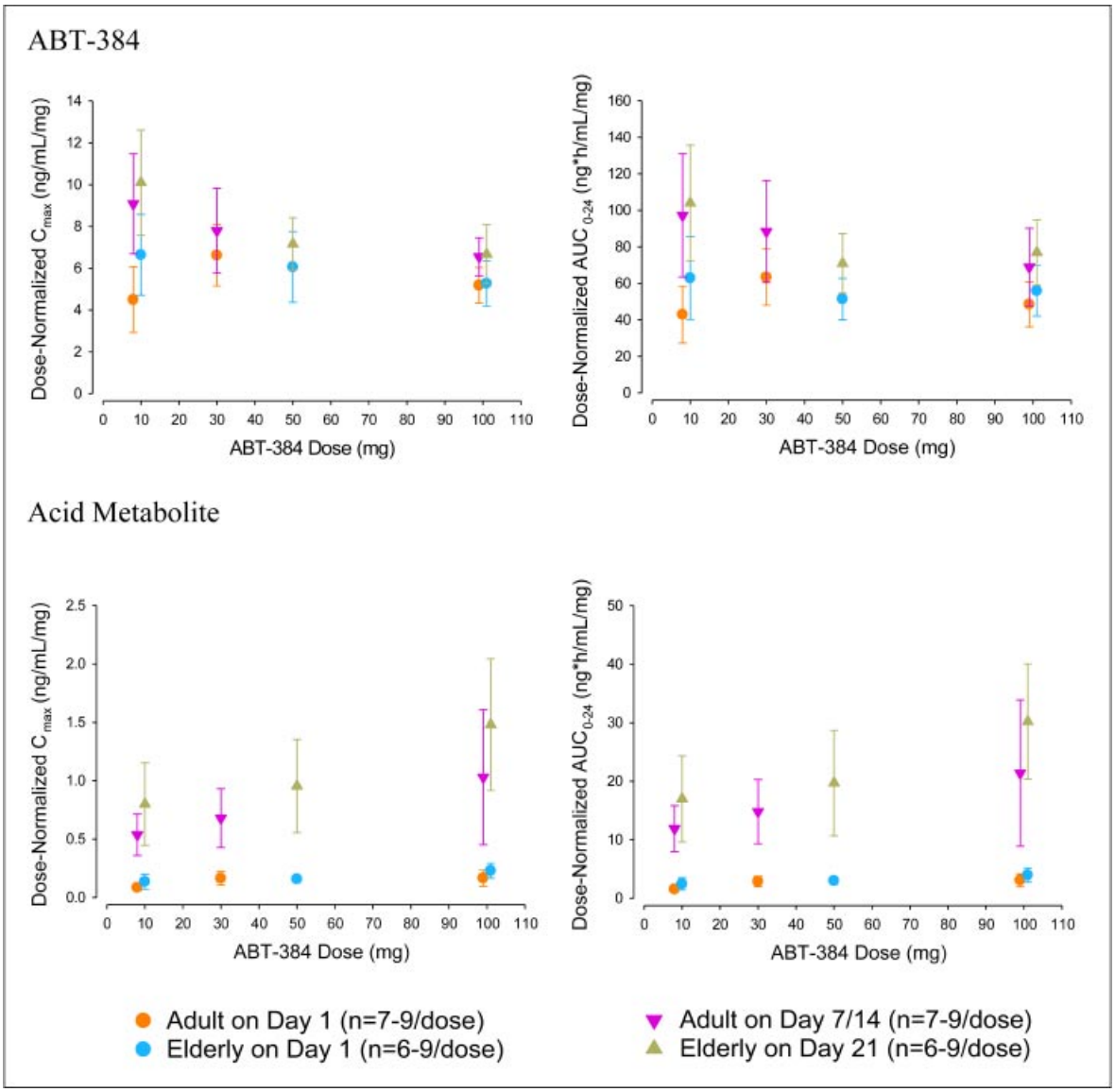

Figure 7. Mean ± SD exposures of ABT-384 and its acid metabolite in elderly versus adult subjects.

androgen and estradiol levels were observed. The long-term consequences, if any, of these changes are not known. In rat and monkey toxicity studies (AbbVie, data on file), there were no adverse effects that were likely attributable to increased steroid levels. As well, no such adverse effects were reported in clinical trials of other HSD-1 inhibitors with which similar effects on ACTH and steroids were observed.[3,4]

Overall, ABT-384 demonstrated favorable, pharmacokinetic, and pharmacodynamic profiles in the dose range tested in Phase 1 studies. A maximum-tolerated dose was not defined in the clinical studies. Combined with pharmacokinetic properties of its acid metabolite, ABT-384 is suitable for once daily administration. ABT-384 showed similar pharmacokinetic and safety profiles between elderly and adult subjects. Pharmacologic effects, including increases of ACTH levels, cortisol production and androgen and estradiol levels, confirmed the targeted HSD-1 inhibition. The safety, pharmacokinetic, and pharmacodynamic results of these 2 clinical studies support future research on the therapeutic potential of ABT-384.

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Declaration of Conflicting Interests

Yi Wang is a former AbbVie employee. All other authors are AbbVie employees.

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