Pre-clinical Characterization of Absorption, Distribution, Metabolism and Excretion Properties of TAK-063

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Abstract: TAK-063 is currently being developed to treat schizophrenia. In this study, we investigated the absorption, distribution, metabolism and excretion (ADME) properties of TAK-063 using several paradigms. Following oral administration of TAK-063 at 0.3 mg/kg, bioavailability of TAK-063 was 27.4% in rats and 49.5% in dogs with elimination half-lives of 3.1 hr in rats and 3.7 hr in dogs. TAK-063 is a highly permeable compound without P-glycoprotein (P-gp) or breast cancer resistance protein substrate liability and can be readily absorbed into systemic circulation via the intestine. TAK-063 can also cross the blood–brain barrier. TAK-063 was metabolized mainly by CYP2C8 and CYP3A4/5, while incubation with human liver microsomes produced the major human metabolite, M-I as well as several unknown minor metabolites. Metabolism of TAK-063 to M-I occurs through hydroxylation of the mono-substituted pyrazole moiety. In vitro, TAK-063 was observed to inhibit CYP2C8, CYP2C19 and P-gp with IC50 values of 8.4, 12 and 7.13 µM, respectively. TAK-063 was primarily excreted in the faeces in rats and dogs with M-I as a predominant component. The pre-clinical data from these ADME studies demonstrate a favourable pharmacokinetic profile for TAK-063 with good brain distribution supporting the feasibility of targeting central nervous system regions involved in schizophrenia pathophysiology. TAK-063 has recently been investigated in a phase 2 clinical trial (NCT02477020).

Phosphodiesterase 10A (PDE10A) acts as an important regulator of signal transduction by degrading the second messengers cyclic adenosine monophosphate and cyclic guanosine monophosphate [1,2]. PDE10A is preferentially expressed in the medium spiny neurons of the striatum, a region of critical importance for domains that are disrupted in patients with schizophrenia [3–5].

Current treatments often do not adequately address the multifaceted clinical symptomatology of schizophrenia [6]. In addition, antipsychotics are associated with cardiometabolic disturbances and cardiovascular morbidity that worsen long-term outcomes [7]. Because of the shortcomings in current treatment options for schizophrenia, PDE10A inhibition by TAK-063 is being evaluated as a new therapeutic strategy. TAK-063 is a potent and highly selective PDE10A inhibitor [8,9]. Furthermore, TAK-063 has demonstrated pre-clinical efficacy in animal models of schizophrenia. TAK-063 produced dose-dependent antipsychotic-like effects in rodent models of induced psychosis [10]. In rodents, PDE10A inhibition by TAK-063 also improved cognitive functions impaired in schizophrenia [11]. TAK-063 exhibits a favourable safety profile in a rodent study [12]. To understand the mechanism of these efficacy and safety profiles of TAK-063, the absorption, distribution, metabolism and excretion (ADME) properties of TAK-063 were investigated.

In general, ADME properties using radiolabelled materials in animals and human beings are required for inclusion in the New Drug Application for new molecular entities. This report helps describe the disposition and elimination mechanisms of TAK-063 and its related metabolites in animal models and human beings and can be used to compare the exposure of TAK-063-related entities in animals used in the toxicity programmes during drug development with data from human studies and to investigate human-specific metabolite(s).

Here, we report the pre-clinical pharmacokinetic (PK) properties of TAK-063 including ADME. The results of the current study are useful in complementing the PK profile of TAK-063 delineated in the clinical programme.

Materials and Methods

Chemical and biological reagents. All reagents and solvents were of analytical grade or equivalent. TAK-063 (1-[2-fluoro-4-(1H-pyrazol-1-yl)phenyl]-5-methoxy-3-(1-phenyl-1H-pyrazol-5-yl)pyridazin-4(1H)-one) and M-I (1-[2-Fluoro-4-(4-hydroxy-1H-pyrazol-1-yl)phenyl]-5-methoxy-3-[1-phenyl-1H-pyrazol-5-yl]pyridazin-4(1H)-one) were synthesized by Takeda Pharmaceutical Company Limited (Kanagawa, Japan). [14C] TAK-063 was supplied by Drug Metabolism and Pharmacokinetics Research Laboratories (Takeda Pharmaceutical Company, Ltd.). See the Supporting information for a complete list of reagents.

Animals and dosing formulations. All experiments using animals were reviewed and approved by the Experimental Animal Ethical Committee, Nemoto Science Co., Ltd. Male Sprague Dawley (SD) (Charles River Laboratories, Kanagawa, Japan) and Iar: Long Evans (Institute for Animal Reproduction, Ibaraki, Japan) rats were 8 weeks old and 251.4–342.7 g at the time of drug administration. Male beagle dogs (Kitayama Labes Co., Ltd.; Hongo-machi, Japan) were 7–10 months old and 9.6–10.5 kg at drug administration. They were fed laboratory chow (CR-LPF for rats, Oriental Yeast Co., Ltd., Tokyo, Japan; CD-5M for dogs, CLEA Japan, Inc., Tokyo, Japan), had free access to water and were housed in temperature and humidity-
controlled rooms (18–28°C, 36–73%), with 12-hr light/dark cycles, for up to 1 week before use. TAK-063 and [14C]TAK-063 (specific activity: 6.75 MBq/kg) were suspended in 0.5% MC solution, and N, N-dimethylacetamide (DMAA)/PEG400 (1:1, v/v) for oral and intravenous administration, respectively. For intraduodenal administration, TAK-063 and [14C]TAK-063 were dissolved in water followed by dimethylsulphoxide (DMSO) and methanol (1:9, v/v). M-I was dissolved in DMSO/acetonitrile for infusion.

**Rat studies.** Pharmacokinetic, tissue distribution, mass balance and metabolite profiling studies of TAK-063 or M-I were investigated in intact or bile duct-cannulated (BDC) male rats. After oral administration of [14C]TAK-063 to rats, blood was collected from the tail vein at the following time-points: 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 32, 48 and 72 hr, while blood was collected at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 32 and 48 hr after oral administration of TAK-063. After intravenous administration of TAK-063, blood was collected at the following time-points: 0.083, 0.167, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 32 and 48 hr. The blood was immediately cooled under ice-chilled conditions and centrifuged at approximately 10,000 × g at 4°C for 3 min. to obtain the plasma. Concentrations of TAK-063 and M-I were analysed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). The total radioactivity in the plasma was determined by liquid scintillation counter (LSC).

Urine and faecal samples were collected separately from the intact rats treated with [14C]TAK-063 under dry ice-chilled conditions and room temperature in metabolic cages up to 168 hr after dosing. In addition, expired air was collected and separated into a CO2-absorbent solution of 2-aminoethanol/2-methoxyethanol (1:2, v/v) at room temperature up to 24 hr after dosing. The total radioactivity in urine and faeces was determined by LSC.

For the BDC rat study, the common bile duct of rats was cannulated with polyethylene tubing (PE10, Becton Dickinson and Company, Franklin Lakes, NJ, USA) under anaesthesia with isoflurane. After efflux of bile was confirmed, a single dose of [14C]TAK-063 (1 mg/kg) was given intraduodenally and the rats were housed in Ballman cages. Bile samples were collected under dry ice-chilled conditions, and urine and faecal samples were collected at room temperature during the 24 hr after dosing.

In vivo tissue distribution was determined after administration of [14C]TAK-063 at a dose of 1 mg/kg to fed male albino and pigmented rats. At 1, 3, 8, 24, 48 and 168 hr after administration of [14C]TAK-063, the rats were killed and tissue was collected. Concentrations of tissue radioactivity were determined by the combustion method of radioactive measurement.

To assess brain penetration of M-I, male rats received M-I intravenously at a dose of 0.1 mg/kg/h for a total of 4 hr. After 4 hr, the rats were killed and brains were excised to produce a 20% brain homogenate, which was analysed for M-I by LC-MS/MS.

**Dog studies.** Pharmacokinetic, mass balance and metabolite profiling studies of TAK-063 were undertaken in male fed or fasted dogs. After oral or IV administration of TAK-063 and/or [14C]TAK-063 to dogs, blood was collected from the cephalic vein at the following time-points (oral): 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 32 and 48 hr, and (IV): 0.083, 0.167, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 32 and 48 hr. The blood was immediately cooled under ice-chilled conditions and centrifuged at approximately 1850 × g at 4°C for 10 min. to obtain the plasma. Concentrations of TAK-063 and M-I were analysed by LC-MS/MS. The total radioactivity in the plasma was determined by LSC. A portion of the plasma samples was used for metabolite identification studies.

Urine and faecal samples were collected under dry ice-chilled conditions and room temperature, respectively, in separate amber glass vials up to 24 or 72 hr after dosing. The total radioactivity in urine and faecal samples was determined by LSC.

**Permeability study of [14C]TAK-063 across Caco-2 cells.** Caco-2 cells were cultured at 37°C in a 5% CO2 incubator for 23 days to achieve cell layers sufficient to perform a permeability study to determine the absorption of TAK-063. The passage medium was composed of Dulbecco’s modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA, USA) containing 9% foetal bovine serum (FBS; Invitrogen), 100 μM non-essential amino acids (Invitrogen), 2 mM L-glutamine (Invitrogen), 100 μM penicillin G (Invitrogen), 100 μg/mL streptomycin (Invitrogen) and 250 ng/mL amphotericin B (Invitrogen). For analysis of apical (A) to basal (B) or basal (B) to apical (A) transport, the donor side of Caco-2 cells was incubated at 37°C in the presence of buffer containing 3 μM [14C]TAK-063, 3 μM [3H]digoxin (PerkinElmer, Walling, MA, USA), 0.1 μM [3H]HE85 (PerkinElmer), 10 μM [14C]antipyrine or 10 μM [14C]mannitol (PerkinElmer). After a 1- to 2-hr incubation, samples were collected from the receiver side and total radioactivity was measured by scintillation counting. The permeation coefficient (P_app) was calculated according to the following equation:

\[
P_{app}(\text{cm/sec}) = \left(\frac{dQ}{dt}\right) \frac{|A|}{|C|}
\]

where dQ/dt, A and C represent the transport rate, membrane area and initial concentration of TAK-063 in the donor side, respectively.

The efflux ratio of P_app was equal to the P_app value for B to A transport (P_app B → A) divided by the P_app value for A to B transport (P_app A → B), as in the following equation:

\[	ext{Efflux ratio of } P_{app} = \frac{P_{app} B \rightarrow A}{P_{app} A \rightarrow B} \times 100\%
\]

The inhibitory effect of TAK-063 on P-glycoprotein (P-gp) activity was also investigated in Caco-2 cell monolayers. The experimental conditions were similar to those of the permeability study. [3H]digoxin (3 μM), a substrate of P-gp, was incubated in the absence or presence of TAK-063 (0.3, 1, 3, 10 and 30 μM) or in the absence or presence of quinidine (1, 3, 10 and 30 μM), a specific inhibitor of P-gp.

Per cent (%) of control values were estimated according to the following equation:

\[
\% \text{ of control} = \frac{1}{1 + IC_{50}} \times 100\%
\]

IC_{50} values were calculated from the relationship between TAK-063 or quinidine concentration and the per cent of the control according to the following equation:

\[
\% \text{ of control} = \frac{1}{1 + I[1]} \times 100\%
\]

[I] is the concentration of TAK-063 or quinidine.

In vitro distribution of [14C]TAK-063 in blood cells of rats, dogs and human beings. Pooled blood samples of rats, dogs or human beings were spiked with 0.05, 0.5 or 5 μg/mL [14C]TAK-063 and incubated at 37°C for 30 min. Samples were then separated by centrifugation at 1850 × g for 10 min., and the radioactivity of the resultant plasma was measured by the combustion method.

In vitro plasma protein binding of TAK-063 and TAK-063 M-I. Pooled plasma specimens (4 mL) from rats, dogs or human beings were incubated with [14C]TAK-063 at a dose of 1 mg/kg to fed male albino and pigmented rats, and urine and faecal samples were collected at room temperature during the 24 hr after dosing. The total radioactivity in urine and faecal samples was determined by LSC.
beings were spiked with \(^{14}C\)TAK-063 or non-radio-labelled TAK-063 M-I at 0.05, 0.5 and 5 \(\mu\)g/mL. A 100 \(\mu\)L aliquot of the spiked sample was transferred into a polyethylene vial, and concentrations of \(^{14}C\)TAK-063 and M-I were determined by LSC and LC-MS/MS, respectively. A 3-ml aliquot of the spiked sample was transferred into an ultracentrifugation tube and centrifuged at approximately 274,000 or 316,000 \(\times\) g at 4°C for 14 hr. After centrifugation, a 500-\(\mu\)L aliquot of the supernatant just under the lipid layer was transferred into a polyethylene vial and concentrations of \(^{14}C\)TAK-063 and M-I were determined by LSC and LC-MS/MS, respectively. In addition, the protein-binding ratios of radiolabelled TAK-063 in solutions of 4% human serum albumin (HSA), 0.05% zl-acid glycoprotein (AGP) and 4% HSA/0.05% AGP were calculated to quantify the protein bound by TAK-063 in human plasma. The protein binding (R) of TAK-063 in human plasma. The protein binding (R) of TAK-063 or M-I was calculated by the following equation:

\[
R(\%) = \left( \frac{C_p - C_f}{C_p} \right) \times 100
\]

where \(C_p\) and \(C_f\) represent protein-binding ratio (%) of tested compound, concentration of tested compound in the spiked sample (dpm/mL) and concentration of tested compound in supernatant after ultracentrifugation (dpm/mL), respectively.

**CYP reaction phenotyping studies.** A study of CYP-expressing microsomes and correlation analysis between the elimination rates of TAK-063 or formation rates of metabolites and rates of metabolism of marker substrates using individual human liver microsomes from 16 donors (mixed) was conducted for CYP reaction phenotyping.

\(^{14}C\)TAK-063 (10 \(\mu\)M) was incubated with human liver microsomes (0.5 mg protein/mL) or CYP-expressing microsomes (100 pmol P450/mL) in 50 mM potassium phosphate buffer (pH 7.4) to a final volume of 300 \(\mu\)L. The reactions were initiated by adding a nicotinamide-adenosine dinucleotide phosphate (NADPH)-generating system (final concentration of 5 mM MgCl\(_2\), 5 mM glucose 6-phosphate, 0.5 mM \(\beta\)-NADP\(^+\) and 1.5 units/mL glucose-6-phosphate dehydrogenase). After incubation at 37°C for 30 min. in a shaking water bath, the reaction was terminated by adding 150 \(\mu\)L acetonitrile. For CYP2C9-expressing microsomes, the incubation was conducted in Tris–HCl buffer (50 mM, pH 7.4) instead of potassium phosphate buffer. After centrifuging at 1500 \(\times\) g for 10 min., the supernatant was applied to LSC and high-performance liquid chromatography (HPLC) analysis. The HPLC conditions were conducted as described in the rat and dog studies sections with minor modifications in the gradient programme. Correlation analyses and calculation of the Pearson product–moment correlation coefficient were performed between the logarithmic metabolic rates of the respective CYP isomeric-specific substrates and the logarithmic elimination amount of TAK-063 or the logarithmic formation amounts of M-I, and unidentified metabolites UK-1 and UK-2 with SAS System version 8.02.

**Evaluation of CYP inhibition by TAK-063 or M-I in human liver microsomes.** The inhibitory effects of TAK-063 and M-I on the activities of seven CYP isoforms (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5) were examined in human liver microsomes. The markers used to assay activity were phenacetin-O-deethylation for CYP1A2, bupropion hydroxylation for CYP2B6, paclitaxel 6\(\alpha\)-hydroxylation for CYP2C8, diclofenac 4\'-hydroxylation for CYP2C9, (S)-mephenytoin 4\'-hydroxylation for CYP2C19, bufuralol 1\'-hydroxylation for CYP2D6 and midazolam 1\'-hydroxylation and testosterone 6\(\beta\)-hydroxylation for CYP3A4/5. Each CYP substrate was incubated in human liver microsomes with or without pre-incubation of TAK-063 (0, 1, 3, 10 and 20 \(\mu\)M) or M-I (0, 0.3, 1, 3 and 10 \(\mu\)M). For the pre-incubation study, TAK-063 (0, 3, 10 and 20 \(\mu\)M) or M-I (0, 0.3, 1, 3 \(\mu\)M) was incubated with an NADPH-generating system and human liver microsomes (2 mg/mL for TAK-063, 0.2 mg/mL for M-I) at 37°C for 30 min. Reactions were incubated at 37°C for 5 to 30 min. depending on the CYP substrate. After the reactions were terminated, samples were analysed by LC-MS/MS.

Inhibition was calculated according to the following equation:

\[
\text{Inhibition} = \frac{\text{Formation rate in positive inducer-treated sample} - \text{Formation rate in control-treated sample}}{\text{Formation rate in positive inducer-treated sample}} \times 100
\]

If the remaining CYP activity in the presence of TAK-063 (up to 20 \(\mu\)M) was 50.0% or less, the activity was analysed by Pharmacodynamic model 103 (Inhibitory effect \(E_{\text{max}}\) model) using WinNonlin version 5.2.1 (Pharsight Corporation, Cary, NC, USA) to determine the concentration required to achieve 50% inhibition (IC\(_{50}\)). If the remaining CYP activity in the presence of the highest tested concentration of TAK-063 or M-I was greater than 50.0%, IC\(_{50}\) was expressed as greater than the highest tested concentration of TAK-063 or M-I.

**Evaluation of CYP induction by TAK-063 or M-I in human hepatocytes.** Induction of CYP1A2, CYP2B6 and CYP3A4/5 activities by TAK-063 or M-I was examined using human primary hepatocytes from three donors (mixed sex). Three preparations of cryopreserved human hepatocytes from three separate livers were treated once daily for 1 to 4 consecutive days with DMSO (0.1% \(v/v\), vehicle control), TAK-063 (1, 3, 10 or 20 \(\mu\)M) or M-I (0.2, 0.5, 1 or 2 \(\mu\)M), negative control or one of the following positive inducers: 50 \(\mu\)M omeprazole (CYP1A2), 750 or 1000 \(\mu\)M phenobarbital (CYP2B6) or 10 or 20 \(\mu\)M rifampin (CYP3A4/5). Enzyme induction by TAK-063 and M-I in cultured human hepatocytes was assessed by measuring marker enzyme activity or target gene mRNA, respectively. For TAK-063, after treatment for 1 or 4 days, hepatocytes were rinsed with fresh medium and then incubated with phenacetin (100 \(\mu\)M, CYP1A2), bupropion (500 \(\mu\)M, CYP2B6) or testosterone (250 \(\mu\)M) in the CO2 incubator at 37°C for 2 hr. After incubation, the medium for each well was stored at –80°C until analysis. Marker enzyme activities of CYP1A2 (phenacetin-O-deethylation), CYP2B6 (bupropion hydroxylation) and CYP3A4/5 (testosterone 6\(\beta\)-hydroxylation) were analysed by HPLC. After 3-day treatment with M-I, the hepatocytes were harvested to isolate RNA, which was analysed by quantitative real-time polymerase chain reaction to assess the effect of TAK-063 M-I on CYP1A2, CYP2B6 and CYP3A4 mRNA levels.

Enzyme induction ratios (%) for TAK-063 and M-I were calculated as follows:

\[
\text{Induction ratio} (%) = \frac{\text{Formation rate in TAK-063- or control-treated sample}}{\text{Formation rate in positive inducer-treated sample}} \times 100
\]

\[
\text{Control ratio} (%) = \frac{\text{fold change in positive inducer-treated sample}}{\text{fold change in M-I-treated sample} - 1} \times 100
\]

**Metabolite identification study.** Metabolite identification in dog plasma was performed with a Prominance HPLC system (Shimadzu Corporation, Kyoto, Japan) coupled to a hybrid mass spectrometer (LTQ Orbitrap, Thermo Fisher Scientific, Inc., Waltham, MA, USA) equipped with an electrospray ionization (ESI) interface system in a positive ion mode and online flow scintillation analyses (Radiomatic 505TR, PerkinElmer). Pooled dog plasma (3 hr after \(^{14}C\)TAK-063 dosing) was extracted with 3 volumes of acetonitrile and centrifuged at 1500 \(\times\) g at 10°C for 10 min. The supernatant was evaporated to
dryness under a stream of nitrogen gas at room temperature. The residue was dissolved in a 4:1 (v/v) mixture of the mobile phases (MP)-A, and MP-B (MP-A and MP-B are mixtures of 10 mM ammonium acetate [pH 4] and acetonitrile [9:1 and 1:9, by volume, respectively]). The sample was then subjected to LC/MS and online flow scintillation analyses. For separation of analytes, a Develosil C30 UG-5 column (5 μm, 250 × 4.6 mm i.d., Nomura Chemical Co., Ltd., Hinode-cho Seto, Japan) at a flow rate of 1.0 mL/min. was used. The column temperature was maintained at 40°C. The samples were eluted with the following gradients: the concentration of MP-B was initially increased from 10 to 50% over 35 min. and from 50 to 90% in 0.1 min., then held at 90% for 9.9 min. and finally cycled back to the initial conditions.

Metabolite profile studies. Metabolite profiles in plasma, brain, excreta and liver microsomes were elucidated by HPLC equipped with online RI detector. The preparation method for plasma samples and brain and faecal homogenates was similar to that described in the metabolite identification study with minor modification. Urine and bile samples were diluted with the mixture of MP-A and MP-B (9:1) under ice-chilled conditions at ratios of 1:1 and 1:4, respectively. After 1-min. vortex, the samples were centrifuged at approximately 1850 × g at 10°C for 10 min. Each supernatant was then subjected to HPLC-RI analysis.

[14C]TAK-063 (10 μM) was incubated with liver microsomes (rats: 2 mg/mL; human beings and dogs: 0.5 mg/mL) obtained from rats, dogs or human beings. After a 30-min. incubation at 37°C, the reaction was terminated by adding a half volume acetonitrile. After vortex and centrifugation, the supernatant was injected into the HPLC system under conditions described in the metabolite identification study. After LC-MS/MS using an Agilent 1100 HPLC system and TSQ Quantum Discovery MAX (Thermo Electron, Waltham, MA, USA) using ESI as an interface. The plasma samples (50 μL) collected from rats and dogs treated with TAK-063 were added to 10 μL of internal standard (IS) working solution, followed by 400 μL distilled water. After vortex, the samples were applied onto a solid-phase extraction (SPE) plate previously equilibrated with 200 μL methanol and distilled water, successively. The SPE plate was then washed with 200 μL 5% methanol. The analytes were eluted twice with 50 μL of the elution solution, followed by the addition of 300 μL aliquots of 10 mM ammonium acetate. After mixing, 20 μL aliquots of the diluted eluates were injected into the LC-MS/MS column. Chromatography was carried out using a CAPCELL PAK C18 MGH column (4.6 mm i.d. × 150 mm, 5 μm) at 40°C and two mobile phases (50 mM ammonium acetate and acetonitrile) delivered at a flow rate of 1 mL/min. In the mass spectrometric analysis, analytes were detected via the selected reaction monitoring method. The monitoring ions of TAK-063 were m/z 429.1→217.1, and the calibration curve of TAK-063 ranged from 0.3 to 1000 ng/mL.

Concentrations of M-I in the plasma and brain of rats administered TAK-063 at 10 μM (former Nordic Pharmacological Society) at a flow rate of 1.0 mL/min. was used. The column temperature was maintained at 40°C. The samples were eluted with the following gradients: the concentration of MP-B was initially increased from 10 to 50% over 35 min. and from 50 to 90% in 0.1 min., then held at 90% for 9.9 min. and finally cycled back to the initial conditions.

Quantification of TAK-063 and M-1 in plasma and/or brain. Plasma concentrations of TAK-063 in rats and dogs were quantitated by LC-MS/MS using an Agilent 1100 HPLC system and TSQ Quantum Discovery MAX (Thermo Electron, Waltham, MA, USA) using ESI as an interface. The plasma samples (50 μL) collected from rats and dogs treated with TAK-063 were added to 10 μL of internal standard (IS) working solution and 10-μL aliquots of DMSO/methanol (1:9, v/v), followed by 400 μL distilled water. After vortex, the samples were applied onto a solid-phase extraction (SPE) plate previously equilibrated with 200 μL methanol and distilled water, successively. The SPE plate was then washed with 200 μL 5% methanol. The analytes were eluted twice with 50 μL of the elution solution, followed by the addition of 300 μL aliquots of 10 mM ammonium acetate. After mixing, 20 μL aliquots of the diluted eluates were injected into the LC-MS/MS column. Chromatography was carried out using a CAPCELL PAK C18 MGH column (4.6 mm i.d. × 150 mm, 5 μm) at 40°C and two mobile phases (50 mM ammonium acetate and acetonitrile) delivered at a flow rate of 1 mL/min. In the mass spectrometric analysis, analytes were detected via the selected reaction monitoring method. The monitoring ions of TAK-063 were m/z 429.1→217.1, and the calibration curve of TAK-063 ranged from 0.3 to 1000 ng/mL.

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Measurement of radioactivity. Radioactivity in plasma, urine, bile and organic solvent extracts was measured by a direct method using liquid scintillation counters (LSC-6500, LSC-600iC and LSC-6100, Beckman Coulter Inc., Danvers, MA, USA, and Tri-carb 2100TR or 2900 TR (Packard Instrument Co., Inc., Meriden, CT, and PerkinElmer, Waltham, MA, USA), with Hionic-Fluor™ (PerkinElmer), Permafluor E’ and Ultima Flo AP (PerkinElmer) or liquid scintillator A (Wako Pure Chemical) as the liquid scintillator. Radioactivity in faecal homogenates and tissues was measured by the combustion method using an A030701 Sample Oxidizer (PerkinElmer). Carbo-Sorb™E (PerkinElmer) and Permafluor™E’ (PerkinElmer) were used as the carbon dioxide absorbent and liquid scintillator, respectively. The lower limit of quantitation was defined as twice the background value.

Data analysis. Cmax, Tmax and C0.083 h were taken from the actual values. Other PK parameters were derived using non-compartmental analysis methods with WinNonlin version 6.3 (Pharsight Corporation). Absolute bioavailability (BA) was calculated as

\[ BA = \frac{AUC_{oral}}{AUC_{iv}} \times \frac{Dose_{oral}}{Dose_{iv}} \]

Results

Pharmacokinetics. The PK parameters of TAK-063 in rats and dogs after a single oral and IV administration are summarized in table 1. After oral administration at a single dose of 0.3 mg/kg, TAK-063 was moderately absorbed with time to reach the maximum observed plasma concentration (Cmax) values of 2.0 and 3.5 hr in fed male rats and dogs, respectively. The oral absolute BA of TAK-063 was 27.4% in fed male rats and 49.5% in fed male dogs. After oral administration, the elimination half-life (T1/2) values were 3.1 and 3.7 hr in the rat and dog, respectively.

After IV administration to rats, the mean plasma clearance (CLp) and volume of distribution at steady-state (Vss) were 122.8 mL/h/kg and 733.9 mL/kg, respectively. The CLp value was lower than the hepatic blood flow (3310 mL/h/kg) and the value of Vss was similar to total body water (668 mL/kg) in rats, suggesting that TAK-063 has a low hepatic extraction ratio and volume of distribution in rats. After IV administration to dogs, the mean CLp was 1229.4 mL/h/kg, which approached the hepatic blood flow (1850 mL/h/kg), indicating that TAK-063 has a high hepatic extraction ratio in dogs [13]. The value of Vss (246.5 mL/kg) was approximately fourfold greater than total body water (604 mL/kg) in dogs, suggestive of moderate to high tissue distribution. Compared with rats,
the shorter $T_{1/2}$ (1.6 hr) in dogs after IV administration is probably due to a higher CLp (fig. 1 and table 1).

Overall, systemic exposure of TAK-063 as measured by area under the plasma drug concentration–time curve (AUC) and peak plasma concentration ($C_{\text{max}}$) increased in an approximately dose proportional manner after oral administration to fed rats and dogs over a dose range of 0.3 mg/kg to 1.0 mg/kg. Food effect on the PK of TAK-063 was observed in dogs. Compared with data in fasted dogs after a single oral dose of 0.3 mg/kg, the $C_{\text{max}}$ and AUC$_{0-24}$ values in fed dogs

| Species | Feeding condition | IV | PO |
|---------|------------------|----|----|
|         | CLp (mL/h/kg)    | Vss (mL/kg) | $T_{1/2}$ (h) | $T_{1/2}$ (h) | $C_{\text{max}}$ (ng/mL) | $T_{\text{max}}$ (h) | AUC$_{0-24}$ (ng-h/mL) | Absolute BA (%) |
| Rat     | Fed              | 122.8 | 733.9 | 4.9 | 3.1 | 134.1 | 2.0 | 784.7 | 27.4 |
| Dog     | Fed              | 1229.4 | 2464.5 | 1.6 | 3.7 | 15.5 | 3.5 | 135.3 | 49.5 |
| Dog     | Fasted           | –   | –   | –   | 2.4 | 53.2 | 1.0 | 231.4 | –   |

AUC$_{0-24}$, area under the plasma concentration–time curve from time 0 to 24 h after dosing; BA, bioavailability; CLp, mean plasma clearance; $C_{\text{max}}$, maximum observed plasma concentration; IV, intravenous administration; PO, oral administration; $T_{\text{max}}$, time to reach the maximum observed plasma concentration; $T_{1/2}$, elimination half-life; Vss, volume of distribution at steady-state.

Dash (–) indicates not determined; data are expressed as mean values (rats, n = 3; dogs, n = 4).

Fig. 1. Concentration–time profile of TAK-063 after single oral administration at 0.3 mg/kg (closed circles) and 1 mg/kg (open circles) to male rats (A) and dogs (B)$^{a,b}$. $^a$Values below the lower limit of quantitation (LOQ) were graphed as zero. $^b$For fig. 1A and 1B, points and vertical bars represent the mean and standard deviation of 3 and 4 animals, respectively.

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were reduced by approximately 70 and 40%, respectively, and the $T_{\text{max}}$ value increased from 1 to 3.5 hr. A longer $T_{1/2}$ was also observed (table 1).

The apparent permeability coefficient ($P_{\text{app}}$) of $[^{14}\text{C}]$TAK-063 from the apical to the basal side in Caco-2 cell monolayers was $29.7 \times 10^{-6}$ cm/sec, which is comparable with that of antipyrine, a high permeability marker (table 2), suggesting that TAK-063 has high permeability. The $P_{\text{app}}$ ratio of $[^{14}\text{C}]$TAK-063 was 1.1, indicating that TAK-063 is not a substrate of P-gp and/or breast cancer resistance protein (BCRP).

The inhibitory effect of TAK-063 on P-gp activity was also investigated in Caco-2 cell monolayers. TAK-063 decreased the $P_{\text{app}}$ efflux ratio of the P-gp substrate digoxin in a concentration-dependent manner with an IC$_{50}$ value of 7.13 μM (fig. 2).

**Distribution.**

**Tissue distribution in rats.** TAK-063 was widely distributed throughout albino rat tissues after oral administration of a single dose of 1 mg/kg of $[^{14}\text{C}]$TAK-063, including the brain (table 3). The concentrations of radioactivity attained their maxima at 3 hr after administration for all tissue compartments except for stomach ($C_{\text{max}}$ at 1 hr). After 3 hr, the highest concentration was found in the liver (1.584 μg equiv/g). The concentrations in all tissues decreased to low levels or below the limit of quantitation (LOQ) after 168 hr. A metabolite profile study indicated that TAK-063 was the major component in the rat brain (>99% of total radioactivity) at 3 hr after dosing. In addition, the study indicated that TAK-063 and/or its metabolites may have a weak affinity for melanin (data on file).

M-I brain penetration was evaluated in rats in a separate study. After IV infusion of M-I for 4 hr at a dose of 0.1 mg/kg, the M-I brain-to-plasma ratio ranged from 0.04 to 0.07, suggesting minimal brain penetration for M-I in rats. In comparison, the ratio for TAK-063 at 3 hr after oral administration of $[^{14}\text{C}]$TAK-063 was approximately 1.

**Distribution of $[^{14}\text{C}]$TAK-063 into blood cells.** The distribution ratios of $[^{14}\text{C}]$TAK-063 in blood cells at 0.05, 0.5 and 5.0 μg/mL were ≤12.0% in rats, ≤42.0% in dogs and ≤33.0% in human beings (table 4). These results indicate that TAK-063 distribution into blood cells was relatively low, with especially low values in rats (table 4). For all species tested, the distribution of TAK-063 in blood cells was independent of concentration over the experimental dose range of 0.05–5 μg/mL.

**Plasma protein binding.** In all species examined, TAK-063 and M-I exhibited high plasma protein binding independent of concentration (table 5). TAK-063 mainly bound to HSA in human plasma. Plasma protein-binding ratios of TAK-063 were comparable to those of M-I in rats and dogs, whereas plasma protein binding of M-I in human plasma was higher than that of parent drug TAK-063 (>99.6% versus 96.4%).

**Metabolism.** The metabolism of TAK-063 to the metabolite M-I was postulated to occur by hydroxylation of the mono-substituted pyrazole moiety (fig. 3). The chemical structures of unchanged TAK-063 and M-I were identified by LC-MS/MS analysis as the mass spectra and HPLC retention time of TAK-063 and M-I in dog plasma coincided with those of the authentic standards.

**In vivo metabolism.** Profiling of plasma samples collected from rats or dogs treated with $[^{14}\text{C}]$TAK-063 at a single oral dose of 1 mg/kg indicated that TAK-063 was the major

| Compound          | Concentration ($\mu$M) | $P_{\text{app}}$ ($\times 10^{-6}$ cm/sec) | Efflux ratio ($P_{\text{app}}$ A→B/$P_{\text{app}}$ B→A) |
|-------------------|------------------------|------------------------------------------|--------------------------------------------------|
| $[^{14}\text{C}]$TAK-063 | 3                      | 29.7 ± 0.50                             | 1.1                                              |
| $[^{3}\text{H}]$Digoxin  | 3                      | 1.77 ± 0.14                             | 5.5                                              |
| $[^{3}\text{H}]$Digoxin  | 0.1                    | 0.79 ± 0.01                             | 35.6                                             |
| $[^{14}\text{C}]$Antipyrine | 10                     | 41.6 ± 0.80                             | 1.1                                              |
| $[^{14}\text{C}]$Mannitol  | 10                     | 0.39 ± 0.03                             | 0.9                                              |

$P_{\text{app}}$, permeation coefficient.

>Data are expressed as mean values ± standard deviation (n = 3).
In vitro distribution of \([^{14}C]\)TAK-063 in rats after oral administration (1 mg/kg).

| Concentration (ng equivalents of \([^{14}C]\)TAK-063/g or mL) | 1 h | 3 h | 8 h | 24 h | 48 h | 168 h |
|---|---|---|---|---|---|---|
| Blood | 179 | 191 | 146 | 8 | 2 | 2 |
| Plasma | 236 | 261 | 194 | 9 | 1 | 0 |
| Brain | 110 | 121 | 106 | 21 | 3 | 1 |
| Spinal cord | 90 | 101 | 84 | 11 | 1 | 0 |
| Hypophysis | 304 | 393 | 280 | 45 | 18 | 5 |
| Eyes | 54 | 65 | 61 | 9 | 2 | 1 |
| Harderian glands | 539 | 637 | 503 | 34 | 10 | 3 |
| Submaxillary glands | 350 | 388 | 282 | 8 | 3 | 3 |
| Thyroid gland | 269 | 350 | 294 | 78 | 44 | 14 |
| Thymus | 164 | 190 | 142 | 9 | 2 | 1 |
| Heart | 371 | 419 | 346 | 68 | 27 | 9 |
| Lung | 271 | 315 | 245 | 15 | 6 | 2 |
| Liver | 1456 | 1584 | 1278 | 86 | 32 | 8 |
| Spleen | 187 | 241 | 182 | 12 | 3 | 2 |
| Pancreas | 358 | 419 | 323 | 48 | 21 | 5 |
| Adrenals | 744 | 875 | 630 | 39 | 10 | 2 |
| Kidneys | 585 | 671 | 507 | 60 | 22 | 8 |
| Testes | 227 | 257 | 225 | 118 | 63 | 21 |
| Skeletal muscle | 156 | 176 | 143 | 18 | 7 | 2 |
| Skin | 158 | 191 | 147 | 8 | 4 | 1 |
| Fat | 194 | 257 | 216 | 7 | LOQ | LOQ |
| Femur | 39 | 46 | 32 | 2 | 0 | 0 |
| Bone marrow | 182 | 214 | 167 | 8 | 1 | 1 |
| Stomach | 494 | 493 | 258 | 28 | 10 | 3 |
| Intestine | 445 | 549 | 411 | 30 | 6 | 2 |
| Bladder | 156 | 215 | 161 | 11 | 3 | 1 |

Table 4.

In vitro distribution ratio of \([^{14}C]\)TAK-063 into red blood cells in rats, dogs and human beings.

| Species | Concentration (µg/mL) | Distribution ratio (%) |
|---|---|---|
| Rats | | |
| 0.05 | 11.6 |
| 0.5 | 9.7 |
| 5 | 9.5 |
| Dogs | | |
| 0.05 | 42.4 |
| 0.5 | 42.3 |
| 5 | 40.2 |
| Human beings | | |
| 0.05 | 33.3 |
| 0.5 | 33.3 |
| 5 | 32.2 |

Table 5.

In vitro protein binding of \([^{14}C]\)TAK-063 and M-I.

| Species | Concentrations (µg/mL) | % Protein binding |
|---|---|---|
| Rat | 0.05 | 99.2 |
| 0.5 | 99.2 |
| 5 | 99.2 |
| Dog | 0.05 | 96.0 |
| 0.5 | 95.8 |
| 5 | 95.6 |
| Human beings | 0.05 | 96.4 |
| 0.5 | 96.5 |
| 5 | 96.4 |

M-I, TAK-063 metabolite.

1Data are expressed as mean values (n = 3).

2\(^{14}C\)TAK-063 is bound primarily to human serum albumin (87–90%) and α1-acid glycoprotein (44–68%) at the concentration range from 0.05 to 5 µg/mL.

component, accounting for 95.2 and 71.4% of total radioactivity, respectively. On average, the plasma concentrations of M-I accounted for 11.2% of total radioactivity in dogs, but were below the LOQ at all time-points examined in rats.

As shown in table 6, after oral administration to intact rats, 91.9% of dosed radioactivity was associated with faeces, with 4.7% being excreted via urine within 48 hr of administration. Unchanged TAK-063, M-I and other unidentified minor metabolites accounted for 41.5, 24.8 and 25.6% of the dose in faeces, and LOQ, 1.3% and 3.4% of the dose in urine, respectively. These minor metabolites were various conjugates of oxidative metabolites that were detected in urine, and a number of oxidative metabolites including PK-5 that were detected in faeces (fig. 4). After intraduodenal administration to BDC rats, 37.8% of dose radioactivity was associated with bile. Unchanged TAK-063, M-I and other unidentified minor metabolites (various conjugates of oxidative metabolites) accounted for 0.3, 2.2 and 35.3% of the dose in bile (table 6), respectively.

After oral administration to dogs, 95.6% of dosed radioactivity was associated with faeces, with 1.6% excreted via urine within 24 hr of administration (table 6). Unchanged TAK-063, M-I and various oxidative metabolites that were observed in urine and faeces accounted for 60.8, 18.4 and 16.5% of the dose in faeces, respectively (table 6). The amount of parent drug and M-I was less than 0.1% in the urine (table 6).

In vitro metabolism. Incubation of \([^{14}C]\)TAK-063 with human hepatic microsomes generated M-I and unidentified minor metabolites including UK-1 (structure unknown) and UK-2 (oxidative metabolite) (fig. 4). Among these metabolites, M-I was the major metabolite in human beings as well as in animals. No human-specific metabolite was noted in human hepatic microsomes.

CYP reaction phenotyping.

As shown in fig. 5, the CYP-expressing microsomal study suggested that CYP2C8, CYP2D6 and CYP3A4 were the major isoforms involved in the elimination of TAK-063 or formation of M-I, UK-I or UK-2.

The correlation study indicated that elimination of TAK-063 most strongly correlated with CYP2C8 (r = 0.7502, p ≤ 0.01) and CYP3A4/5 activities (r = 0.7074, p ≤ 0.01), followed by CYP1A2 (r = 0.5814, p ≤ 0.01), CYP2D6 (r = 0.7502, p ≤ 0.01) and CYP2C9 (r = 0.7629, p ≤ 0.01).
addition, formation of M-I also correlated with CYP1A2 activity \((r = 0.6205, p \leq 0.05)\). Formation of UK-2 correlated with CYP2C19 \((r = 0.6262, p \leq 0.05)\) and CYP3A4/5 activities \((r = 0.6824, p \leq 0.05)\). There was no significant correlation between the elimination of TAK-063, formation of M-I, UK-1 and UK-2 or any other marker enzyme activity (table 7).  

**CYP inhibition and induction.** TAK-063 inhibited CYP2C8 and CYP2C19 activities with IC₅₀ values of 8.4 and 12 μM, respectively (table 8). TAK-063 did not significantly inhibit CYP1A2, CYP2B6, CYP2C9, CYP2D6 or CYP3A4/5 activities, with IC₅₀ values >20 μM for all of these enzymes. As seen in table 8, M-I did not significantly inhibit any CYPs tested (IC₅₀ >10 μM). In addition, neither TAK-063 up to 20 μM nor M-I exhibited significant time-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 activities. Overall, TAK-063 and M-I exhibited minimal inducing effects on CYP1A2, CYP2B6 or CYP3A4/5 activities in vitro with average induction ratios <19% for TAK-063 and ≤8% for M-I (table 9). For CYP2B6, in the three-day treatment with 20-μM TAK-063, the induction ratio was 21% in one donor. In the two-day treatment with 20-μM TAK-063, the induction ratios of CYP2B6 in three donors ranged from 20 to 23%.

**Excretion.** After oral administration of [¹⁴C]TAK-063 to fed male rats and dogs, the mean total recovery of radioactivity at 168 hr after dosing was 97.3 and 97.9%, respectively (table 10). Faeces and urine accounted for 92.6% and 4.7% of the dose in rats, and 96.1 and 1.9% of the dose in dogs, indicating that the primary route of elimination of drug-derived radioactivity was via faeces. Unchanged TAK-063 accounted for <1% of the dose in urine and/or bile of rats and dogs, suggesting that urinary and/or biliary excretion of TAK-063 is minimal. After a single oral dose of [¹⁴C]TAK-063 at 1 mg/kg, the total

**Table 6.** Mean levels of TAK-063 and M-I in the excreta of rats and dogs after a single oral dose of [¹⁴C]TAK-063 at 1 mg/kg.¹²

| Compound   | % of administered dose | Rat         | Dog         |
|------------|------------------------|-------------|-------------|
|            |                        | Urine (0–48 h) | Faeces (0–48 h) | Bile (0–24 h) | Urine (0–24 h) | Faeces (0–72 h) |
| Total radioactivity |                        | 4.7         | 91.9        | 37.8         | 1.6         | 95.6            |
| TAK-063    |                        | LOQ         | 41.5        | 0.3          | LOQ         | 60.8            |
| M-I        |                        | 1.3         | 24.8        | 2.2          | 0.0         | 18.4            |
| Others¹    |                        | 3.4         | 25.6        | 35.3         | 1.6         | 16.5            |

LOQ, below the lower limit of quantification; M-I, TAK-063 metabolite.

¹Other unknown minor metabolites.

²For rats, data are expressed as the mean and standard deviation (n = 3, urine and faeces and n = 4, bile). For dogs, each value represents the mean ± standard deviation (n = 4).

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Correlation of elimination rates of TAK-063 or formation rates of metabolites with marker enzyme activities in microsomes of 16 human livers.

| Isoform   | Isoform-specific activity          | TAK-063 elimination | M-I formation | UK-1 formation | UK-2 formation |
|-----------|-----------------------------------|---------------------|---------------|----------------|---------------|
| CYP1A2    | Phenacetin-O-deethylation          | 0.5814              | 0.6205        | 0.5463         | -0.2294       |
| CYP2B6    | Bupropion hydroxylation            | 0.2469              | 0.1103        | 0.0106         | 0.0143        |
| CYP2C8    | Paclitaxel 6a-hydroxylation        | 0.7502              | 0.7431        | 0.7629         | 0.0936        |
| CYP2C9    | Diclofenac 4’-hydroxylation        | 0.2294              | 0.1688        | 0.3883         | 0.0197        |
| CYP2C19   | S-Methylenedioxy-4’-hydroxylation  | -0.0331             | -0.2307       | -0.3682        | 0.6262        |
| CYP2D6    | Dextromethorphan O-demethylation   | 0.1047              | -0.1085       | 0.4890         | 0.4659        |
| CYP3A4/5  | Midazolam 1’-hydroxylation         | 0.7074              | 0.3164        | 0.4683         | 0.6824        |

M-I, TAK-063 metabolite; UK-1, unidentified metabolite-1; UK-2, unidentified metabolite-2.

1$^P \leq 0.05$.
2$^P \leq 0.01$.

Discussion

In the present study, pre-clinical ADME properties of TAK-063 were characterized in animals, and these properties were subsequently studied in human beings. TAK-063 exhibited a favourable profile for efficacious brain tissue distribution and exposure in rats. After oral administration, TAK-063 was moderately absorbed in fed male rats and dogs and was highly permeable in intestinal cell assays while showing no P-gp or BCRP liability. The result is consistent with a previous report, which demonstrated that TAK-063 effectively crossed the blood–brain barrier and increased second messenger concentrations in the mouse striatum [9].

TAK-063 is characterized as a low clearance compound with a small volume of distribution in rats, but a high clearance compound with a moderate to high volume of distribution in dogs. These differences could be explained by higher plasma protein binding of TAK-063 in rats (>99%) than in dogs and human beings (95–96%). In a phase I clinical study of safety, tolerability and PK, TAK-063 exposure increased with dose within the 3 mg to 1000 mg dose range [14]. Clearance (CL/F) and volume of distribution (V/F) of TAK-063 also increased with dose, ranging from 14.0 to 232.0 L/h and from 407 to 5570.7 L, respectively [14]. The rat tissue distribution study indicated that radioactivity was distributed rapidly into tissues including the brain after oral administration of $^{[14]}$C]TAK-063. The ratio of brain-to-plasma TAK-063 was approximately 1 at 3 hr after dosing. In a human $^{[14]}$C]T-773 PET study of PDE10A binding after TAK-063 oral administration, TAK-063 bound PDE10A in a plasma concentration-dependent manner [15]. These data demonstrate that TAK-063 was well distributed in the brains of animal models and human beings.

TAK-063 is mainly metabolized to M-I by hydroxylation of the mono-substituted pyrazole moiety. In addition, low levels of miscellaneous oxidative metabolites and their conjugates were detected in rats and dogs. Approximately 20–30% of the dose was eliminated as M-I via faeces and urine in rats and dogs treated with $^{[14]}$C]T-773, suggesting that the formation of M-I is one of the primary elimination pathways of TAK-063, with no difference in species noted. However, exposure levels of M-I were much lower than those of parent drug TAK-063 in dogs and rats. In contrast, M-I in human beings showed similar PK properties to TAK-063, with M-I-to-parent ratios of approximately 1 for $C_{\text{max}}$, $AUC_{0-\text{inf}}$ and $AUC_{0-\text{inf}}$ (Tsai et al. 2016). Interestingly, plasma protein binding of M-I is higher than TAK-063 (≥99% versus 96%, table 5), suggesting that the free fraction of TAK-063 is significantly higher.
DMSO, dimethylsulphoxide; M-I, TAK-063 metabolite.

Data are expressed as a mean of three lots of hepatocytes, except for CYP2B6, in which the values represent one lot and an average of two lots of hepatocytes for TAK-063 at 0 and 1 μM, respectively.

0.1% DMSO.

Enzyme activities and mRNA were assayed for TAK-063 (two- or three-day treatment) and M-I (three-day treatment), respectively.

Rifampin at 10 and 20 μM was used for TAK-063 and M-I assays, respectively. Only CYP3A4 was determined for the mRNA assay.

Table 9.
Induction of CYP1A2, CYP2B6 and CYP3A4/5 by TAK-063 or M-I in human hepatocytes.

| Treatment | (μM) | CYP1A2<sup>4</sup> | CYP2B6<sup>5</sup> | CYP3A4/5<sup>6</sup> |
|-----------|------|-------------------|-------------------|-------------------|
| TAK-063   | 0    | 5.2 ± 2.7         | 11.2              | 2.3 ± 1.1         |
|           | 1    | 5.5 ± 2.7         | 11.1              | 2.6 ± 1.3         |
|           | 3    | 5.7 ± 2.8         | 11.6 ± 1.4        | 3.2 ± 1.4         |
|           | 10   | 6.0 ± 2.8         | 13.3 ± 0.6        | 5.5 ± 2.9         |
|           | 20   | 5.8 ± 3.0         | 18.8 ± 2.0        | 9.2 ± 3.4         |
| M-I       | 0    | 0.0               | 0.0               | 0.0               |
|           | 0.2  | -0.560 ± 0.462    | -0.00844 ± 1.14866| 1.02 ± 0.74      |
|           | 0.5  | -0.539 ± 0.788    | 1.72 ± 4.23       | 2.43 ± 2.86      |
|           | 1    | 0.129 ± 1.122     | 5.51 ± 6.56       | 5.85 ± 4.45      |
|           | 2    | -0.217 ± 1.200    | 7.90 ± 8.55       | 7.92 ± 5.80      |

Table 10.
Recovery of total radioactivity after single oral dose of [14C]TAK-063 at 1 mg/kg.<sup>1</sup>

| Species | Time after dose (hr) | Recovery of radioactivity (% of administered dose) |
|---------|----------------------|-----------------------------------------------|
|         |                      | Urine | Faeces | Bile | Total  |
| Rat<sup>2</sup> (n = 3) | 4       | 1.1   | –      | –    | NE     |
|        | 8       | 2.0   | –      | –    | NE     |
|        | 24      | 4.6   | 77.9   | –    | 82.5   |
|        | 48      | 4.7   | 91.9   | –    | 96.6   |
|        | 72      | 4.7   | 92.3   | –    | 97.0   |
|        | 96      | 4.7   | 92.4   | –    | 97.1   |
|        | 120     | 4.7   | 92.5   | –    | 97.2   |
|        | 144     | 4.7   | 92.6   | –    | 97.3   |
|        | 168     | 4.7   | 92.6   | –    | 97.3   |
| Rat<sup>3</sup> (n = 4) | 4       | 1.0   | –      | –    | NE     |
|        | 8       | 1.9   | –      | 26.0 | NE     |
|        | 24      | 3.3   | 56.8   | –    | 37.8   |
| Dog (n = 4) | 4     | –     | –      | –    | NE     |
|          | 8      | 0.8   | –      | –    | NE     |
|          | 24     | 1.6   | 54.2   | –    | 55.8   |
|          | 48     | 1.8   | 94.3   | –    | 96.1   |
|          | 72     | 1.9   | 95.6   | –    | 97.5   |
|          | 96     | 1.9   | 95.8   | –    | 97.7   |
|          | 120    | 1.9   | 96.0   | –    | 97.8   |
|          | 144    | 1.9   | 96.0   | –    | 97.9   |
|          | 168    | 1.9   | 96.1   | –    | 97.9   |

NE, not estimated.

Dash (–) indicates not determined; data are expressed as mean values (n = 3 or 4).

Expired air was collected at 4, 8 and 24 hr after dosing. Radioactivity was not detectable in all expired air samples collected.

Bile duct-cannulated rats after a single intraduodenal administration.

be low despite showing similar pharmacological potency to unchanged TAK-063 in vitro. One reason led us to this conclusion. The brain distribution of M-I was confirmed after intravenous administration to rats, but the brain-to-plasma concentration ratio of M-I (K<sub>P</sub> = 0.014) was markedly lower than that of TAK-063.

The correlation and CYP-expressing microsomal studies suggested that metabolism of TAK-063 is mediated by multiple CYPs. CYP2C8 and CYP3A4/5 appear to be the major metabolizing enzymes of TAK-063. TAK-063 inhibited CYP2C8, CYP2C19 and P-gp activities with IC<sub>50</sub> values of 8.4, 12 and 7.13 μM, respectively. Thus, these findings suggest that potential is low for TAK-063 inhibition of CYP2C8, CYP2C19 and P-gp.

After oral administration of [14C]TAK-063 to rats and dogs, recovery of administered radioactivity was nearly complete (>97% of the dose), with >92% of the dose being excreted into faeces in both species. The concentrations of unchanged parent drug were below the lower limit of quantification in rat and dog urine, and <1% of the dose in rat bile, suggesting negligible importance of urinary and biliary excretion of TAK-063 in both species. This was in agreement with the low renal clearance of TAK-063 (CL<sub>R</sub> < 7 mL/h) observed in a phase 1 study of TAK-063 at a dose range of 3 to 1000 mg (Tsai et al. 2016). Unchanged TAK-063 accounted for 41.5 and 60.8% of the dose in faeces of fed rats and dogs, respectively, after oral administration, indicating that TAK-063 may have poor solubility. A food effect on the PK of TAK-063 was observed in dogs as drug absorption could be reduced by approximately 50% through co-administration with food in these species.

Pre-clinical data from these ADME studies demonstrate a favourable pharmacokinetic profile in animals for TAK-063 including wide distribution in the brain and other organs and targeting CNS domains involved in schizophrenia.
pathophysiology. A phase 2 clinical trial of TAK-063 in subjects with an acute exacerbation of schizophrenia was recently completed (NCT02477020).

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Author responsibility

Tagawa, Tohyama, Sudo, Morohashi, Kato and Takahashi participated in research design; Tohyama, Sudo and Kato conducted experiments; Tagawa, Tohyama, Sudo, Morohashi and Kato performed data analysis; Tagawa, Tohyama, Sudo, Morohashi, Kato and Takahashi wrote or contributed to the writing of the manuscript.

Chemical structure disclosure

All authors certify that the chemical structure of all new compounds cited or discussed in this manuscript is presented or a citation to the published structure is provided, in accordance with the journal’s editorial policy.

Disclosure statement

Kimio Tohyama, Miyako Sudo, Suguru Kato and Yoshihiko Tagawa are employees of Takeda Pharmaceutical Company Limited. Akio Morohashi and Junzo Takahashi were employees of Takeda Pharmaceutical Company Limited at the time of this study.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Methods.